

# Extensive Copy Number Variation in Fermentation-Related Genes Among *Saccharomyces cerevisiae* Wine Strains

Jacob Steenwyk and Antonis Rokas<sup>1</sup>

Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee 37235

ORCID IDs: 0000-0002-8436-595X (J.S.); 0000-0002-7248-6551 (A.R.)

**ABSTRACT** Due to the importance of *Saccharomyces cerevisiae* in wine-making, the genomic variation of wine yeast strains has been extensively studied. One of the major insights stemming from these studies is that wine yeast strains harbor low levels of genetic diversity in the form of single nucleotide polymorphisms (SNPs). Genomic structural variants, such as copy number (CN) variants, are another major type of variation segregating in natural populations. To test whether genetic diversity in CN variation is also low across wine yeast strains, we examined genome-wide levels of CN variation in 132 whole-genome sequences of *S. cerevisiae* wine strains. We found an average of 97.8 CN variable regions (CNVRs) affecting ~4% of the genome per strain. Using two different measures of CN diversity, we found that gene families involved in fermentation-related processes such as copper resistance (*CUP*), flocculation (*FLO*), and glucose metabolism (*HXT*), as well as the *SNO* gene family whose members are expressed before or during the diauxic shift, showed substantial CN diversity across the 132 strains examined. Importantly, these same gene families have been shown, through comparative transcriptomic and functional assays, to be associated with adaptation to the wine fermentation environment. Our results suggest that CN variation is a substantial contributor to the genomic diversity of wine yeast strains, and identify several candidate loci whose levels of CN variation may affect the adaptation and performance of wine yeast strains during fermentation.

## KEYWORDS

domestication  
genomics  
structural  
variation  
carbohydrate  
metabolism  
alcoholic  
fermentation

*Saccharomyces cerevisiae*, commonly known as baker's or brewer's yeast, has been utilized by humans for the production of fermented beverages since at least 1350 B.C.E. but may go as far back as the Neolithic period 7000 yr ago (Mortimer 2000; Cavalieri *et al.* 2003). Phylogenetic analyses and archaeological evidence suggest wine strains originated from Mesopotamia (Bisson 2012), and were domesticated in a single event around the same time as the domestication of grapes (Schacherer *et al.* 2009; Sicard and Legras 2011). Further phylogenetic, population structure and identity-by-state analyses of single nucleotide

polymorphism (SNP) data reveal close affinity and low genetic diversity among wine yeast strains across the globe, consistent with a domestication-driven population bottleneck (Liti *et al.* 2009; Schacherer *et al.* 2009; Sicard and Legras 2011; Cromie *et al.* 2013; Borneman *et al.* 2016). These low levels of genetic diversity have led some to suggest that further wine strain development should be focused on introducing new variation into wine yeasts rather than exploiting their standing variation (Borneman *et al.* 2016).

Many wine strains have characteristic variants that have presumably been favored in the wine-making environment (Marsit and Dequin 2015). For example, adaptive point mutations, deletions, and rearrangements in the promoter and coding sequence of *FLO11* contribute to flocculation and floating, thereby increasing yeast cells' ability to obtain oxygen in the hypoxic environment of liquid fermentations (Fidalgo *et al.* 2006). Similarly, duplications of *CUP1* are strongly associated with resistance to copper (Warringer *et al.* 2011), which, at high concentrations, can cause stuck fermentations, and *THI5*, a gene involved in thiamine metabolism whose expression is associated with an undesirable rotten-egg sensory perception in wine, is absent or downregulated among wine strains and their derivatives (Bartra *et al.* 2010; Brion *et al.*

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<sup>1</sup>Corresponding author: Department of Biological Sciences, Vanderbilt University, VU Station B 351634, Nashville, TN 37235. E-mail: [antonis.rokas@vanderbilt.edu](mailto:antonis.rokas@vanderbilt.edu)

2014). As these examples illustrate, the mutations underlying these, as well as many other, presumably adaptive, traits are not only SNPs, but also genomic structural variants, such as duplications, insertions, inversions, and translocations (Pretorius 2000; Marsit and Dequin 2015).

Copy number (CN) variants, a class of structural variants defined as duplicated or deleted loci ranging from 50 bp to whole chromosomes (Zhang *et al.* 2009; Arlt *et al.* 2014), have received considerable attention due to their widespread occurrence (Sudmant *et al.* 2010; Bickhart *et al.* 2012; Axelsson *et al.* 2013; Pezer *et al.* 2015) as well as their influence on gene expression and phenotypic diversity (Freeman *et al.* 2006; Henrichsen *et al.* 2009). Mechanisms of CN variant evolution include nonallelic homologous recombination (Lupski and Stankiewicz 2005) and retrotransposition (Kaessmann *et al.* 2009). CN variants are well studied in various mammals, including humans (*Homo sapiens*; Sudmant *et al.* 2015), cattle (*Bos taurus*; Bickhart *et al.* 2012), the house mouse (*Mus musculus*; Pezer *et al.* 2015), and the domestic dog (*Canis lupus familiaris*; Axelsson *et al.* 2013), where they are important contributors to genetic and phenotypic diversity.

Relatively few studies have investigated whole-genome CN profiles in fungi (Hu *et al.* 2011; Farrer *et al.* 2013; Steenwyk *et al.* 2016). For example, the observed CN variation of chromosome 1 in the human pathogen *Cryptococcus neoformans* results in the duplications of *ERG11*, a lanosterol-14- $\alpha$ -demethylase and target of the triazole antifungal drug fluconazole (Lupetti *et al.* 2002), and *AFR1*, an ATP binding cassette (ABC) transporter (Sanguinetti *et al.* 2006), leading to increased fluconazole resistance (Sionov *et al.* 2010). Similarly, resistance to itraconazole, a triazole antifungal drug, is attributed to the duplication of cytochrome P-450-dependent C-14 lanosterol  $\alpha$ -demethylase (*pdmA*)—a gene whose product is essential for ergosterol biosynthesis—in the human pathogen *Aspergillus fumigatus* (Oshero *et al.* 2001). Finally, in the animal pathogen *Batrachochytrium dendrobatidis*, the duplication of Supercontig V is associated with increased fitness in the presence of resistance to an antimicrobial peptide, although the underlying genetic elements involved remain elusive (Farrer *et al.* 2013).

Similarly understudied is the contribution of CN variation to fungal domestication (Gibbons and Rinker 2015; Gallone *et al.* 2016). Notable examples of gene duplication being associated with microbial domestication include those of  $\alpha$ -amylase in *Aspergillus oryzae*, which is instrumental in starch saccharification during the production of sake (Hunter *et al.* 2011; Gibbons *et al.* 2012), and of the *MAL1* and *MAL3* loci in beer-associated strains of *S. cerevisiae* that metabolize maltose—the most abundant sugar in the beer wort (Gallone *et al.* 2016; Gonçalves *et al.* 2016). Beer strains of *S. cerevisiae* often contain additional duplicated genes associated with maltose metabolism, including *MPH2* and *MPH3*, two maltose permeases, and the putative maltose-responsive transcription factor, *YPR196W* (Gonçalves *et al.* 2016). Adaptive gene duplication in *S. cerevisiae* has also been detected in experimentally evolved populations (Dunham *et al.* 2002; Gresham *et al.* 2008; Dunn *et al.* 2012). Specifically, duplication of the locus containing the high affinity glucose transporters *HXT6* and *HXT7* has been observed in adaptively evolved asexual strains (Kao and Sherlock 2008), as well as in populations grown in a glucose-limited environment (Brown *et al.* 1998; Dunham *et al.* 2002; Gresham *et al.* 2008). Altogether, these studies suggest that CN variation is a significant contributor to *S. cerevisiae* evolution and adaptation.

To determine the contribution of CN variation to genome evolution in wine strains of *S. cerevisiae*, we characterized patterns of CN variation across the genomes of 132 wine strains, and determined the functional impact of CN variable genes in environments reflective of wine-making. Our results suggest that there is substantial CN variation

among wine yeast strains, including in gene families (such as *CUP*, *FLO*, *HXT*, and *MAL*) known to be associated with adaptation in the fermentation environment. More generally, it raises the hypothesis that CN variation is an important contributor to adaptation during microbial domestication.

## MATERIALS AND METHODS

### Data mining, quality control, and mapping

Raw sequence data for 132 *S. cerevisiae* wine strains were obtained from three studies (Borneman *et al.* 2016, 127 strains, Bioproject ID: PRJNA303109; Dunn *et al.* 2012, two strains, Bioproject ID: SRA049752; Skelly *et al.* 2013, three strains, Bioproject ID: PRJNA186707) (Supplemental Material, File S1 and Figure S1 in File S2). Altogether, these 132 strains represent a diverse set of commercial and noncommercial isolates from the “wine” yeast clade (Borneman *et al.* 2016).

Sequence reads were quality-trimmed using Trimmomatic, version 0.36 (Bolger *et al.* 2014), with the following parameters and values: leading:10, trailing:10, slidingwindow:4:20, minlen:50. Reads were then mapped to the genome sequence of the *S. cerevisiae* strain S288c (annotation release: R64.2.1; <http://www.yeastgenome.org/>) using Bowtie 2, version 1.1.2 (Langmead and Salzberg 2012), with the “sensitive” parameter on. For each sample, mapped reads were converted to the bam format, sorted and merged using SAMtools, version 1.3.1. Sample depth of coverage was obtained using the SAMtools depth function (Li *et al.* 2009).

### CN variant identification

To facilitate the identification of single nucleotide polymorphisms (SNPs), we first generated mpileup files for each strain using SAMtools, version 1.3.1 (Li *et al.* 2009). Using the mpileup files as input to VarScan, version 2.3.9 (Koboldt *et al.* 2009, 2012), we next identified all statistically significant SNPs (Fisher’s Exact test;  $P < 0.05$ ) present in the 132 strains that had a read frequency of at least 0.75 and minimum coverage of 8 $\times$ . This step enabled us to identify 149,782 SNPs. By considering only SNPs that harbored a minor allele frequency of at least 10%, we retained 43,370 SNPs. These SNPs were used to confirm the evolutionary relationships among the strains using Neighbor-Net phylogenetic network analyses in SplitsTree, version 4.14.1 (Huson 1998) as well as the previously reported low levels of SNP diversity (Figure S2 in File S2, and Borneman *et al.* 2016).

To detect and quantify CN variants we used Control-FREEC, version 9.1 (Boeva *et al.* 2011, 2012), which we chose because of its low false positive rate, and high true positive rate (Duan *et al.* 2013). Importantly, the average depth of coverage or read depth of the 132 strains was  $30.1 \pm 14.7\times$  (minimum: 13.0 $\times$ , maximum: 104.5 $\times$ ; Figure S3 in File S2), which is considered sufficient for robust CNV calling (Sims *et al.* 2014).

Control-FREEC uses LOESS modeling for GC-bias correction and a LASSO-based algorithm for segmentation. Implemented Control-FREEC parameters included window = 250, minExpectedGC = 0.35, maxExpectedGC = 0.55, and telocentromeric = 7000. To identify statistically significant CN variable loci ( $P < 0.05$ ), we used the Wilcoxon Rank Sum test. The same Control-FREEC parameters, but with a window size of 25 bp, were used to examine CN variation within the intragenic Serine/Threonine-rich sequences of *FLO11* (Lo and Dranginis 1996). Bedtools, version 2.25 (Quinlan and Hall 2010) was used to identify duplicated or deleted genic loci (*i.e.*, CN variable loci) that overlapped with genes by at least one nucleotide. The CN of each gene (genic CN) was then calculated as the average CN of the 250 bp windows that overlapped with the gene’s location coordinates in the genome. The same

method was used to determine nongenic CN for loci that did not overlap with genes (*i.e.*, nongenic CN variable loci). To identify statistically significant differences between CN variable loci that were duplicated *vs.* those that were deleted, we employed the Mann-Whitney *U*-test (Wilcoxon rank-sum test) with continuity correction (Wallace 2004).

### Diversity in CN variation and GO enrichment

To identify CN diverse loci across strains, we used two different measures. The first measure calculates the statistical variance ( $s^2$ ) for each locus where CN variants were identified in one or more strains;  $s^2$  values were subsequently  $\log_{10}$  normalized.  $\log_{10}(s^2)$  accounts for diversity in raw CN values, but not for diversity in CN allele frequencies. Thus, we also employed a second measure based on the Polymorphic Index Content (PIC) algorithm, which has previously been used to identify informative microsatellite markers for linkage analyses by taking into account both the number of alleles present and their frequencies (Keith *et al.* 1990; Risch 1990). PIC has also been used to quantify population-level diversity of simple sequence repeat loci and restriction fragment length polymorphisms in maize (Smith *et al.* 1997). PIC values were calculated for each locus harboring at least one CN variant based on the following formula:

$$\text{PIC} = 1 - \sum_{i=a}^z i^2$$

where  $i^2$  is the squared frequency of  $a$  to  $z$  CN values (Smith *et al.* 1997). PIC values may range from 0 (no CN diversity) to 1 (all CN alleles are unique). Both  $\log_{10}(s^2)$  and PIC are measurements of CN diversity between strains, and do not account for any sequence variation between copies of the same locus.

To create a list of loci exhibiting high CN diversity for downstream analyses, we retained only those loci that fell within the 50th percentile of  $\log_{10}(s^2)$  values (min =  $-2.12$ , median =  $-1.02$ , and max =  $2.40$ ), or the 50th percentile of PIC values (min =  $0.02$ , median =  $0.14$ , and max =  $0.96$ ).

Genes overlapping with loci exhibiting high CN diversity were used for Gene ontology (GO) enrichment analysis with AmiGO2, version 2.4.24 (Carbon *et al.* 2009) using the PANTHER Overrepresentation Test (release 20160715) with default settings. This test uses the PANTHER GO database, version 11.0 (Thomas *et al.* 2003; release date July 15, 2016), which is directly imported from the GO Ontology database, version 1.2 (Gene Ontology Consortium2004; release date 27 October, 2016), a reference gene list from *S. cerevisiae*, and a Mann-Whitney *U*-test (Wilcoxon rank-sum test) with Bonferroni multi-test corrected *P*-values to identify over- and under-represented GO terms (Mi *et al.* 2013). Statistical analyses and figures were created using pheatmap, version 1.0.8 (Kolde 2012), gplots, version 3.0.1, ggplot2 (Wickham 2009), or standard functions in R, version 3.2.2 (R Development Core Team 2011).

### Identifying loci absent in the reference strain

To identify loci absent from the reference strain but present in other strains, we assembled unmapped reads from the 20 strains with the lowest percentage of mapped reads. The percentage of mapped reads was determined using SAMtools (Li *et al.* 2009); its average across strains was 96% (min = 70.5% and max = 99%; Figure S4 in File S2). Unmapped reads from the 20 strains with the lowest percentage of mapped reads were assembled using SPAdes, version 3.8.1 (Bankevich *et al.* 2012). The identity of scaffolds longer than the average length of a *S. cerevisiae*'s gene ( $\sim 1400$  bp) was determined using blastx from NCBI's BLAST, version 2.3.0 (Madden 2013) against a local copy of

the GenBank nonredundant protein database (downloaded on January 5, 2017).

### Data availability

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article.

## RESULTS

### Descriptive statistics of CN variation

To examine CN variation across wine yeasts, we generated whole genome CN profiles for 132 strains (File S1 and Figure S5 in File S2). Across all strains, we identified a total of 2820 CNVRs that overlapped with 2061 genes and spanned 3.7 Mb. The size distribution of CNVRs was skewed toward CN variants that were  $< 1$  kb in length (Figure 1A, and Figure S6A and Table S1 in File S2). Most CNVRs stemming from duplications were  $< 1$  kb in size, whereas most CNVRs stemming from deletions were between 5 and 7 kb (Figure S7 in File S2). These deletions were flanked by Ty transposable elements, which have been shown to frequently induce deletions (Xu and Boeke 1987). Strains had an average of  $97.8 \pm 9.5$  CNVRs (median = 86) (Figure S6B in File S2) that affected an average of  $4.3 \pm 0.1\%$  of the genome (median = 4.1%) (Figure S6C in File S2).

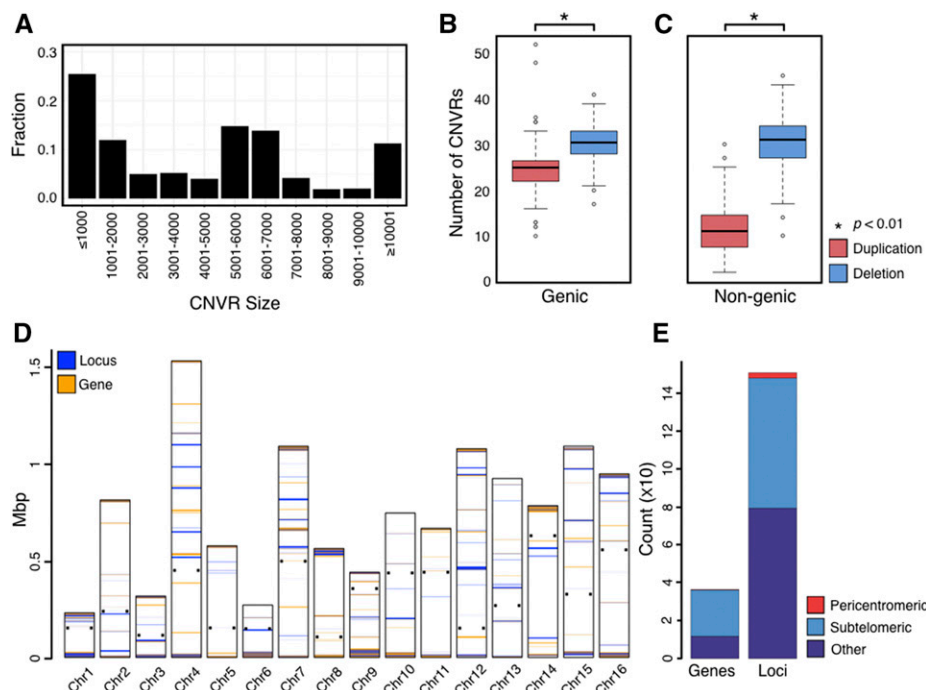
Due to the known influence of CN variable genes (Henrichsen *et al.* 2009; Orozco *et al.* 2009), we next quantified the number of genic and nongenic CNVRs (Figure 1, B and C). We found statistically significant differences in the number of duplicated and deleted loci that are genic or nongenic (Mann-Whitney *U*-test;  $P < 0.01$  for both genic and nongenic comparisons), revealing that there were significantly more deleted genic and nongenic CNVRs than duplicated ones.

### CN diversity in subtelomeres

To identify loci that exhibited high CN diversity, we retained only those loci that fell within the 50th percentile of at least one of our two different measures [ $\log_{10}(s^2)$  and PIC] across the 132 strains. These loci covered  $\sim 6\%$  of the yeast genome. The distributions of the two measures (Figure S8 in File S2) were similar, with 1326 loci (Figure S8C in File S2) and 291 genes (Figure S8D in File S2) identified in the top 50% of CN diverse genes by both measures.

In addition, the  $\log_{10}(s^2)$  measure identified an additional 85 loci and 54 genes in its set of top 50% genes, and PIC an additional 85 loci and 18 genes. In total, our analyses identified 1502 loci and 363 genes showing high CN diversity. Among the genes harboring the highest  $\log_{10}(s^2)$  and PIC values were *YLR154C-G* [PIC = 0.96;  $\log_{10}(s^2)$  = 2.16], *YLR154W-A* [PIC = 0.96;  $\log_{10}(s^2)$  = 2.16], *YLR154W-B* [PIC = 0.96;  $\log_{10}(s^2)$  = 2.16], *YLR154W-C* [PIC = 0.96;  $\log_{10}(s^2)$  = 2.16], *YLR154W-E* [PIC = 0.96;  $\log_{10}(s^2)$  = 2.16], *YLR154W-F* [PIC = 0.96;  $\log_{10}(s^2)$  = 2.16] and *YLR154C-H* [PIC = 0.93;  $\log_{10}(s^2)$  = 2.40]; these genes are all encoded within the 25S rDNA or 35S rDNA locus. The rDNA locus is known to be highly CN diverse (Gibbons *et al.* 2015), thereby demonstrating the utility and efficacy of our CN calling protocol as well as our two measures of CN diversity. We next generated CN diversity maps for all 16 *S. cerevisiae* chromosomes (Figure 1D; Figure S9 in File S2). CN diversity was higher in loci and genes located in subtelomeres (defined as the 25 kb of DNA immediately adjacent to the chromosome ends; Barton *et al.* 2003). Specifically, 684/1502 (45.5%) of CN diverse loci and 243/363 (66.9%) CN diverse genes were located in the subtelomeric regions. Conducting the same analysis using an alternative definition of subtelomere (defined as the DNA between the chromosome's end to the first essential gene; Winzeler *et al.* 1999) showed similar results.





**Figure 1** Size distribution and location of CN variable loci. (A) The fraction of CNVRs (y-axis) for a given size range. Most CNVRs are  $\leq 1000$  bp. (B, C) Deleted genic (B) and nongenic (C) CNVRs are more prevalent than duplicated ones ( $P < 0.01$  for both comparisons). Note that 26.23% of genic duplications occurred in multiples of three. (D) Location of CN variable loci across the 16 yeast chromosomes. The small, black squares on either side of each chromosome denote centromere location. Chromosomes are oriented with the start of the chromosome on the bottom and the end on top. Loci (blue bars) and genes (orange bars) harboring high  $\log_{10}(s^2)$  or PIC values are shown. (E) A total of 684 of the 1502 CN diverse loci and 243 of the 363 CN diverse genes reside in subtelomeric regions of the yeast genome; in contrast, very few are found in pericentromeric regions (28 loci and three genes).

Specifically, 721/1502 (48%) of CN diverse loci and 233/363 (64.2%) of CN diverse genes were located in the subtelomeric regions.

### GO enrichment of CN diverse genes

To determine the functional categories over- and under-represented in the 363 genes showing high CN diversity, we performed GO enrichment analysis. The majority of enriched GO terms were associated with metabolic functions such as  $\alpha$ -GLUCOSIDASE ACTIVITY ( $P < 0.01$ ) and CARBOHYDRATE TRANSPORTER ACTIVITY ( $P < 0.01$ ) (Figure 2 and File S3 in File S1).

Genes associated with these GO terms include *SUC2* (*YIL162W*, involved in hydrolyzing sucrose), all six members from the *MAL* gene family (involved in the fermentation of maltose and other carbohydrates), and all five members of the *IMA* gene family (involved in isomaltose, sucrose, and turanose metabolism). Other enriched categories were associated with multi-cellular processes such as the FLOCCULATION ( $P < 0.01$ ) and AGGREGATION OF UNICELLULAR ORGANISMS ( $P = 0.03$ ). All members of the *FLO* gene family (involved in flocculation) and *YHR213W* (a flocculin-like gene) were associated with these GO enriched terms.

Contrary to over-represented GO terms, under-represented terms were associated with genes whose protein products are part of the interactome or protein-protein interactions such as PROTEIN COMPLEX ( $P < 0.01$ ), MACROMOLECULAR COMPLEX ASSEMBLY ( $P = 0.03$ ), TRANSFERASE COMPLEX ( $P < 0.01$ ), and RIBONUCLEOPROTEIN COMPLEX BIOGENESIS ( $P = 0.04$ ). Our finding of under-represented GO terms being associated with multi-unit protein complexes supports the gene balance hypothesis, which states that the stoichiometry of genes contributing to multi-subunit complexes must be maintained to conserve kinetics and assembly properties (Birchler and Veitia 2010, 2012). Thus, genes associated with multi-unit protein complexes are unlikely to exhibit CN variation.

### Genic CN diversity

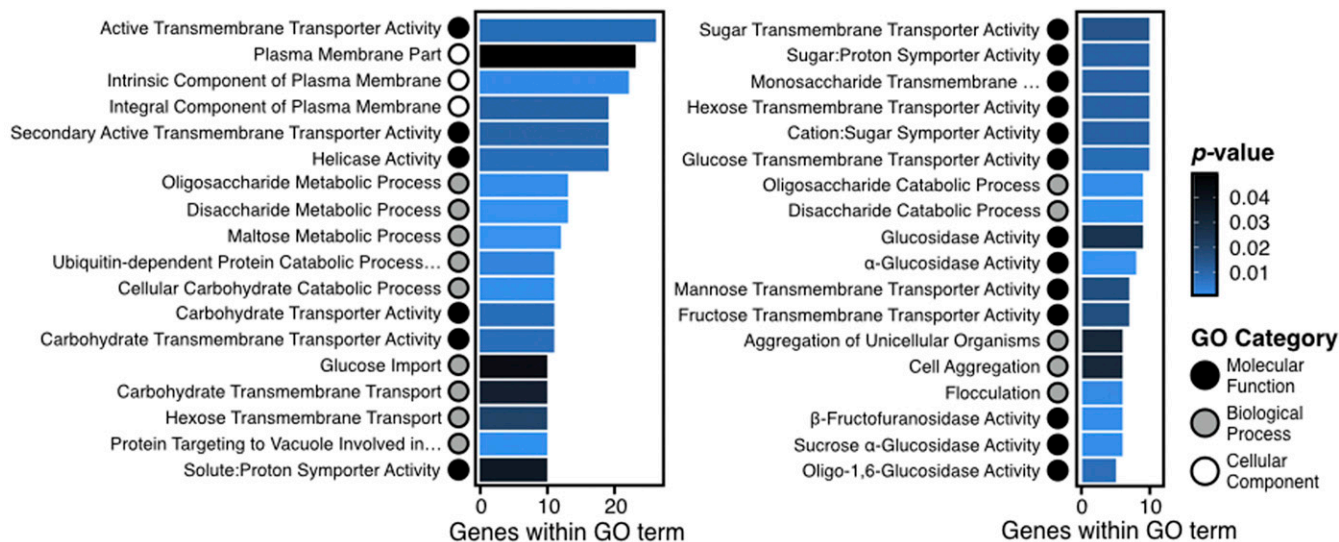
To further understand the structure of CN variation in highly diverse CN genes, we first calculated the absolute CN of 23 genes associated with GO enriched terms related to wine fermentation processes (e.g., metabolic functions; Figure 2 and File S3 in File S1) as well as 57 genes with

the highest PIC or  $\log_{10}(s^2)$  values (File S4 in File S1 and Figure S10 in File S2; 69 total unique genes). Among these 69 genes, gene CN ranged from 0 to 92; both the highest CN diversity and absolute CN values were observed in segments of the rDNA locus (mentioned above).

Importantly, 35 of the 69 genes have also been reported to have functional roles in fermentation-related processes. The remaining 34 genes with high scores for CN diversity, to our knowledge, have no known association with wine fermentation (e.g., *YAL064W-B*, *FRK1/YPL141C*, and *ENA5/YDR038C* among others). All genes with high CN diversity scores are listed in File S4 in File S1 and summarized in Figure S10 in File S2. For the purposes of this manuscript, we chose to focus on genes related to fermentation. For example, the CNs of *PAU3* (*YCR104W*), a gene active during alcoholic fermentation, and its gene neighbor *ADH7* (*YCR105W*), an alcohol dehydrogenase, both varied between zero and three. Similarly, the absolute CN of the locus containing both *CUP1-1* (*YHR053C*; PIC = 0.868) and its paralog *CUP1-2* (*YHR055C*; PIC = 0.879) ranged from 0 and 14 (Figure 3 and File S4 in File S1), with 90 strains (68.2%) showing duplications (i.e., a CN  $> 1$ ), and another 11 strains (8.3%) a deletion (i.e., a CN of 0). Interestingly, multiple copies of *CUP1* confer copper resistance to wine strains of *S. cerevisiae*, with CN variation at this locus thought to be associated with domestication (Warringer *et al.* 2011; Marsit and Dequin 2015).

The expression of *SNO* family members is induced just prior to, or after, the diauxic shift as a response to nutrient limitation, and is associated with vitamin B acquisition (Padilla *et al.* 1998; Rodríguez-Navarro *et al.* 2002). We found that *SNO2* (*YNL334C*) and *SNO3* (*YFL060C*) were among the 363 genes with highest CN diversity. *SNO2* was duplicated in 14 strains (10.6%) and deleted in nine strains (6.8%), while *SNO3* was deleted in 117 strains (88.6%) (Figure 3). The other two members of the *SNO* gene family, *SNO1* (*YMR095C*) and *SNO4* (*YMR322C*), both showed a CN of 1 in all strains.

Another gene family whose members show high CN diversity is the *THI* gene family, which is responsible for thiamine metabolism, and is activated at the end of the growth phase during fermentation



**Figure 2** GO enriched terms from high CN diverse genes. Molecular function (black), biological process (gray), and cellular component (white) GO categories are represented by circles, and are enriched among the 363 genes that overlap with CN diverse loci. Enriched terms are primarily related to metabolic function, such as  $\alpha$ -GLUCOSIDASE ACTIVITY ( $P < 0.01$ ), CARBOHYDRATE TRANSPORTER ACTIVITY ( $P < 0.01$ ) and FLOCCULATION ( $P < 0.01$ ).

(Brion *et al.* 2014). Specifically, *THI13* (*YDL244W*; PIC = 0.759) was among the 57 genes with the highest CN diversity (File S4 in File S1), and *THI5* (*YFL058W*) and *THI12* (*YNL332W*) among the 363 most CN diverse genes (File S3 in File S1). *THI13* was duplicated in 82 strains (62.1%) and deleted in two strains (1.5%) (Figure 3). In contrast, *THI5* was deleted in 121 strains (91.67%), whereas *THI12* was deleted in 23 strains (17.42%), and duplicated in only three strains (2.27%). Lastly, the CN of the last *THI* gene family member, *THI11* (*YJR156C*), did not exhibit CN variation.

In addition to the high CN diversity observed in all six members of the *MAL1* and *MAL3* loci responsible for maltose metabolism and growth on sucrose (Stambuk *et al.* 2000; Gallone *et al.* 2016), *MAL13* (*YGR288W*; PIC = 0.53) was among the 57 genes with the highest CN diversity (File S4 in File S1). Evaluation of the absolute CN of all *MAL1* locus genes (Figure 3) showed that *MAL11*, *MAL12* (*YGR292W*), and *MAL13* were deleted in 65 (49.2%), 86 (65.2%), and 61 strains (46.2%), respectively. In contrast, the *MAL3* locus genes *MAL31* (*YBR298C*), *MAL32* (*YBR299W*), and *MAL33* (*YBR297W*) were duplicated in 100 (75.8%), 99 (75%), and 98 strains (74.2%), respectively. Interestingly, we did not observe any deletions in any of the *MAL3* locus genes across the 132 strains. When considering all members of the *MAL* gene family, we found that the 132 strains differed widely in their degree to which the locus had undergone expansion or contraction (Figure S11 in File S2).

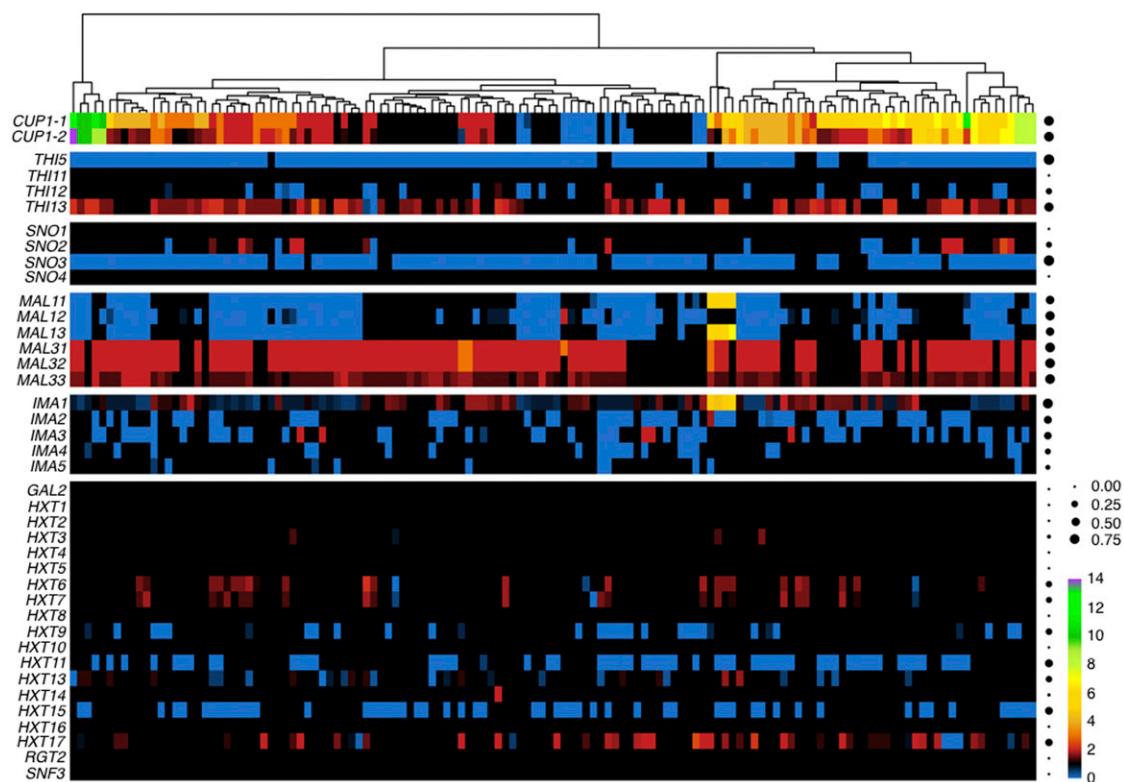
All members of the *IMA* gene family, composed of genes aiding in sugar fermentation (Teste *et al.* 2010), were among the 363 genes with high CN diversity (File S3 in File S1) and *IMA1* (*YGR287C*; PIC = 0.87) was among the top 57 genes with the highest CN diversity (File S4 in File S1). *IMA1* was deleted in 54 strains (40.9%) and duplicated in 50 strains (37.9%) (Figure 3). Although many duplications or deletions did not span the entirety of *IMA1*, there were four strains that harbored high CNs between four and six. These same four strains also had similar and unique duplications of *MAL11* and *MAL13*, suggesting that *IMA1*, *MAL11*, and *MAL13*, which are adjacent to each other in the genome, may have been duplicated as one locus. The other isomaltases (*IMA2-5*; *YOL157C*, *YIL172C*, *YJL221C*, and *YJL216C*) were deleted in at least 11 strains (8.3%) and at most 55 strains (41.7%). No duplications in *IMA2-5* were detected, and only rarely in *IMA3* (five strains, 3.8%).

Altogether, the 132 strains exhibited both expansions and contractions of the *IMA* gene family (Figure S11 in File S2).

We identified seven members of the *HXT* gene family (*HXT6*/*YDR343C*, *HXT7*/*YDR342C*, *HXT9*/*YJL219W*, *HXT11*/*YOL156W*, *HXT13*/*YEL069C*, *HXT15*/*YDL245C*, and *HXT17*/*YNR072W*), which is involved in sugar transport, that were among the 363 CN diverse genes (File S3 in File S1). Members of the *HXT* gene family were duplicated, deleted, or had mosaic absolute CN values across the 132 strains. For example, *HXT6* and *HXT7* were primarily duplicated in 25 (18.9%) and 22 strains (16.7%), respectively, while only three strains (2.3%) had deletions in either gene (Figure 3). *HXT9*, *HXT11*, and *HXT15* were deleted in 32 (24.2%), 57 (43.2%), and 53 strains (40.2%), respectively, while no strains had duplications. Finally, *HXT13* was duplicated in 12 strains (9.1%) and deleted in 17 strains (12.9%), and *HXT17* was duplicated in 37 strains (28%) and deleted in nine strains (6.8%).

As expansions in the *HXT* gene family are positively correlated with aerobic fermentation in *S. paradoxus* and *S. cerevisiae* (Lin and Li 2011), we also examined the absolute CN of all other 10 members (*GAL2*/*YLR081W*, *HXT1*/*YHR094C*, *HXT2*/*YMR011W*, *HXT4*/*YHR092C*, *HXT5*/*YHR096C*, *HXT8*/*YJL214W*, *HXT10*/*YFL011W*, *HXT16*/*YJR158W*, *RGT2*/*YDL138W*, and *SNF3*/*YDL194W*) of the *HXT* gene family (Figure 3). Interestingly, all remaining 10 members of the *HXT* gene family were not CN variable. Altogether, examination of the *HXT* family CN diversity patterns across the 132 strains suggests that wine yeast strains typically exhibit minor contractions (*i.e.*, *HXT* gene deletions exceed those of duplications) relative to the S288c reference strain (Figure S11 in File S2).

All five members of the *FLO* gene family, which is responsible for flocculation (Govender *et al.* 2008), a trait shown to aid in the escape of oxygen limited environments during liquid fermentation (Fidalgo *et al.* 2006; Govender *et al.* 2008), were found to be among the 363 most CN diverse genes. Furthermore, *FLO5* (*YHR211W*; PIC = 0.82) and *FLO11* (*YIR019C*; PIC = 0.88) were among the 57 genes with the highest CN diversity (File S4 in File S1). Due to the importance of site-directed CN variation in *FLO* family genes (Fidalgo *et al.* 2006), we modified our representation of CN variation to display intragenic



**Figure 3** CN variation of genes and gene families. Heat map of the CN profiles the *CUP*, *THI*, *SNO*, *MAL*, *IMA*, and *HXT* gene families; rows correspond to genes and columns to strains. Blue-colored cells correspond to deletions, black-colored cells to no CN variation and red-to-purple-colored cells to duplications (ranging from 2 to 14). Dots on the right side of the figure represent the proportion of individual strains that harbor CN variation in that gene—the larger the dot, the greater the proportion of the strains that is CN variable for that gene.

CN variation using a 250 bp window (Figure S12 in File S2). *FLO5* was partially duplicated in 57 strains (43.2%), partially deleted in 47 strains (35.6%), and 115 strains (87.1%) had at least one region of the gene unaffected by CN variation. Duplications and deletions were primarily observed in the Threonine-rich region or Serine/Threonine-rich region located in the center or end of the *FLO5* gene, respectively. To better resolve intragenic CN variation of *FLO11*, whose repeat unit is shorter than that of *FLO5*, we recalled CN variants with a smaller window size of 25 bp and re-evaluated CN variation (Figure S13 in File S2). Using this window size, we found extensive duplications in 97 strains (73.5%) between gene coordinates 250–350 bp. Furthermore, duplications were observed in the hydrophobic Serine/Threonine-rich regions (Figure S13 in File S2), which are associated with the flocculation phenotype (Fidalgo *et al.* 2006; Ramsook *et al.* 2010).

In contrast to *FLO5* and *FLO11*, other members of the *FLO* gene family did not exhibit intragenic CN variation. For example, CN variation in *FLO1* (*YAR050W*) and *FLO9* (*YAL063C*) typically spanned most or all of the sequence of each gene. Specifically, 125 strains (99.2%) had deletions spanning  $\geq 80\%$  of the gene in *FLO1*, and only two strains (1.5%) had the entirety of the gene intact. *FLO9* had deletions in 99 strains (75%) that spanned  $\geq 75\%$  of the gene, 11 strains (8.3%) that had a partial deletion spanning  $< 75\%$  of the gene, whereas one strain (0.8%) had a CN of two, and the remaining 21 strains (15.9%) had a CN of one. In contrast, *FLO10* (*YKR102W*) showed limited CN variation. Specifically, 108 strains (81.8%) had no CN variation while six strains (4.5%) had deletions spanning the entirety of the gene. No duplications spanned the entirety of the gene but partial

duplications were observed in 17 strains (12.9%) and were located in, or just before, the Serine/Threonine-rich region.

### Functional implications CN variable genes

To determine the functional impact of deleted CN variable genes, we examined the relative growth of deleted CN variable genes (denoted with the  $\Delta$  symbol) relative to the wild-type (WT) *S. cerevisiae* strain S288c across 418 conditions using the Hillenmeyer *et al.* (2008) data (File S5 in File S1 and Figure S14 in File S2). To determine the impact of duplicated genes, we examined growth fitness of the WT strain with low ( $\sim 2$ –3 gene copies) or high ( $\sim 8$ –24 gene copies) plasmid CN, where each plasmid contained a single gene of interest from previously published data, relative to WT (File S6 in File S1, Figure S15 in File S2, and Payen *et al.* 2016).

Among deleted genes, 42/69 genes for which data exist showed negative and positive fitness effects in at least one tested condition in the S288c genetic background. Furthermore, we found that 12/42 genes that are commonly deleted among wine strains typically resulted in a fitness gain in conditions that resembled the fermentation environment. These conditions include growth at 23° and at 25°, temperatures within the 15–28° range in which wine is fermented (Molina *et al.* 2007), and growth in minimal media, which is commonly used to understand fermentation-related processes (Seki *et al.* 1985; Govender *et al.* 2008; Vilela-Moura *et al.* 2008).

When examining fitness effects when grown at 23° or at 25° for five or 15 generations for the 12 commonly deleted genes, we observed at least one deletion that resulted in a fitness gain or loss for each



condition. However, we observed extensive deletions in the *MAL1* locus (Figure 3), and therefore prioritized reporting the fitness impact of deletions in *MAL11*, *MAL12*, and *MAL13*.  $\Delta$ *MAL11* resulted in a fitness gain for growth at 23° and 25° for 5 (0.45× and 0.27×, respectively) and 15 generations (0.20× and 0.52×, respectively).  $\Delta$ *MAL12* resulted in a gain of fitness at only 25° after 15 generations (0.46×) and in a loss of fitness ranging from  $-0.36\times$  to  $-1.29\times$  in the other temperature conditions. Similarly,  $\Delta$ *MAL13* resulted in fitness gains and losses dependent on the number of generations. For example, when grown for 15 generations at 25° a fitness gain of 0.50× was observed, while a fitness loss of  $-0.82\times$  was observed at 23°.

We next determined the fitness effect of deleted genes in minimal media after 0, 5, and 10 generations. Similar patterns of complex fitness gain and loss were observed as for the other conditions. For example,  $\Delta$ *THI12* resulted in a loss of fitness of  $-4.13\times$  and  $-1.97\times$  after 0 and 5 generations, but a fitness gain of 0.63× after 10 generations. In contrast, other genes resulted in positive fitness effects. For example,  $\Delta$ *MAL12* resulted in a fitness gain of 7.25× and 10.41× for 0 generations and 10 generations.

Among duplicated genes, we focused on growth in glucose- and phosphate-limited conditions because glucose becomes scarce toward the end of fermentation prior to the diauxic shift and phosphate limitation is thought to contribute to stuck fermentations (Bisson 1999; Marsit and Dequin 2015). Among the 35 of the 69 genes where data were available, 14 genes had duplications among the 132 strains.

When examining the fitness effects of duplicated genes in a glucose-limited environment in the S288c background, we found that fitness effects were small in magnitude, and dependent on condition and plasmid CN (File S6 in File S1). For example, *MAL32* low CN increased growth fitness by 0.02× but decreased fitness by  $-0.01\times$  at a high CN (Figure S15 in File S2). Interestingly, the most prevalent CN for *MAL32* across the 132 strains was two (96 strains, 72.7%), with only three strains showing a CN of three and none a higher CN. Another gene found at low CN in 37 strains (28%) was *HXT17*. Low plasmid CN in a glucose-limited conditions resulted in a fitness gain of 0.06×. In contrast, *MAL13* low or high plasmid CN resulted in a negative growth fitness of  $-0.02\times$  and  $-0.01\times$ , respectively. Interestingly, *MAL13* duplication is observed in only four strains (3%), and deletions are observed in 61 strains (46.2%).

Similar to the glucose-limited condition, we found fitness was dependent on high or low plasmid CN in the phosphate-limited condition. For example, *MAL31*, a gene present at low CN in 100 strains, had a fitness gain of 0.04× at high plasmid CN, but low plasmid CN resulted in a fitness loss of  $-0.02\times$ . In contrast, *MAL32*, which was present at low CN in 99 strains, had a small fitness gain of 0.002× at low plasmid CN, and a fitness loss at a high plasmid CN of  $-0.02\times$ . A total of six genes resulted in a disadvantageous growth effect when present at low CN, such as *DDR48*, which resulted in a fitness loss of  $-0.04\times$ . Altogether, our results suggest that the deleted and duplicated CN variable genes we observe (Figure 4) modulate cellular processes that result in advantageous fitness effects in conditions that resemble the fermentation environment.

### Identifying loci absent from CN variation analysis

The present study was able to capture loci represented in the WT/S288c laboratory strain. To identify loci absent from the reference strain, we assembled unmapped reads for 20 strains with the lowest percentage of reads mapped and determined their identity (see *Materials and Methods*; Figure S4 in File S2). Across the 20 strains, we identified 429 loci absent from S288c but present in other sequenced *S. cerevisiae* strains.

These loci had an average length of 6.9 kb and an average coverage of 107.2×. The 20 loci with the highest bitscore alongside with the number of strains containing the locus are shown in Table S2 in File S2. All but two of these loci were present only in one of the 20 strains we examined. The two exceptions were the EC1118\_1N26\_0012p locus, which we found in 8/20 strains, which originates from horizontal gene transfer from *Zygosaccharomyces rouxii* to the commercial EC1118 wine strain of *S. cerevisiae* (Novo *et al.* 2009); and the EC1118\_1O4\_6656p locus, which we found in 7/20 strains. This locus was also originally found in the EC1118 strain (Novo *et al.* 2009), and contains a gene similar to a conserved hypothetical protein found in *S. cerevisiae* strain AWRI1631 (Borneman *et al.* 2008).

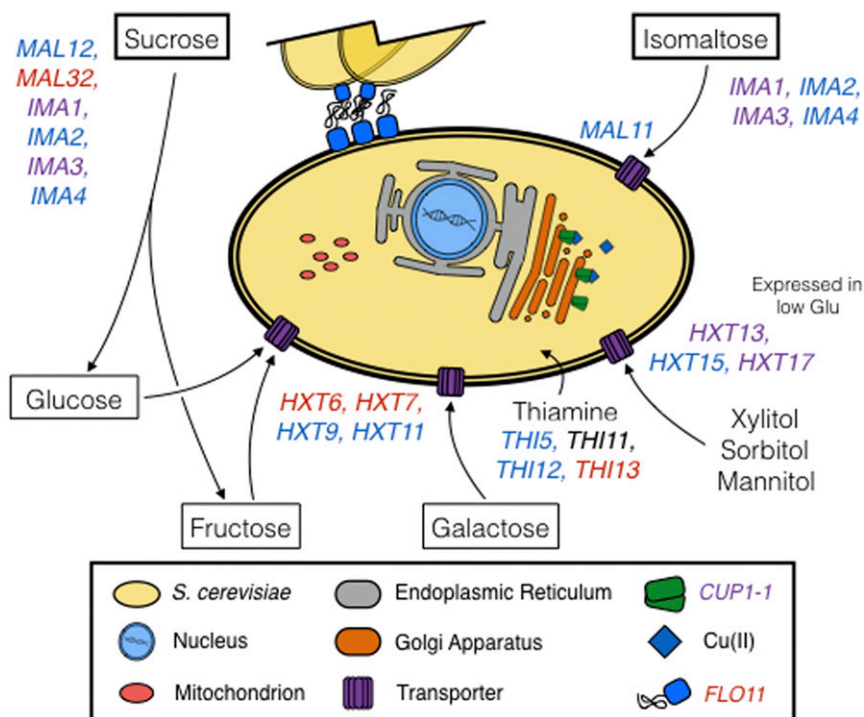
## DISCUSSION

CN variant loci are known to contribute to genomic and phenotypic diversity (Perry *et al.* 2007; Cutler and Kassner 2008; Orozco *et al.* 2009). However, the extent of CN variation in wine strains of *S. cerevisiae*, and its impact on phenotypic variation remains less understood. Our examination of structural variation in 132 yeast strains representative of the “wine clade” showed that CN variants are a significant contributor to the genomic diversity of wine strains of *S. cerevisiae*. Importantly, CN variant loci overlap with diverse genes and gene families functionally related to the fermentation environment such as *CUP*, *FLO*, *THI*, *MAL*, *IMA*, and *HXT* (summarized in Figure 4).

The characteristics of CN variation in wine yeast (Figure 1A, and Figure S6 and Table S1 in File S2) were found to be similar to those of the recently described beer yeast lineage (Gallone *et al.* 2016). For example, both lineages exhibited a similar size range of CNVRs (Figure 1A, and Figure S6 and Table S1 in File S2), as well as a higher prevalence of CNVRs in the subtelomeric regions (Figure 1D). However, wine strains had a smaller fraction of their genome affected by CN variation (Figure S6 in File S2) than beer strains (Gallone *et al.* 2016).

Wine yeast strains are thought to be partially domesticated due to the seasonal nature of wine-making, which allows for outcrossing with wild populations (Marsit and Dequin 2015; Gallone *et al.* 2016; Gonçalves *et al.* 2016). One human-driven signature of domestication is thought to be the duplication of the *CUP1* locus, because multiple copies confer copper resistance and copper sulfates have been used to combat powdery mildews in vineyards since the early 1800s (Warringer *et al.* 2011; Marsit and Dequin 2015). Consistent with this “partial domestication” view (Marsit and Dequin 2015; Gallone *et al.* 2016; Gonçalves *et al.* 2016), many wine strains were not CN variable for *CUP1-1* and *CUP1-2*, or had one or both genes deleted (Figure 3).

An alternative, albeit not necessarily conflicting, hypothesis is that wine yeasts underwent domestication for specific but diverse wine flavor profiles (Hyma *et al.* 2011). Consistent with this view is the deletion (in >90% of the strains) of the *THI5* gene (Figure 3), whose activity is known to produce an undesirable rotten-egg sensory perception via higher SH<sub>2</sub> production and is associated with sluggish fermentations (Bartra *et al.* 2010). In contrast to wine strains, duplications of *THI5* have been observed across the *Saccharomyces* genus, including in several strains of *S. cerevisiae* (CBS1171, two copies; S288c, four copies; EM93, five copies), *S. paradoxus* (five copies), and the lager brewing yeast hybrid *S. pastorianus* (syn. *S. carlsbergensis*; 2+ copies) (Wightman and Meacock 2003). In contrast, *THI13*, which is duplicated in 62.1% of strains, shows an increase in its expression of 6- to 100-fold in *S. cerevisiae* when grown on medium containing low concentrations of thiamine, allowing for the compensation of low thiamine levels (Li *et al.* 2010). Low levels of thiamine in wine fermentation have been associated with stuck or slow fermentations (Ough *et al.* 1989;



**Figure 4** Model summary of CN variable genes in wine yeast strains and their cellular functions. Genes that are deleted among wine strains are blue, whereas those that are duplicated are in red. Genes that were observed to be both duplicated and deleted (*IMA1*, *IMA3*, *HXT13*, and *CUP1-1*) are purple. Disaccharides are in thick-lined boxes, monosaccharides in thin-lined boxes, and alcohols are unboxed.

Bataillon *et al.* 1996). Similar to *THI5* deletions, *THI13* duplications may have also been driven by human activity due to the advantageous effect of increased expression within the fermentation environment.

Two other gene families subject to CN variation were the *MAL* and *HXT* gene families. The S288c strain that we used as a reference contained two *MAL* loci (*MAL1* and *MAL3*), each containing three genes—a maltose permease (*MALx1*), a maltase (*MALx2*), and an *MAL* trans-activator (*MALx3*)—and located near the ends of different chromosomes (Michels *et al.* 1992). *MAL1* has been observed to be duplicated in beer strains of *S. cerevisiae* (Gallone *et al.* 2016; Gonçalves *et al.* 2016), while wine strains primarily lack this locus (Figure 3; Gonçalves *et al.* 2016). In contrast to the deletion of the *MAL1* locus, *MAL3* duplication in wine yeasts (Figure 3; Gonçalves *et al.* 2016) is surprising because maltose is absent from the grape must (Gallone *et al.* 2016). However, knockout studies have demonstrated *MAL32* is necessary for growth on turanose, maltotriose, and sucrose (Brown *et al.* 2010), which are present in small quantities in wines (Victoria and Carmen 2013). Due to the prominent duplication of *MAL3*, in particular the enzymatic genes *MAL31* and *MAL32*, we speculate that the *MAL3* locus may be utilized to obtain sugars less prevalent in the wine environment, or serve other purposes.

The *HXT* gene family in the S288c strain that we used as a reference contains 16 *HXT* paralogs, *GAL2*, *SNF3*, and *RGT2*. The expansion of the *HXT* gene family is positively correlated with aerobic fermentation in *S. paradoxus* and *S. cerevisiae* (Lin and Li 2011). *HXT6* and *HXT7* are high-affinity glucose transporters expressed at low glucose levels, and repressed at high glucose levels (Reifenberger *et al.* 1995). In contrast to the recently described Asia (Sake), Britain (Beer) and Mosaic lineages (Gallone *et al.* 2016), we detected duplications in the *HXT6* and *HXT7* genes in wine yeasts (Figure 3). This may confer an advantage toward the end of fermentation and before the diauxic shift when glucose becomes a scarce resource. Evidence potentially supporting this hypothesis is that *HXT6* and *HXT7* are upregulated by 9.8- and 5.6-fold, respectively, through wine fermentation in the *S. cerevisiae* strain Vin13 (Marks *et al.* 2008). Furthermore, *HXT6* or *HXT7* is found

to be duplicated in experimentally evolved populations in glucose-limited environments (Dunham *et al.* 2002; Gresham *et al.* 2008; Dunn *et al.* 2012).

In summary, these results, together with recent studies of CN variation in beer yeast strains (Gallone *et al.* 2016; Gonçalves *et al.* 2016), suggest that this type of variation significantly contributes to the genomic diversity of domesticated yeast strains. Furthermore, as most studies of CN variation, including ours, use reference strains, they are likely conservative in estimating the amount of CN variation present in populations. This caveat notwithstanding, examination of publically available data regarding the functional impact of duplicated or deleted genes (again in the context provided by the reference strain's genetic background) suggests that CN variation in several, but not all, of the wine yeast genes confer fitness advantages in conditions that resemble the fermentation environment. Our results raise the questions of the extent to which CN variation contributes to fungal, and more generally microbial, domestication as well as whether the importance of CN variants in natural yeast populations, including those of other *Saccharomyces* yeasts, is on par to their importance in domestication environments.

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#### LITERATURE CITED

- Arlt, M. F., S. Rajendran, S. R. Birkeland, T. E. Wilson, and T. W. Glover, 2014 Copy number variants are produced in response to low-dose ionizing radiation in cultured cells. *Environ. Mol. Mutagen.* 55: 103–113.
- Axelsson, E., A. Ratnakumar, M.-L. Arendt, K. Maqbool, M. T. Webster *et al.*, 2013 The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 495: 360–364.



- Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin *et al.*, 2012 SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19: 455–477.
- Barton, A. B., Y. Su, J. Lamb, D. Barber, and D. B. Kaback, 2003 A function for subtelomeric DNA in *Saccharomyces cerevisiae*. *Genetics* 165: 929–934.
- Bartra, E., M. Casado, D. Carro, C. Campamà, and B. Piña, 2010 Differential expression of thiamine biosynthetic genes in yeast strains with high and low production of hydrogen sulfide during wine fermentation. *J. Appl. Microbiol.* 109: 272–281.
- Bataillon, M., A. Rico, J. M. Sablayrolles, J. M. Salmon, and P. Barre, 1996 Early thiamin assimilation by yeasts under enological conditions: impact on alcoholic fermentation kinetics. *J. Ferment. Bioeng.* 82: 145–150.
- Bickhart, D. M., Y. Hou, S. G. Schroeder, C. Alkan, M. F. Cardone *et al.*, 2012 Copy number variation of individual cattle genomes using next-generation sequencing. *Genome Res.* 22: 778–790.
- Birchler, J. A., and R. A. Veitia, 2010 The gene balance hypothesis: implications for gene regulation, quantitative traits and evolution. *New Phytol.* 186: 54–62.
- Birchler, J. A., and R. A. Veitia, 2012 Gene balance hypothesis: connecting issues of dosage sensitivity across biological disciplines. *Proc. Natl. Acad. Sci. USA* 109: 14746–14753.
- Bisson, L. F., 1999 Stuck and sluggish fermentations. *Am. J. Enol. Vitic.* 50: 107–119.
- Bisson, L. F., 2012 Geographic origin and diversity of wine strains of *Saccharomyces*. *Am. J. Enol. Vitic.* 63: 165–176.
- Boeva, V., A. Zinovyev, K. Bleakley, J. P. Vert, I. Janoueix-Lerosey *et al.*, 2011 Control-free calling of copy number alterations in deep-sequencing data using GC-content normalization. *Bioinformatics* 27: 268–269.
- Boeva, V., T. Popova, K. Bleakley, P. Chiche, J. Cappo *et al.*, 2012 Control-FREEC: a tool for assessing copy number and allelic content using next-generation sequencing data. *Bioinformatics* 28: 423–425.
- Bolger, A. M., M. Lohse, and B. Usadel, 2014 Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.
- Borneman, A. R., A. H. Forgan, I. S. Pretorius, and P. J. Chambers, 2008 Comparative genome analysis of a *Saccharomyces cerevisiae* wine strain. *FEMS Yeast Res.* 8: 1185–1195.
- Borneman, A. R., A. H. Forgan, R. Kolouchova, J. A. Fraser, and S. A. Schmidt, 2016 Whole genome comparison reveals high levels of inbreeding and strain redundancy across the spectrum of commercial wine strains of *Saccharomyces cerevisiae*. *G3* 6: 957–971.
- Brion, C., C. Ambroset, P. Delobel, I. Sanchez, and B. Blondin, 2014 Deciphering regulatory variation of THI genes in alcoholic fermentation indicate an impact of Thi3p on PDC1 expression. *BMC Genomics* 15: 1085.
- Brown, C. A., A. W. Murray, and K. J. Verstrepen, 2010 Rapid expansion and functional divergence of subtelomeric gene families in yeasts. *Curr. Biol.* 20: 895–903.
- Brown, C. J., K. M. Todd, and R. F. Rosenzweig, 1998 Multiple duplications of yeast hexose transport genes in response to selection in a glucose-limited environment. *Mol. Biol. Evol.* 15: 931–942.
- Carbon, S., A. Ireland, C. J. Mungall, S. Shu, B. Marshall *et al.*, 2009 AmiGO: online access to ontology and annotation data. *Bioinformatics* 25: 288–289.
- Cavalieri, D., P. E. McGovern, D. L. Hartl, R. Mortimer, and M. Polsinelli, 2003 Evidence for *S. cerevisiae* fermentation in ancient wine. *J. Mol. Evol.* 57: S226–S232.
- Cromie, G. A., K. E. Hyma, C. L. Ludlow, C. Garmendia-Torres, T. L. Gilbert *et al.*, 2013 Genomic sequence diversity and population structure of *Saccharomyces cerevisiae* assessed by RAD-seq. *G3* 3: 2163–2171.
- Cutler, G., and P. D. Kassner, 2008 Copy number variation in the mouse genome: implications for the mouse as a model organism for human disease. *Cytogenet. Genome Res.* 123: 297–306.
- Duan, J., J.-G. Zhang, H.-W. Deng, and Y.-P. Wang, 2013 Comparative studies of copy number variation detection methods for next-generation sequencing technologies. *PLoS One* 8: e59128.
- Dunham, M. J., H. Badrane, T. Ferea, J. Adams, P. O. Brown *et al.*, 2002 Characteristic genome rearrangements in experimental evolution of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 99: 16144–16149.
- Dunn, B., C. Richter, D. J. Kvittek, T. Pugh, and G. Sherlock, 2012 Analysis of the *Saccharomyces cerevisiae* pan-genome reveals a pool of copy number variants distributed in diverse yeast strains from differing industrial environments. *Genome Res.* 22: 908–924.
- Farrer, R. A., D. A. Henk, T. W. Garner, F. Balloux, D. C. Woodhams *et al.*, 2013 Chromosomal copy number variation, selection and uneven rates of recombination reveal cryptic genome diversity linked to pathogenicity. *PLoS Genet.* 9: e1003703.
- Fidalgo, M., R. R. Barrales, J. I. Ibeas, and J. Jimenez, 2006 Adaptive evolution by mutations in the FLO11 gene. *Proc. Natl. Acad. Sci. USA* 103: 11228–11233.
- Freeman, J. L., G. H. Perry, L. Feuk, R. Redon, S. A. McCarroll *et al.*, 2006 Copy number variation: new insights in genome diversity. *Genome Res.* 16: 949–961.
- Gallone, B., J. Steensels, T. Pahl, L. Soriaga, V. Saels *et al.*, 2016 Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. *Cell* 166: 1397–1410.e16.
- Gene Ontology Consortium, 2004 The gene ontology (GO) database and informatics resource. *Nucleic Acids Res.* 32: D258–D261.
- Gibbons, J. G., and D. C. Rinker, 2015 The genomics of microbial domestication in the fermented food environment. *Curr. Opin. Genet. Dev.* 35: 1–8.
- Gibbons, J. G., L. Salichos, J. C. Slot, D. C. Rinker, K. L. McGary *et al.*, 2012 The evolutionary imprint of domestication on genome variation and function of the filamentous fungus *Aspergillus oryzae*. *Curr. Biol.* 22: 1403–1409.
- Gibbons, J. G., A. T. Branco, S. A. Godinho, S. Yu, and B. Lemos, 2015 Concerted copy number variation balances ribosomal DNA dosage in human and mouse genomes. *Proc. Natl. Acad. Sci. USA* 112: 2485–2490.
- Gonçalves, M., A. Pontes, P. Almeida, R. Barbosa, M. Serra *et al.*, 2016 Distinct domestication trajectories in top-fermenting beer yeasts and wine yeasts. *Curr. Biol.* 26: 2750–2761.
- Govender, P., J. L. Domingo, M. C. Bester, I. S. Pretorius, and F. F. Bauer, 2008 Controlled expression of the dominant flocculation genes FLO1, FLO5, and FLO11 in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 74: 6041–6052.
- Gresham, D., M. M. Desai, C. M. Tucker, H. T. Jenq, D. A. Pai *et al.*, 2008 The repertoire and dynamics of evolutionary adaptations to controlled nutrient-limited environments in yeast. *PLoS Genet.* 4: e1000303.
- Henrichsen, C. N., E. Chaignat, and A. Reymond, 2009 Copy number variants, diseases and gene expression. *Hum. Mol. Genet.* 18: R1–R8.
- Hillenmeyer, M. E., E. Fung, J. Wildenhain, S. E. Pierce, S. Hoon *et al.*, 2008 The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science* 320: 362–365.
- Hu, G., J. Wang, J. Choi, W. H. Jung, I. Liu *et al.*, 2011 Variation in chromosome copy number influences the virulence of *Cryptococcus neoformans* and occurs in isolates from AIDS patients. *BMC Genomics* 12: 526.
- Hunter, A. J., B. Jin, and J. M. Kelly, 2011 Independent duplications of alpha-amylase in different strains of *Aspergillus oryzae*. *Fungal Genet. Biol.* 48: 438–444.
- Huson, D. H., 1998 SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* 14: 68–73.
- Hyma, K. E., S. M. Saerens, K. J. Verstrepen, and J. C. Fay, 2011 Divergence in wine characteristics produced by wild and domesticated strains of *Saccharomyces cerevisiae*. *FEMS Yeast Res.* 11: 540–551.
- Kaessmann, H., N. Vinckenbosch, and M. Long, 2009 RNA-based gene duplication: mechanistic and evolutionary insights. *Nat. Rev. Genet.* 10: 19–31.
- Kao, K. C., and G. Sherlock, 2008 Molecular characterization of clonal interference during adaptive evolution in asexual populations of *Saccharomyces cerevisiae*. *Nat. Genet.* 40: 1499–1504.
- Keith, T. P., P. Green, S. T. Reeders, V. A. Brown, P. Phipps *et al.*, 1990 Genetic linkage map of 46 DNA markers on human chromosome 16. *Proc. Natl. Acad. Sci. USA* 87: 5754–5758.
- Koboldt, D. C., K. Chen, T. Wylie, D. E. Larson, M. D. McLellan *et al.*, 2009 VarScan: variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics* 25: 2283–2285.

- Koboldt, D. C., Q. Zhang, D. E. Larson, D. Shen, M. D. McLellan *et al.*, 2012 VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res.* 22: 568–576.
- Kolde, R., 2012 Package 'pheatmap'. *Bioconductor* 1–6. Available at: <https://CRAN.R-project.org/package=pheatmap>.
- Langmead, B., and S. L. Salzberg, 2012 Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9: 357–359.
- Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan *et al.*, 2009 The sequence alignment/map format and SAMtools. *Bioinformatics* 25: 2078–2079.
- Li, M., B. J. Petteys, J. M. McClure, V. Valsakumar, S. Bekiranov *et al.*, 2010 Thiamine biosynthesis in *Saccharomyces cerevisiae* is regulated by the NAD<sup>+</sup>-dependent histone deacetylase Hst1. *Mol. Cell. Biol.* 30: 3329–3341.
- Lin, Z., and W. H. Li, 2011 Expansion of hexose transporter genes was associated with the evolution of aerobic fermentation in yeasts. *Mol. Biol. Evol.* 28: 131–142.
- Liti, G., D. M. Carter, A. M. Moses, J. Warringer, L. Parts *et al.*, 2009 Population genomics of domestic and wild yeasts. *Nature* 458: 337–341.
- Lo, W. S., and A. M. Dranginis, 1996 FLO11, a yeast gene related to the STA genes, encodes a novel cell surface flocculin. *J. Bacteriol.* 178: 7144–7151.
- Lupetti, A., R. Danesi, M. Campa, M. Del Tacca, and S. Kelly, 2002 Molecular basis of resistance to azole antifungals. *Trends Mol. Med.* 8: 76–81.
- Lupski, J. R., and P. Stankiewicz, 2005 Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. *PLoS Genet.* 1: e49.
- Madden, T., 2013 The BLAST sequence analysis tool, pp. 1–17 in *The NCBI Handbook*. Ed. 2. National Center for Biotechnology Information, Bethesda, MD.
- Marks, V. D., S. J. Ho Sui, D. Erasmus, G. K. Van Der Merwe, J. Brumm *et al.*, 2008 Dynamics of the yeast transcriptome during wine fermentation reveals a novel fermentation stress response. *FEMS Yeast Res.* 8: 35–52.
- Marsit, S., and S. Dequin, 2015 Diversity and adaptive evolution of *Saccharomyces* wine yeast: a review. *FEMS Yeast Res.* 15: fov067.
- Mi, H., A. Muruganujan, J. T. Casagrande, and P. D. Thomas, 2013 Large-scale gene function analysis with the PANTHER classification system. *Nat. Protoc.* 8: 1551–1566.
- Michels, C. A., E. Read, K. Nat, and M. J. Charron, 1992 The telomere-associated MAL3 locus of *Saccharomyces* is a tandem array of repeated genes. *Yeast* 8: 655–665.
- Molina, A. M., J. H. Swiegers, C. Varela, I. S. Pretorius, and E. Agosin, 2007 Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Appl. Microbiol. Biotechnol.* 77: 675–687.
- Mortimer, R. K., 2000 Evolution and variation of the yeast (*Saccharomyces*) genome. *Genome Res.* 10: 403–409.
- Novo, M., F. Bigey, E. Beyne, V. Galeote, F. Gavory *et al.*, 2009 Eukaryote-to-eukaryote gene transfer events revealed by the genome sequence of the wine yeast *Saccharomyces cerevisiae* EC1118. *Proc. Natl. Acad. Sci. USA* 106: 16333–16338.
- Orozco, L. D., S. J. Cokus, A. Ghazalpour, L. Ingram-Drake, S. Wang *et al.*, 2009 Copy number variation influences gene expression and metabolic traits in mice. *Hum. Mol. Genet.* 18: 4118–4129.
- Oshero, N., D. P. Kontoyiannis, A. Romans, and G. S. May, 2001 Resistance to itraconazole in *Aspergillus nidulans* and *Aspergillus fumigatus* is conferred by extra copies of the A. *nidulans* P-450 14 $\alpha$ -demethylase gene, pdmA. *J. Antimicrob. Chemother.* 48: 75–81.
- Ough, C., M. Davenport, and K. Joseph, 1989 Effects of certain vitamins on growth and fermentation rate of several commercial active dry wine yeasts. *Am. J. Enol. Vitic.* 40: 208–213.
- Padilla, P. A., E. K. Fuge, M. E. Crawford, A. Errett, and M. Werner-Washburne, 1998 The highly conserved, coregulated SNO and SNZ gene families in *Saccharomyces cerevisiae* respond to nutrient limitation. *J. Bacteriol.* 180: 5718–5726.
- Payen, C., A. B. Sunshine, G. T. Ong, J. L. Pogachar, W. Zhao *et al.*, 2016 High-throughput identification of adaptive mutations in experimentally evolved yeast populations. *PLoS Genet.* 12: e1006339.
- Perry, G. H., N. J. Dominy, K. G. Claw, A. S. Lee, H. Fiegler *et al.*, 2007 Diet and the evolution of human amylase gene copy number variation. *Nat. Genet.* 39: 1256–1260.
- Pezer, Z., B. Harr, M. Teschke, H. Babiker, and D. Tautz, 2015 Divergence patterns of genic copy number variation in natural populations of the house mouse (*Mus musculus domesticus*) reveal three conserved genes with major population-specific expansions. *Genome Res.* 25: 1114–1124.
- Pretorius, I. S., 2000 Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast* 16: 675–729.
- Quinlan, A. R., and I. M. Hall, 2010 BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26: 841–842.
- R Development Core Team, 2011 *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Ramsook, C. B., C. Tan, M. C. Garcia, R. Fung, G. Soybelman *et al.*, 2010 Yeast cell adhesion molecules have functional amyloid-forming sequences. *Eukaryot. Cell* 9: 393–404.
- Reifenberger, E., K. Freidel, and M. Ciriacy, 1995 Identification of novel HXT genes in *Saccharomyces cerevisiae* reveals the impact of individual hexose transporters on glycolytic flux. *Mol. Microbiol.* 16: 157–167.
- Risch, N., 1990 Linkage strategies for genetically complex traits. II. The power of affected relative pairs. *Am. J. Hum. Genet.* 46: 229–241.
- Rodríguez-Navarro, S., B. Llorente, M. T. Rodríguez-Manzanque, A. Ramne, G. Uber *et al.*, 2002 Functional analysis of yeast gene families involved in metabolism of vitamins B1 and B6. *Yeast* 19: 1261–1276.
- Sanguinetti, M., B. Posteraro, M. La Sorda, R. Torelli, B. Fiori *et al.*, 2006 Role of *AFRI*, an ABC transporter-encoding gene, in the in vivo response to fluconazole and virulence of *Cryptococcus neoformans*. *Infect. Immun.* 74: 1352–1359.
- Schacherer, J., J. A. Shapiro, D. M. Ruderfer, and L. Kruglyak, 2009 Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*. *Nature* 458: 342–345.
- Seki, T., E. H. Choi, and D. Ryu, 1985 Construction of killer wine yeast strain. *Appl. Environ. Microbiol.* 49: 1211–1215.
- Sicard, D., and J. L. Legras, 2011 Bread, beer and wine: yeast domestication in the *Saccharomyces sensu stricto* complex. *C. R. Biol.* 334: 229–236.
- Sims, D., I. Sudbery, N. E. Ilott, A. Heger, and C. P. Ponting, 2014 Sequencing depth and coverage: key considerations in genomic analyses. *Nat. Rev. Genet.* 15: 121–132.
- Sionov, E., H. Lee, Y. C. Chang, and K. J. Kwon-Chung, 2010 *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog.* 6: e1000848.
- Skelly, D. A., G. E. Merrihew, M. Riffle, C. F. Connelly, E. O. Kerr *et al.*, 2013 Integrative phenomics reveals insight into the structure of phenotypic diversity in budding yeast. *Genome Res.* 23: 1496–1504.
- Smith, J. S. C., E. C. L. Chin, H. Shu, O. S. Smith, S. J. Wall *et al.*, 1997 An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPS and pedigree. *Theor. Appl. Genet.* 95: 163–173.
- Stambuk, B. U., A. S. Batista, and P. S. De Araujo, 2000 Kinetics of active sucrose transport in *Saccharomyces cerevisiae*. *J. Biosci. Bioeng.* 89: 212–214.
- Steenwyk, J. L., J. S. Soghigian, J. R. Perfect, and J. G. Gibbons, 2016 Copy number variation contributes to cryptic genetic variation in outbreak lineages of *Cryptococcus gattii* from the North American Pacific Northwest. *BMC Genomics* 17: 700.
- Sudmant, P. H., J. O. Kitman, F. Antonacci, C. Alkan, M. Malig *et al.*, 2010 Diversity of human copy number variation and multicopy genes. *Science* 330: 641–646.
- Sudmant, P. H., S. Mallick, B. J. Nelson, F. Hormozdiari, N. Krumm *et al.*, 2015 Global diversity, population stratification, and selection of human copy-number variation. *Science* 349: aab3761.

- Teste, M. A., J. Marie François, and J. L. Parrou, 2010 Characterization of a new multigene family encoding isomaltases in the yeast *Saccharomyces cerevisiae*, the *IMA* family. *J. Biol. Chem.* 285: 26815–26824.
- Thomas, P. D., M. J. Campbell, A. Kejariwal, H. Mi, B. Karlak *et al.*, 2003 PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res.* 13: 2129–2141.
- Victoria, M.M.-A., and P. M. Carmen, 2013 Wine chemistry and biochemistry. *J. Chem. Inf. Model.* 53: 1689–1699.
- Vilela-Moura, A., D. Schuller, A. Mendes-Faia, and M. Côrte-Real, 2008 Reduction of volatile acidity of wines by selected yeast strains. *Appl. Microbiol. Biotechnol.* 80: 881–890.
- Wallace, D., 2004 The Mann-Whitney test. *J. Am. Soc. Inf.* 55: 93.
- Warringer, J., E. Zörgö, F. A. Cubillos, A. Zia, A. Gjuvslund *et al.*, 2011 Trait variation in yeast is defined by population history. *PLoS Genet.* 7: e1002111.
- Wickham, H., 2009 *ggplot2*. Available at: <http://ggplot2.org>.
- Wightman, R., and P. A. Meacock, 2003 The THI5 gene family of *Saccharomyces cerevisiae*: distribution of homologues among the hemiascomycetes and functional redundancy in the aerobic biosynthesis of thiamin from pyridoxine. *Microbiology* 149: 1447–1460.
- Winzler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson *et al.*, 1999 Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285: 901–906.
- Xu, H., and J. D. Boeke, 1987 High-frequency deletion between homologous sequences during retrotransposition of Ty elements in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 84: 8553–8557.
- Zhang, F., W. Gu, M. E. Hurles, and J. R. Lupski, 2009 Copy number variation in human health, disease, and evolution. *Annu. Rev. Genomics Hum. Genet.* 10: 451–481.

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