

# The 100-genomes strains, an *S. cerevisiae* resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen

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*Saccharomyces cerevisiae*, a well-established model for species as diverse as humans and pathogenic fungi, is more recently a model for population and quantitative genetics. *S. cerevisiae* is found in multiple environments—one of which is the human body—as an opportunistic pathogen. To aid in the understanding of the *S. cerevisiae* population and quantitative genetics, as well as its emergence as an opportunistic pathogen, we sequenced, de novo assembled, and extensively manually edited and annotated the genomes of 93 *S. cerevisiae* strains from multiple geographic and environmental origins, including many clinical origin strains. These 93 *S. cerevisiae* strains, the genomes of which are near-reference quality, together with seven previously sequenced strains, constitute a novel genetic resource, the “100-genomes” strains. Our sequencing coverage, high-quality assemblies, and annotation provide unprecedented opportunities for detailed interrogation of complex genomic loci, examples of which we demonstrate. We found most phenotypic variation to be quantitative and identified population, genotype, and phenotype associations. Importantly, we identified clinical origin associations. For example, we found that an introgressed *PDR5* was present exclusively in clinical origin mosaic group strains; that the mosaic group was significantly enriched for clinical origin strains; and that clinical origin strains were much more copper resistant, suggesting that copper resistance contributes to fitness in the human host. The 100-genomes strains are a novel, multipurpose resource to advance the study of *S. cerevisiae* population genetics, quantitative genetics, and the emergence of an opportunistic pathogen.

[Supplemental material is available for this article.]

Research on *Saccharomyces cerevisiae*, the most extensively characterized model eukaryote, has historically focused on a very small number of strains, or genetic backgrounds. In particular, most research has focused on the laboratory strain S288c, the first eukaryotic genome to be completely sequenced, assembled, and annotated (Goffeau et al. 1996) and thus the reference *S. cerevisiae* genome (Engel et al. 2014). However, as with all species, there is more to *S. cerevisiae* than one strain. For example, array analyses (Muller and McCusker 2009b, 2011; Schacherer et al. 2009; Muller et al. 2011; Dunn et al. 2012), low coverage sequencing (Liti et al. 2009), and higher coverage sequencing (Wei et al. 2007; Doniger et al. 2008; Dowell et al. 2010; Skelly et al. 2013; Bergstrom et al. 2014) of a limited number of additional *S. cerevisiae* strains identified extensive sequence variation. Studies of *S. cerevisiae* genetic variation and its influence on phenotypic variation have been limited by the modest number of high quality, complete, assembled, and annotated genome sequences. To address these limitations, we describe here the sequencing, and subsequent de novo, high quality, and extensively manually edited assembly and annotation of the genomes of 93 *S. cerevisiae* strains of multiple geographic and environmental origins.

In addition to isolation from traditional, often human-associated environments (Mortimer and Johnston 1986; Mortimer and Polsinelli 1999; Sniegowski et al. 2002; Cromie et al. 2013), *S. cer-*

*visiae* is isolated clinically, consistent with its being an emerging opportunistic pathogen (Murphy and Kavanagh 1999; Ponton et al. 2000; Silva et al. 2004; Enache-Angoulvant and Hennequin 2005; Munoz et al. 2005; McCusker 2006; Skovgaard 2007; Pfaller and Diekema 2010; Miceli et al. 2011; Chitasombat et al. 2012). Because a reasonable hypothesis is that human environment-associated *S. cerevisiae* give rise to clinical *S. cerevisiae*, we compare 57 nonclinical, mostly human environment-associated strains with 43 clinical strains to gain insight into the emergence of *S. cerevisiae* as an opportunistic pathogen.

These 93 highly accurate, assembled, and annotated genome sequences, together with the genome sequences of S288c (Goffeau et al. 1996), YJM789 (Wei et al. 2007), RM11-1a (RM11 2004), SK1 (Nishant et al. 2010),  $\Sigma$ 1278b (Dowell et al. 2010), YPS163 (Doniger et al. 2008), and M22 (Doniger et al. 2008), constitute a novel, multipurpose genetic resource, the “100-genomes” strains. In addition to describing the sequences of the 93 genomes, we describe for the 100-genomes strains their population structure, multiple types of polymorphisms, chromosome rearrangements, aneuploidy, specific phenotypes, genotype-phenotype associations, as well as phenotypic differentiation between strains varying in population ancestry and in nonclinical vs. clinical origin.

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## Results

### The 100-genomes strains—derivation, genome sequencing, assembly, and annotation

The 100-genomes strains and their parental isolates with geographic and environmental origins are shown in Supplemental Table S1. Definitions of *S. cerevisiae* “isolates” and “strains,” the derivation of strains, and the many advantages of strains over isolates are more fully described in the Supplemental Material. Briefly, isolates, which are *isolated* from different environments, vary in ploidy (Muller and McCusker 2009a) and are frequently heterozygous across much of their genomes (McCusker et al. 1994; Muller and McCusker 2009a; Esberg et al. 2011). Many isolates do not sporulate, and many isolates that do sporulate produce no or very few viable spores (McCusker et al. 1994; Muller and McCusker 2009a). Thus, for many isolates, genome assembly/annotation as well as many types of genetic analysis would be highly problematic. Therefore, rather than isolates, we focus on segregants of sporulation-positive isolates (one segregant per isolate), which we define as strains. Strains greatly simplify genome assembly/annotation and association analysis. The 100-genomes strains were placed into five populations and one mosaic group, as described below. Strains from both natural (e.g., fruit;  $n = 57$ ) and clinical (i.e., human body sites;  $n = 43$ ) environments were chosen to provide insight into the emergence of *S. cerevisiae* as an opportunistic pathogen, as described below.

Genome sequencing, assembly, and annotation of 93 strains are fully described in the Supplemental Material. Briefly, based on 101 base pair paired-end reads, we produced the 93 de novo, high quality genome assemblies from 22- to 650-fold sequencing coverage per strain (average = 183.6-fold; median = 117.4-fold sequencing coverage) (Supplemental Table S2). To insure that the sequences generated corresponded to the initial strains, validation was performed using PCR primer pairs (Supplemental Table S3) to assess restriction fragment length polymorphisms. In the 93 genomes, we identified small numbers of ambiguous sites (N's, ambiguity codons) and unassembled loci, mostly unassembled repetitive telomeric/subtelomeric sequences (Supplemental Table S2). Only a small fraction of the total errors (0.2%–0.6% per genome) occur in protein-coding genes, with the remainder occurring in microsatellite sequences, misassembly of nearly identical repeat sequences, as well as repetitive telomeric/subtelomeric sequences. Approximately 17–114 kb of sequences per genome, consisting primarily of telomeric and subtelomeric repeat sequences, are not included in the assemblies. Thus, with the exception of incompletely assembled repetitive subtelomeric regions, our 93 de novo assemblies approach the quality of the reference S288c genome.

### Genetic assessment of chromosome colinearity/rearrangements in the 100-genomes strains

Due to the limitations of short-read sequencing, chromosomes could not be assembled across multicopy sequences. To determine whether the chromosomes of the 100-genomes strains were colinear (excluding subtelomeric regions lacking essential genes) with or contained chromosome rearrangements relative to the reference S288c genome, we genetically assessed chromosome colinearity/rearrangements (Supplemental Material). Briefly, we crossed haploid spores of all 100 strains with a haploid S288c background strain, sporulated the diploids, dissected tetrads, and assessed spore viabilities and spore viability patterns (Supplemental Table

S4). The high spore viabilities observed in 79 of the 100 crosses (79.7%–97.4%) are consistent with all of the chromosomes of these 79 strains being colinear (excluding subtelomeric regions lacking essential genes) with the S288c reference. In contrast, both the low spore viabilities (5.5%–71.3%) and the diagnostic spore viability patterns observed in the remaining 21 of the 100 crosses are consistent with these 21 strains having chromosome rearrangement(s) relative to the S288c reference (Supplemental Material). For 13 of these 21 strains, both the percent spore viabilities (51.6%–71.3%) and the diagnostic spore viability patterns observed in crosses were consistent with reciprocal translocations with essential genes distal to only one translocation breakpoint. In 12 of these 13 strains, we identified a chromosome 8–16 reciprocal translocation with an *ECM34-SSU1* breakpoint (Supplemental Material; Supplemental Table S4; Perez-Ortin et al. 2002); there are essential genes distal to *SSU1* but not *ECM34*.

### Genome sequence data defines multiple types of genetic variation

We utilized the assembled genomes of the 93 strains to annotate multiple types of sequence variation; identify aneuploidy; identify GO, population, and clinical origin associations; and compile specific sets of sequence variants (Supplemental Material; Supplemental Table S5–S13). Based on sequence read depths, 86 of the 93 strains are euploid and seven strains (7.5%) are aneuploid, with gains of part of a chromosome or for entire chromosomes (Supplemental Fig. S1). YJM326 contains a segmental duplication (i.e., partially aneuploid) for chromosome 7L (1–411 kb), with no polymorphisms between copies of the segmentally duplicated genes. The remaining six strains are aneuploid for one or multiple whole chromosomes, with no heterozygous polymorphisms on the aneuploid chromosomes.

Supplemental Table S8 summarizes single nucleotide polymorphisms (SNPs) and insertions-deletions (indels). The average numbers of SNPs and indels relative to S288c are 78,184 and 7840, respectively. We examined 4522, intron- and indel-free, single copy, protein-encoding genes for open reading frames (ORFs) length polymorphisms arising from frameshift and/or premature stop codon polymorphisms (Supplemental Material; Supplemental Table S12). Five hundred and seventy-six genes had ORF length polymorphisms; of these, in 57 genes the S288c ORF length was a minority allele or private allele. Thirty-five genes with frameshift and/or length polymorphisms were annotated as essential. Relative to the reference S288c genome, we determined that 61 genes were absent from some of the 93 strains, and 219 genes, including many novel genes, were present in some of the 93 strains.

We analyzed 32 genes that have been previously identified as having phenotypically relevant polymorphisms. For six of these 32 genes (*NCS2*, *AQY1*, *AQY2*, *RSF1*, *RME1*, *TAO3*), in addition to the previously identified, phenotypically relevant polymorphisms, other likely inactivating polymorphisms (i.e., frameshift, premature stop, deletions) were identified in some strains (Supplemental Material; Supplemental Table S13). In contrast, many others of these previously identified, phenotypically relevant polymorphisms are found in very few strains or were private to one strain. In particular, previously identified, phenotypically relevant polymorphisms in *MKT1*, *SSD1*, *MIP1*, *AMN1*, *FLO8*, *HAP1*, *BUL2*, and *SAL1* are private to the frequently used, long-term laboratory domesticated S288c and/or its close relative W303 (Supplemental Material).

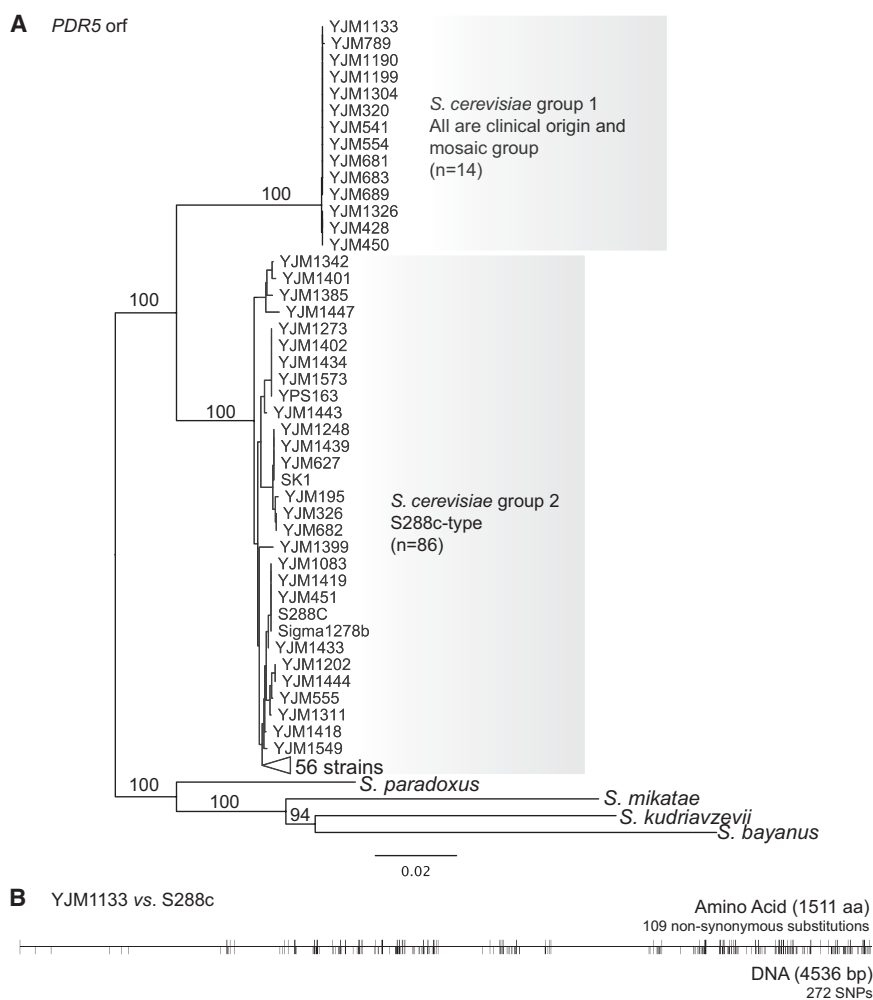
We searched the 93 genomes for highly diverged regions with low (70%–95%) sequence similarity to the syntenic region of the reference S288c genome and found many examples of highly diverged syntenic (putatively introgressed) genes (Supplemental Material). Most chromosomal regions are highly similar (99%–100% identity) to each other and to S288c (for example, see Supplemental Fig. S2A). However, we identified 141 syntenic, highly diverged regions, which ranged in length from 1 kb to 28 kb and contained from one to 16 protein-coding genes, with 70%–95% sequence identity to the corresponding S288c sequences. These 141 syntenic, highly diverged regions contained 401 protein-coding gene sequences, the genomic locations of which are distributed across the genome (Supplemental Table S5). Of the 401 highly diverged genes, 83 genes had low (70%–95%) similarity to sequenced *Saccharomyces* species, suggesting introgression from one or more unsequenced species of *Saccharomyces*. The remaining 318 highly diverged genes had high (>96% identity) similarity to the syntenic region of *S. paradoxus*, consistent with *S. paradoxus* introgression (for example, see Supplemental Fig. S2B). The size of an introgression often varied between strains (Supplemental Fig. S3), consistent with size reduction by recombination.

While 90 of the 93 strains had 1–26 introgressed genes relative to S288c, three strains (YJM1078, YJM1252, and YJM248) had 214–244 introgressed genes (Supplemental Table S5). For these three strains, most of the introgressed genes are shared and have high sequence similarity to *S. paradoxus*, suggesting that these strains derived from a single, recent inter-specific hybridization event. One hundred twenty-four introgressed genes were in only one of the 93 strains; 260 introgressed genes were in 2–8 strains; and 17 introgressed genes were in 12–87 strains. In the case of *YHL008C*, the S288c-like sequence was in only seven of the 100 strains (Supplemental Fig. S2C). In the case of *ZRT1*, polymorphisms in which have been previously described (Engle and Fay 2013), the S288c-like sequence was in only 20 of the 100 strains (Supplemental Fig. S2D).

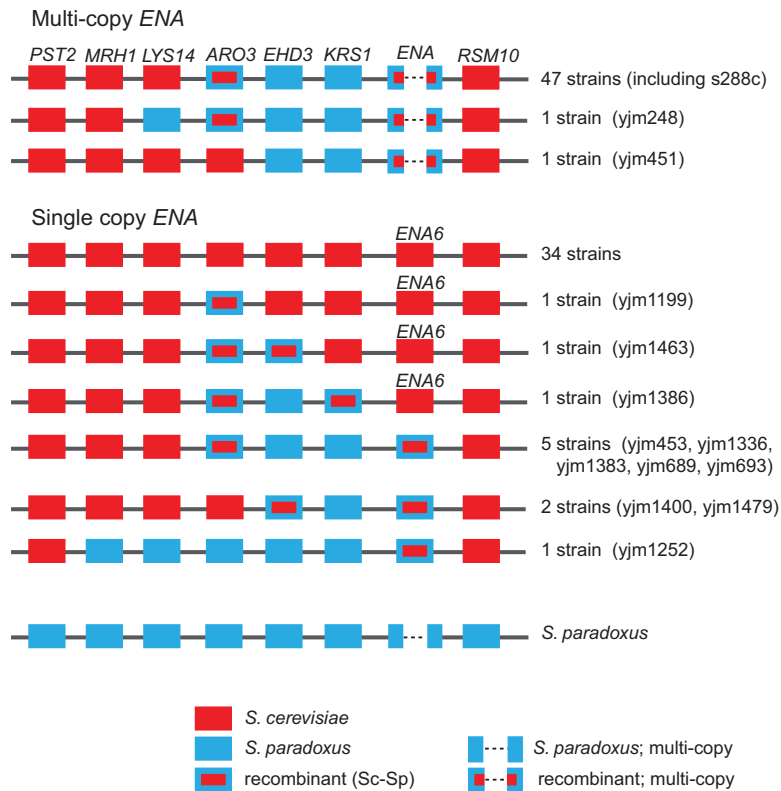
Using the *Saccharomyces* Genome Database Gene Ontology Term Finder, we assessed the Gene Ontology (GO) of the introgressed genes (Supplemental Table S6). Among the introgressed genes, the frequency of eight loci differed significantly between populations (Fisher's exact test, Bonferroni correction; populations defined using *structure* results as described in Supplemental Material), and between strains of clinical and nonclinical origin for an additional locus (Fisher's exact test, Bonferroni correction, no population structure correction) (Supplemental Table S7). In particular, the 14 strains with introgressed *PDR5*, all of which are in the mosaic group (Fig. 1), are of clinical origin ( $P = 0.0120$ ).

## ENA and CUPI tandem copy number variation, sequence variation, and recombination

Our data allowed us to precisely determine copy number of multicopy genes, including the P-type ATPase  $\text{Li}^+/\text{Na}^+$  pump-encoding *ENA* gene, which has been previously determined in very few strains (Haro et al. 1991; Wieland et al. 1995; Daran-Lapujade et al. 2009). Based on sequence coverage relative to flanking single-copy sequences, we determined that *ENA* copy number in the 93 strains ranged from one copy (45 strains) to 14 copies (Supplemental Table S14). Of 49 multicopy *ENA* strains, including S288c, all had the same size *ENA* repeat (3893 bp). Similar to previous lower sequence coverage studies of smaller numbers of strains (Doniger et al. 2008; Warringer et al. 2011), all 49 multicopy *ENA* strains had recombinant (portions of *ENA6* with one of three types of *S. paradoxus* *ENA* genes) *ENA* genotypes (Fig. 2; Supplemental Fig. S4; Supplemental Tables S5, S14). Similar to a previous five-strain study (Doniger et al. 2008; Warringer et al. 2011), *S. paradoxus* sequence similarity in these 49 strains



**Figure 1.** Putative introgressed *PDR5* gene. (A) Neighbor-joining tree of the *PDR5* ORF from 100 *S. cerevisiae* strains and sibling species with bootstrap values shows two clusters of this sequence. The group 1 cluster containing the putative introgressed *PDR5* consists of 14 strains that are both clinical in origin and in the mosaic population. The group 2 cluster consists of sequences from 86 strains including S288c. (B) Comparison of DNA and protein sequences between YJM1133 (with putative introgressed *PDR5*) and S288c. The tick marks indicate polymorphisms.



**Figure 2.** Structure of an introgressed cluster of ORFs. The reference strains S288c and 46 other strains have introgressed two ORFs (*EHD3* and *KRS1*) from *S. paradoxus*, while the other two ORFs (*ARO3* and *ENA*) are recombinant types. There are 34 strains with no *S. paradoxus* type sequences in this region, while one strain (YJM1252) has the most number of ORFs that are introgressed from *S. paradoxus*. The remaining strains have different combinations of introgressed and recombinant ORFs as shown.

extended to the proximal *ARO3-EHD3-KRS1* genes (Fig. 2; Supplemental Fig. S5, S6). In contrast, in the 45 single-copy *ENA* strains, most of the *ENA* genes had high sequence similarity to *ENA6* (Daran-Lapujade et al. 2009), and the contiguous *ARO3-EHD3-KRS1-ENA* genes had, to varying extents, low *S. paradoxus* sequence similarity. Based on our full assemblies and extensive annotation of the region, we identified extensive recombination between introgressed *S. paradoxus* and *S. cerevisiae* sequences in *ENA* and proximal genes (Fig. 2; Supplemental Tables S5, S14).

Similarly, *CUP1* copy number has been precisely determined in very few strains (Fogel and Welch 1982; Hamer et al. 1985; Zhao et al. 2014). Based on *CUP1* sequence coverage relative to flanking single-copy sequences, *CUP1* copy number in the 93 strains varied from one copy (28 strains) to 18 copies (Supplemental Table S15). In the 66 strains, including S288c, with  $\geq 2$  copies of *CUP1*, five different (i.e., breakpoints and repeat lengths) types of *CUP1* repeats were identified (Supplemental Table S15). In the 66 strains with  $\geq 2$  copies of *CUP1*, 57 strains had one type of *CUP1* repeat and nine strains had two types of *CUP1* repeats (Supplemental Table S15), the latter being consistent with recombination between different types of *CUP1* repeats.

### Population structure and principal component analysis

We used model-based clustering, as implemented in *structure* (Pritchard et al. 2000), to analyze the population structure of the 100-genomes strains (Fig. 3). Similar to previous studies (Liti et al.

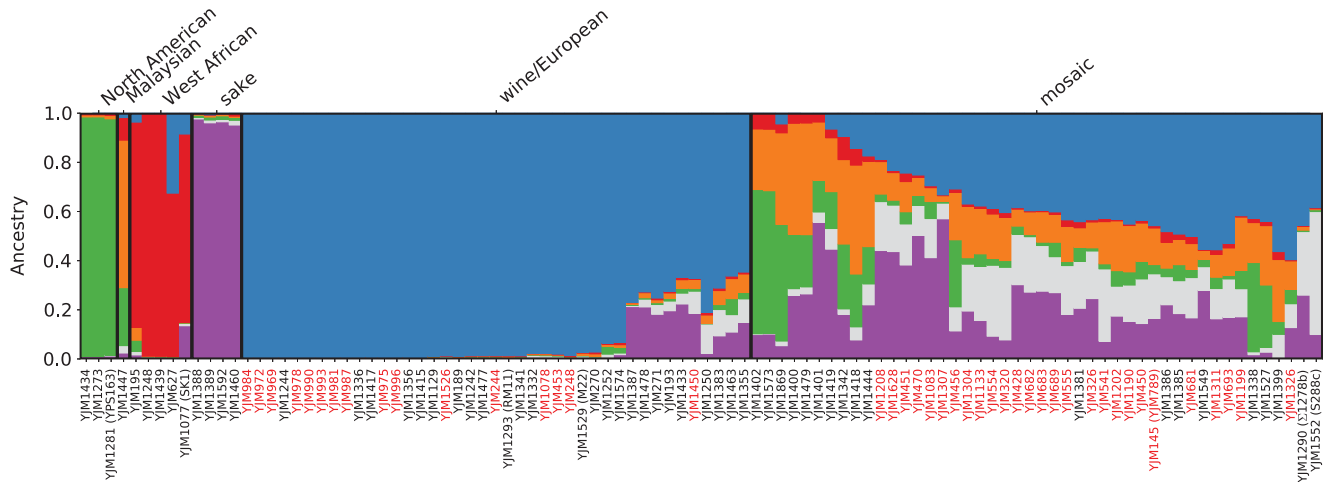
2009; Schacherer et al. 2009), some strains had ancestry corresponding largely to a single population, while other strains were admixed “mosaics” with ancestry from two or more populations (Fig. 3). Five of the populations, and the mosaic group, correspond to those previously reported (Liti et al. 2009), and we use the same names. The sixth population, contributing partial ancestry predominantly to mosaic strains in our data set, was not identified by Liti et al. (2009) but corresponds well with the two human-associated populations identified by Cromie et al. (2013). Specifically, we observed strong correlation (Pearson correlation 0.737, Spearman rank correlation 0.783) between fractional ancestry of our sixth population and summed fractional ancestry of both human-associated populations identified by Cromie et al. (2013). Thus, as per Cromie et al. (2013), we refer to this sixth population as human-associated.

Supplemental Figure S7 imposes population structure assignments on a map of collection locations, where known, for the isolates from which the 100-genomes strains were derived. As reported by Cromie et al. (2013), strain diversity is primarily driven by geography and human-associated admixture. Of the 100-genomes strains, the largest group of strains with ancestry from a single population is the wine/European

population ( $N=41$ ). The majority of the remaining strains ( $N=46$ ) are mosaics; for mosaics, the wine/European population contributed the largest total portion of ancestry (mean 34%), with slightly less ancestry contributed by the sake, Malaysian, human-associated, and North American populations (mean 22%–11%) and little West African population ancestry (mean 2%). Based on sequences extracted from each of the 16 chromosomes of each strain (total = 218 kb; 124 protein-coding genes) (Supplemental Material; Supplemental Table S16), principal component analysis of the sequence identity values (Supplemental Fig. S8), and a phylogeny (Fig. 4) using the SNPs in the 218-kb region, show the clustering of the six populations identified in the population structure analysis and clusters of clinically and nonclinically derived strains. Of the 100-genomes strains, 43 are clinically derived, all of which fell into either the wine/European ( $N=16$ ) population or the mosaic group ( $N=27$ ). Clinically derived strains constituted 59% of the mosaic group ( $P=0.00233$ , Fisher’s exact test).

### Genotype-phenotype associations in the 100 strains

We measured 49 phenotypes in the 100-genomes strains (Supplemental Material). Phenotypic variability was pervasive and in most cases quantitative (Supplemental Table S17; Supplemental Fig. S9). We carried out genotype-phenotype association mapping to identify loci contributing to this phenotypic variation. For this analysis, we focused on common variation (minor allele frequency  $\geq 5\%$ ) and tested  $\sim 158,000$  biallelic SNPs and indels; the



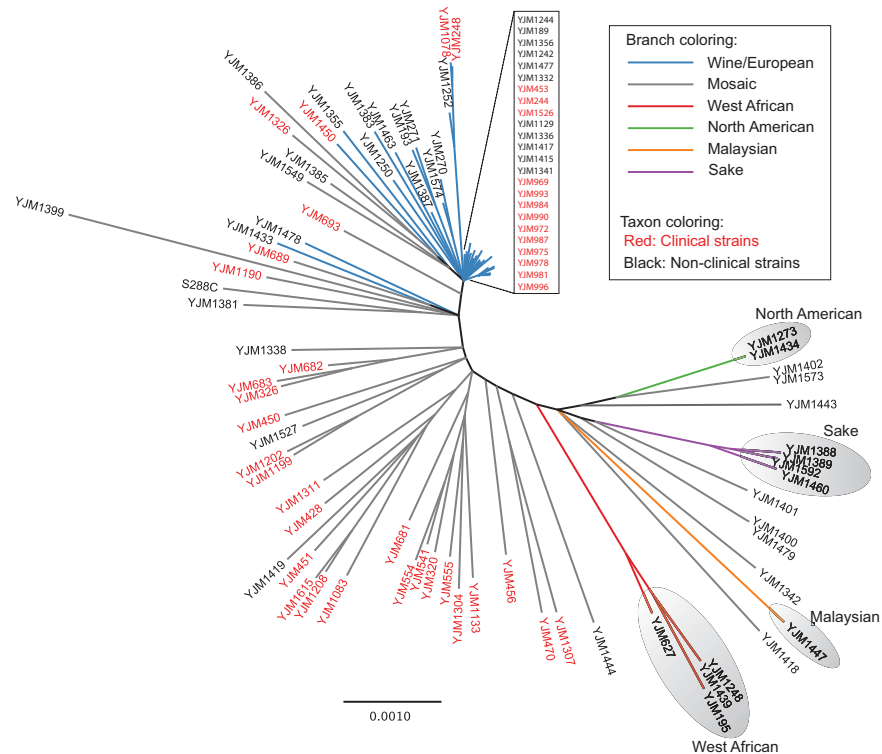
**Figure 3.** Population structure of the 100-omes *S. cerevisiae* strains. Population structure inferred for 100 *S. cerevisiae* strains using the program *struc*-*ture* (Pritchard et al. 2000). Each strain is represented by a single vertical line, which depicts fractional population ancestry from each of  $K = 6$  subpopulations denoted by colors: green (North American), orange (Malaysian), red (West African), purple (sake), blue (wine/European), and gray (human-associated). Black boxes delineate the six strain populations. A threshold of 60% ancestry from any single population was used to construct groupings, except for mosaic strains that have <60% ancestry from any of the other five populations. Strain names in black and red indicate nonclinical and clinical environmental origin strains, respectively.

presence/absence of 28 genes annotated in the reference S288c strain but missing from other members of the 100-omes strains; 32 introgressed loci; 108 genes missing from or not annotated in S288c; and 150 genes with frameshift or premature termination codon polymorphisms. Of the many genotype-phenotype associations (Supplemental Table S18), we focus below on five genotype associations with  $P$ -values lower than an approximate Bonferroni threshold of  $P < 4 \times 10^{-7}$  that also have clear biological connections to the phenotypes. With the exception of Warringer et al. (2011), the cited studies are not population genetic analyses and are cited to demonstrate gene-phenotype biological connections.

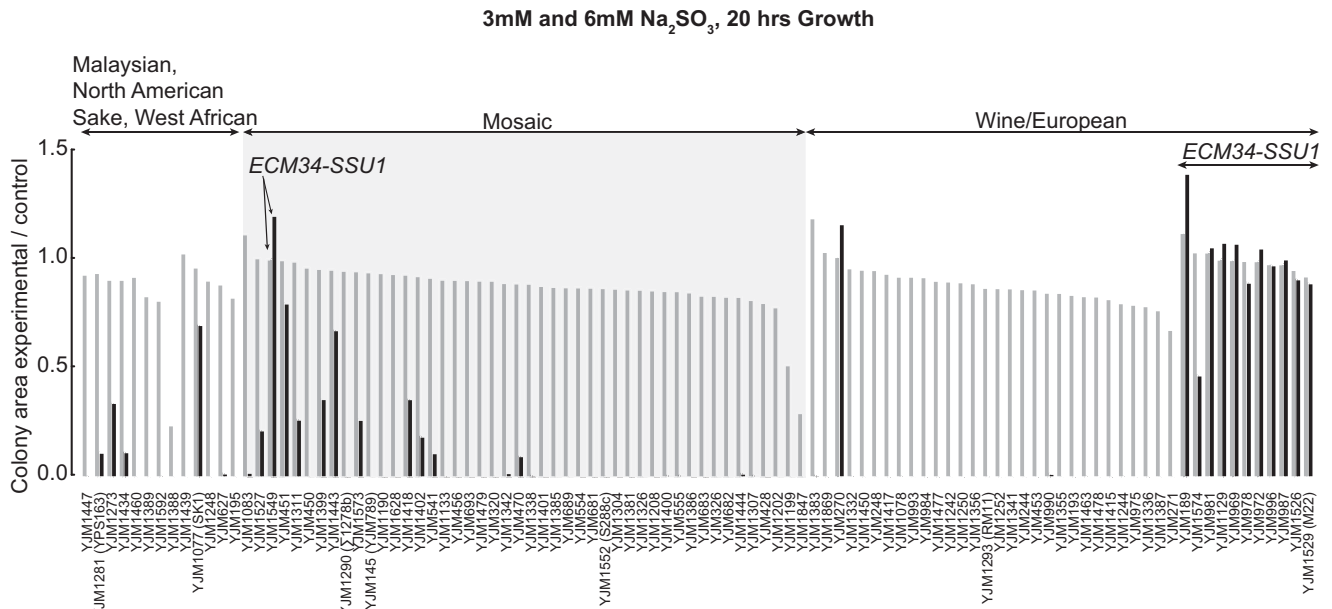
First, growth on 6 mM sulfite was associated ( $P < 10^{-14}$ ) with many SNPs at *SSU1* and *ECM34*, genes implicated in a sulfite resistance-conferring chromosome 8–16 translocation (Perez-Ortin et al. 2002). The *ECM34-SSU1* translocation, which is in 12 strains (Supplemental Material; Supplemental Table S4) and explains ~70% of the 6 mM sulfite growth variation ( $R^2 = 0.71$ ), also showed association ( $P = 6.3 \times 10^{-28}$ ) (Fig. 5; Supplemental Table S18).

Second, consistent with previous work (Haro et al. 1991; Martinez et al. 1991; Wieland et al. 1995; Daran-Lapujade et al. 2009; Warringer et al. 2011), we found lithium resistance association with both SNPs ( $P = 7.4 \times 10^{-8}$ ) and copy number ( $P = 5.0 \times 10^{-10}$ ) of the P-type ATPase  $\text{Li}^+/\text{Na}^+$  pump-encoding *ENA* gene (Supplemental Table S18). *ENA* explains ~40% ( $R^2 = 0.40$ ) of the lithium resistance variation.

Third, consistent with previous work (Fogel and Welch 1982; Hamer et al. 1985; Fogel et al. 1988; Warringer et al. 2011), we found association between *CUP1* copy number (Supplemental Table S18) and copper resistance ( $0.1 \text{ mM}$ ,  $P = 1.9 \times 10^{-17}$ ). Consistent with *CUP1* having undergone five independent amplification events, no linked polymorphisms associated with *CUP1* copy number. In



**Figure 4.** Phylogeny of 94 *S. cerevisiae* strains. Neighbor-joining tree of 93 *S. cerevisiae* strains plus S288c using 218 kb (total) of sequence (excluding introgressions and large indels) gathered from all 16 chromosomes.



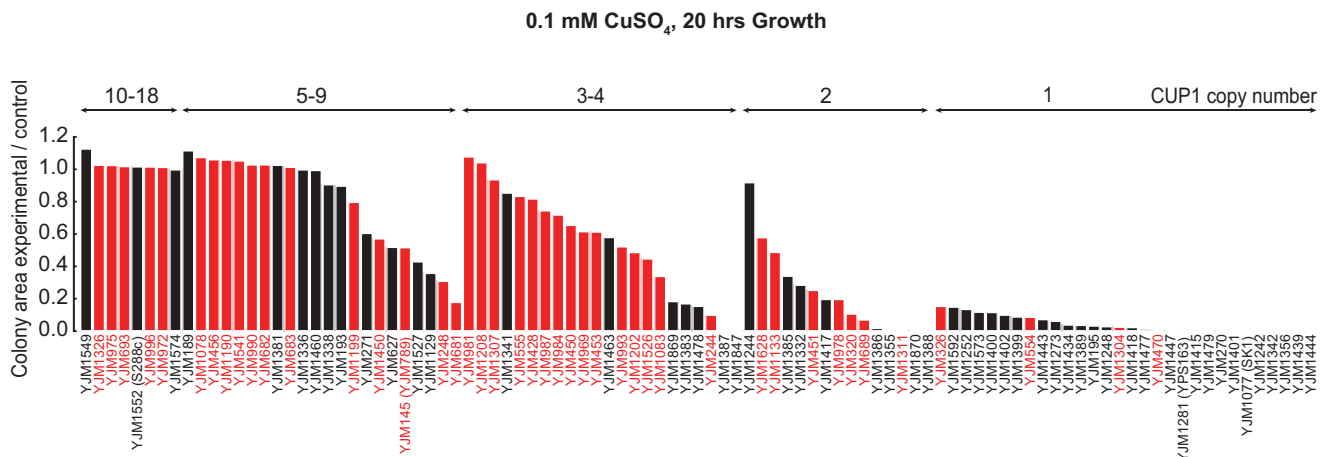
**Figure 5.** Sulfite resistance phenotypes of the 100-genomes *S. cerevisiae* strains. Colony area as a measure of sulfite resistance (3 mM: gray bars; 6 mM: black bars). Strains are arranged by population structure. Strains with the *ECM34-SSU1* translocation show higher sulfite resistance than those without the translocation.

97 strains with known *CUP1* gene copy number (the 93 strains plus YJM1077 and YJM1281, shown by PCR to have one copy of *CUP1*; YJM789 [seven copies of *CUP1*] [Zhao et al. 2014]; and YJM1552 [14 copies of *CUP1* in the S288c background] [Zhao et al. 2014]) (Supplemental Table S15), *CUP1* gene copy number explained ~50% of variation in copper resistance ( $R^2 = 0.52$ ) (Fig. 6).

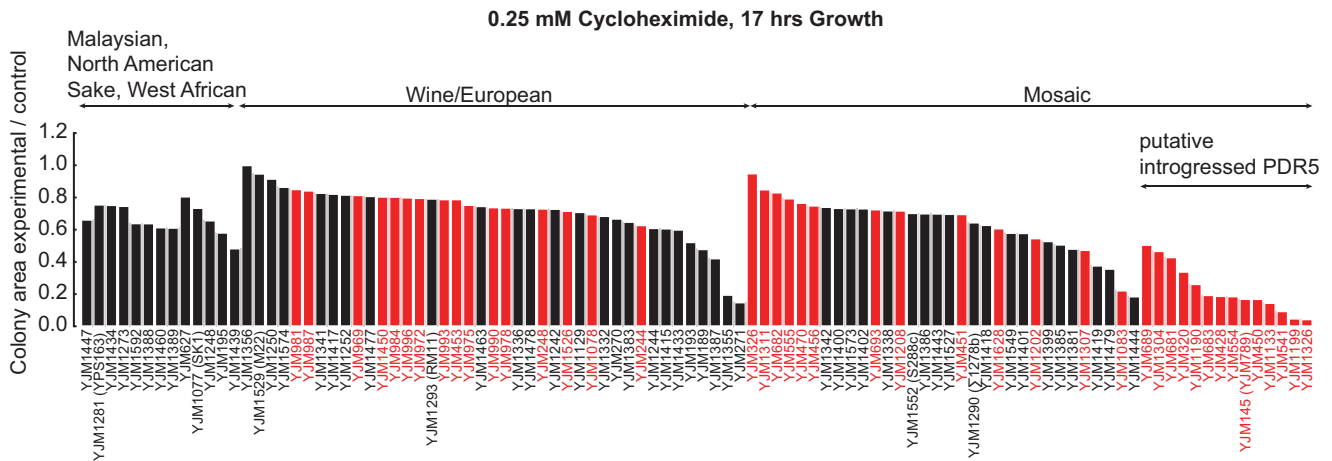
Fourth, consistent with previous work (Winzeler et al. 1998; Wei et al. 2007; Guan et al. 2010), we found association between the multidrug transporter *PDR5* and resistance to the translation inhibitor cycloheximide and to the clinically used ergosterol synthesis inhibitor ketoconazole. In contrast with Guan et al. (2010), we did not detect association between *PDR5* and amphotericin B resistance. However, in agreement with Guan et al. (2010), we

observed association ( $P = 5.3 \times 10^{-9}$ ) between the introgressed *PDR5* and 10 mM ketoconazole sensitivity, with this locus explaining one-third of growth variation between strains ( $R^2 = 0.33$ ). Moreover, we found a strong association ( $P = 4.2 \times 10^{-12}$ ) between the introgressed *PDR5* and 0.25 mM cycloheximide sensitivity (Fig. 7; Supplemental Table S18), with this locus explaining almost half of the variation between strains ( $R^2 = 0.46$ ).

Finally, for growth in medium lacking pyridoxine, we identified association with four SNPs in and surrounding the gene *SNZ1*, the sole copy of *SNZ* required for pyridoxine biosynthesis (Rodriguez-Navarro et al. 2002). The SNP with the strongest association (G53A;  $P = 6.8 \times 10^{-14}$ ) results in an amino acid substitution (G18D) in *SNZ1*. We identified the G18D substitution as



**Figure 6.** Copper resistance phenotypes of the 100-genomes *S. cerevisiae* strains. Colony area as a measure of copper resistance in 96 *S. cerevisiae* strains at 0.1 mM CuSO<sub>4</sub> concentration. Strains are arranged according to the *CUP1* copy number, then to colony size. Increase in *CUP1* copy number increases resistance to copper, although there are also *CUP1* copy number-independent contributions. Red bars denote clinical strains and black bars denote non-clinical strains.



**Figure 7.** Cycloheximide resistance phenotypes of the 100-genomes *S. cerevisiae* strains. Colony area as a measure of cycloheximide resistance in 100 *S. cerevisiae* strains at 0.25 mM cycloheximide concentration. Strains are arranged according to the population structure, then to the presence/absence of the putatively introgressed *PDR5*, then to colony size. Strains having the putatively introgressed *PDR5* are more sensitive to cycloheximide. Red bars denote clinical strains and black bars denote nonclinical strains.

likely to have a strong effect on protein function using computational predictions (Choi et al. 2012), with the G18D substitution ranked as most likely to be deleterious out of 22 nonsynonymous variants in *SNZ1* present among the 100 strains. The G53A SNP explains >40% of variation between strains in growth on media lacking pyridoxine ( $R^2 = 0.44$ ). This is the first report that natural variation in *SNZ1* underlies phenotypic variation among natural yeast strains.

**Phenotypic differentiation between strains varying in population ancestry**

In 99 of the 100-genomes strains (excluding the single Malaysian population strain), we tested for phenotypic differences associated with strains with different population ancestries. Of 49 phenotypes, eight showed significant differences between populations (Table 1). On medium lacking pantothenate, wine/European population strains had lower mean growth (80.1% relative to control) compared to all other populations (mean ranging from 88.1% for mosaic group strains to 100% for sake population strains). Similar to Warringer et al. (2011), on medium containing LiCl, wine/European population strains had higher mean growth (73.2% rel-

ative to control) compared to all other populations (mean ranging from 4.6% for West African population strains to 62.6% for sake population strains). For growth at low and high temperatures in different media, we observed significant phenotypic differences between populations (Table 1).

Lastly, we found significant differences between populations in resistance to ketoconazole, cycloheximide, and amphotericin B that are largely driven by pairwise significant differences between the well-sampled wine/European population and mosaic group strains. Relative to mosaic group strains, wine/European population strains had significantly higher resistance to cycloheximide (mean 71.5% vs. 51.1%) and significantly lower resistance to amphotericin B (mean 54.7% vs. 72.5%). For cycloheximide and ketoconazole sensitivity, the introgressed *PDR5*, which is found only in mosaic group strains (Fig. 7), accounts for the bulk of the phenotypic difference between populations; any additional contribution of population is not significant for cycloheximide and only marginally significant for ketoconazole (ANOVA,  $P = 0.046$ ). Consistent with the lack of association between amphotericin B resistance and *PDR5* (see above), and after accounting for any *PDR5* contribution, population contributes significantly to amphotericin B resistance (ANOVA,  $P = 0.0041$ ) (Table 1).

**Table 1.** Significant phenotypic differences between strains of different population ancestries or clinical/nonclinical origin

Phenotype	Test for significant difference between:	Bonferroni-corrected <i>P</i> -value	Significant pairwise differences (Bonferroni-corrected $P < 0.05$ )
Pantothenate	Populations	0.00319	
Growth on YPEG, 39°C	Populations	0.00342	Wine/European < N. American
Growth on YPD, 15°C and 39°C	Populations	0.00454, 0.0406	Wine/European < sake
Lithium resistance, 50 mM	Populations	0.0119	Wine/European > mosaic (15°C only)
Ketoconazole, 10 mM	Populations	0.0123	Wine/European > mosaic
Amphotericin B, 15 mM	Populations	0.0222	Wine/European < mosaic
Cycloheximide, 0.25 mM	Populations	0.0240	Wine/European > mosaic
% four-spored asci, KAc plates 25°C and 30°C	Clinical/nonclinical	0.00482, 0.0117	Clinical < nonclinical
% sporulation, diet KAc plates 25°C	Clinical/nonclinical	0.00358	Clinical < nonclinical
Copper resistance, 0.075 mM and 0.1 mM	Clinical/nonclinical	0.0116, 0.00531	Clinical > nonclinical

Kruskal–Wallis rank sum test or Mann-Whitney *U* test, Bonferroni-corrected *P*-value < 0.05.

### Phenotypic differentiation between strains of nonclinical vs. clinical origin

We identified two significant phenotypic differences between strains of clinical and nonclinical origin. First, we found that clinical origin strains sporulate at significantly reduced frequencies in three of six tested conditions (Table 1). Second, we found that clinical origin strains are significantly more copper resistant than nonclinical strains (mean 62.1% vs. 31.5% in 0.1 mM CuSO<sub>4</sub>) (Table 1). As expected given the association between copper resistance and *CUP1* copy number, clinical strains had significantly more copies of *CUP1* than nonclinical strains (median 4.0 vs. 2.0; Mann-Whitney *U* test,  $P=0.00023$ ). However, in addition to the *CUP1* copy number contribution, clinical origin contributes significantly to the copper resistance phenotype (ANOVA,  $P=0.0023$ ) (Fig. 6).

## Discussion

### *S. cerevisiae* isolates vs. the 100-genomes strains—genome assembly, annotation, and use

We more fully define *S. cerevisiae* isolates and strains and describe the many advantages of strains over isolates in the Supplemental Material. Briefly, many *S. cerevisiae* isolates are sporulation-deficient and many sporulation-proficient isolates produce few or no viable spores; while useful for population genetic analysis, such *S. cerevisiae* isolates are poorly suited for classical and quantitative genetic analyses. In addition, many isolates are heterozygous across most of their genomes, which would greatly complicate genome assembly and annotation, as well as association studies. However, for those isolates that sporulate and produce viable spores, the resulting haploid and self-diploidized segregants are strains; the lack of heterozygosities in strains greatly simplifies genome assembly and annotation, which for our 93 strains approaches the quality of the reference S288c genome.

Natural selection acts on the phenotype of the isolate. For self-diploidized isolates, and isolates with few heterozygous polymorphisms, a single segregant (strain) identifies all, or almost all, of the genotypic and phenotypic diversity of the parental isolate. For multiply heterozygous isolates, a single segregant (strain) identifies a significant portion of the genotypic and phenotypic diversity of the parental isolate. In-depth phenotypic profiling of isolates and comparisons with multiple segregants (strains) from each isolate will be necessary for a more thorough understanding of the degree to which strain-level variation reflects selection on isolates. However, the significant genotypic and phenotypic associations of strains with populations and clinical/nonclinical origin likely reflect, in part, differences in selection on the parental isolates.

### Chromosomal rearrangements, aneuploidy, and introgression

We find that the reference S288c genome structure is the most common in the 100-genomes strains (79 of 100 strains), although a significant fraction (21 of 100) of strains show evidence of chromosomal rearrangements. In the 93 strains, aneuploidy is less frequent (7.5%; 7 of 93) than chromosomal rearrangements, but our observed rate of aneuploidy may underestimate the true rate in environmental isolates due to our strain selection criteria. Both chromosomal rearrangements and aneuploidy should be considered in population and quantitative genetic studies, which is easily achieved with the 100-genomes strains.

We identified 401 putatively introgressed genes found in at least one of the 93 strains (Supplemental Table S5). The majority of these genes are likely derived from introgression with *S. paradoxus*, but a significant fraction (~20%) of candidate introgressed genes appear to be derived from one or more as yet unsequenced species of *Saccharomyces*. We conclude that introgressed genes are common in *S. cerevisiae* and, in at least two cases (*ENA*, *PDR5*), have major phenotypic effects.

### ENA copy number variation, introgression, and Li<sup>+</sup> resistance in the 100-genomes strains

Relative to Warringer et al. (2011), we determined to a much higher degree of precision the numbers of the P-type ATPase Li<sup>+</sup>/Na<sup>+</sup> pump-encoding *ENA* gene. In 49 strains with ≥2 tandem copies of *ENA*, we identified one type of *ENA*-containing repeat, consistent with one *ENA* amplification event. Similar to Warringer et al. (2011), we observed *ENA* association with Li<sup>+</sup> resistance. Similar to previous studies (Doniger et al. 2008; Warringer et al. 2011), we observed introgression of *S. paradoxus* *ENA* and proximal sequences. However, relative to previous studies (Doniger et al. 2008; Warringer et al. 2011), our sequence coverage, assemblies, detailed annotation, and larger numbers of strains better identified extensive recombination between *S. cerevisiae* and *S. paradoxus* sequences in this region and identified two large classes of strains. First, there was an S288c-like class, consisting of 47 strains with recombinant *ARO3*, *S. paradoxus*-derived *EHD3-KRS1*, and recombinant, multicopy *ENA*. Second, there was a non-S288c-like class, consisting of 34 strains with *ARO3-EHD3-KRS1* having low sequence similarity to *S. paradoxus* and single-copy *ENA6* (Daran-Lapujade et al. 2009). We propose that the non-S288c-like class represents the ancestral *S. cerevisiae* sequences and that the S288c-like class represents *S. paradoxus* introgression.

### *CUP1* copy number variation, types of *CUP1* repeats, and copper resistance in the 100-genomes strains

Relative to Warringer et al. (2011), we determined to a much higher degree of precision the numbers of the copper metallothionein-encoding gene *CUP1*. In 66 strains with ≥2 tandem copies of *CUP1*, we identified five types of *CUP1* repeats (Supplemental Table S15), consistent with independent amplification events. Also consistent with five independent *CUP1* amplification events, there was very little *CUP1* sequence heterogeneity and no *CUP1*-linked SNPs associated with *CUP1* copy number or with copper resistance. We hypothesize that independent *CUP1* gene amplification events, and increased copper resistance, have been selected by the agricultural use of fungicidal copper salts (Borkow and Gabbay 2005; Holb 2009; Mackie et al. 2012) in natural *S. cerevisiae* environments.

Similar to Warringer et al. (2011), we observed *CUP1* copy number association with copper resistance and that *CUP1* copy number was responsible for ~50% of copper resistance. However, in contrast to the two types of *CUP1* repeats identified by Warringer et al. (2011), we identified five types of *CUP1* repeats. Also in contrast to Warringer et al. (2011), we did not observe population association with either the type of *CUP1* repeat or copper resistance. The different *CUP1*-related results of Warringer et al. (2011) and this study are due in part to sequence coverage differences (one- to fourfold [Liti et al. 2009] vs. 22- to 650-fold), and consequently improved assembly and annotation of genomes, as well as numbers of strains; other factors are discussed below.



### Genotype-phenotype associations in the 100-genomes strains

In contrast to previous association studies in yeast, which have been limited in scope (Muller et al. 2011; Connelly and Akey 2012; Diao and Chen 2012) or dependent on additional linkage-based evidence for quantitative trait loci (Warringer et al. 2011), we sought to carry out an unbiased, genome-wide search for quantitative trait loci underlying diverse phenotypes. We considered this analysis to be exploratory in nature, since 100 strains constitute a limited sample size for association mapping. In simulations based on sequenced genomes, we found that the power to detect loci of large effect (>35%) is relatively high (>80% at uncorrected  $P < 1 \times 10^{-7}$ ) (Supplemental Material). Due to the challenges of establishing a single significance threshold, uncorrected  $P$ -values were reported and we focused on loci likely to be true positives; that is, loci with clear mechanisms of action suggested from previous literature and with  $P$ -values much lower than an approximate Bonferroni level of  $P < 4 \times 10^{-7}$ .

For multiple phenotypes, we found few or no associations with  $P$ -values lower than an approximate Bonferroni threshold of  $P < 4 \times 10^{-7}$  (Supplemental Table S18). For some other phenotypes (e.g., inositol and thiamine auxotrophy), we found many associations close to the approximate Bonferroni threshold of  $P < 4 \times 10^{-7}$  but none with associations with much lower  $P$ -values (e.g., minimum  $P$ -value  $> 1 \times 10^{-10}$ ); in all of these cases, the associations had very wide peaks (up to 100 kb) and/or lacked genes with clear biological connections to phenotype (Supplemental Table S18). These results illustrate the challenges of performing association mapping in a small, structured sample and suggest that variation in these phenotypes may not be governed by individual loci of large effect size. However, despite these challenges, we identified five robust genotype-phenotype associations: *ECM34-SSU1* (SNPs, translocation) for sulfite resistance; *ENA* (copy number, SNPs) for  $\text{Li}^+$  resistance; *CUP1* (copy number) for copper resistance; *PDR5* (introgression) for cycloheximide and ketoconazole resistance; and a nonsynonymous SNP in *SNZ1* for pyridoxine auxotrophy. Therefore, the 100-genomes strains can be used to perform unbiased, genome-wide searches for quantitative trait loci.

### Population-phenotype associations in the 100-genomes strains

In contrast to Warringer et al. (2011), we identified relatively few phenotype associations with populations (Table 1), the reasons for which are biological and technical. First, our larger sample size (38 vs. 100 strains) likely includes more phenotypic outlier strains, with consequently increased intra-population phenotype variability and decreased population-phenotype association. Second, the two studies used different phenotyping methods (growth curves vs. colony size) and phenotype replication ( $n = 2$  vs.  $n = 8$  or 24). Third, we tested for an overall effect of population on each phenotype, which is underpowered for phenotypes specific to populations with small sample sizes (sake, North American, and West African), and used a relatively conservative nonparametric test and multiple testing corrections.

### 100-genomes strains' insight into *S. cerevisiae* as an emerging opportunistic pathogen

*S. cerevisiae* is a human colonizer and pathogen. As Pfaller and Diekema (2010) state, "*S. cerevisiae* is a common colonizer of mucosal surfaces and part of the normal flora of the GI tract, the respiratory tract, and the vagina (Salonen et al. 2000). It accounts for

27% of clinical isolates of non-*Candida*, non-*Cryptococcus* yeasts in North America (Pfaller et al. 2007, 2009)." As a likely consequence of being a common colonizer, *S. cerevisiae* also causes infections, the incidence of which has increased (Enache-Angoulvant and Hennequin 2005; Pfaller and Diekema 2010). Like some of its relatives among the more commonly clinically observed *Candida* species, *S. cerevisiae* can be recovered from nonsterile (mucosal) and sterile (e.g., blood) body sites of both immune-deficient and immune-competent patients. *S. cerevisiae* and *Candida* infections have similar predisposing patient factors, such as immune deficiency, antibiotic treatment, and intravascular catheters (Enache-Angoulvant and Hennequin 2005; Pfaller and Diekema 2010; Miceli et al. 2011; Chitasombat et al. 2012). *S. cerevisiae* invasive infections and invasive candidiasis are clinically indistinguishable (Enache-Angoulvant and Hennequin 2005; Pfaller and Diekema 2010). Patient colonization by environmental *S. cerevisiae* can be oral, person-to-person, and nosocomial (Zerva et al. 1996; Salonen et al. 2000; Hennequin et al. 2001; Olver et al. 2002; Clemons et al. 2010). Finally, *S. cerevisiae* infections cause significant mortality (Pfaller and Diekema 2010; Chitasombat et al. 2012). Thus, *S. cerevisiae* is an emerging and often serious opportunistic pathogen (Murphy and Kavanagh 1999; Ponton et al. 2000; Silva et al. 2004; Enache-Angoulvant and Hennequin 2005; McCusker 2006; Pfaller and Diekema 2010; Miceli et al. 2011; Chitasombat et al. 2012).

While *S. cerevisiae* can be isolated from non-human-associated environments (e.g., Wang et al. 2012), nonclinical but human environment-associated *S. cerevisiae* (e.g., food, drink) are the most likely source of clinical *S. cerevisiae*. For this reason, comparison of non-human-associated environment *S. cerevisiae* with clinical *S. cerevisiae* likely would be uninformative and/or misleading as to *S. cerevisiae* becoming an opportunistic pathogen. Therefore, we compared 57 nonclinical, mostly human environment-associated strains with 43 clinical strains to gain insight into the emergence of *S. cerevisiae* as an opportunistic pathogen.

Although host factors clearly exist for *S. cerevisiae* infections (Enache-Angoulvant and Hennequin 2005; Pfaller and Diekema 2010; Miceli et al. 2011; Chitasombat et al. 2012), it seems likely that *S. cerevisiae* genotype and phenotype also play key roles in human colonization and subsequent infection. Consistent with the importance of *S. cerevisiae* genotype for human colonization/infection, environmental and clinical *S. cerevisiae* strains are genotypically distinct. First, clinically derived strains comprised 59% of the mosaic strains ( $P = 0.00233$ , Fisher's exact test). Clinical *S. cerevisiae* isolates tend to be multiply heterozygous (Muller and McCusker 2009a; Magwene et al. 2011), consistent with outcrossing between different *S. cerevisiae* populations and the clinical origin-mosaic group association. Similarly, in the human pathogens *Toxoplasma gondii* and *Cryptococcus gattii*, outcrossing between different populations results in progeny with mosaic genomes that have increased transmission, dissemination, and/or virulence phenotypes (Grigg and Suzuki 2003; Minot et al. 2012; Voelz et al. 2013). Therefore, the clinical origin-mosaic group association suggests that outcrossing between different *S. cerevisiae* populations leads to new allelic combinations that facilitate adaptation to novel niches (Magwene 2014), in this case increased transmission, dissemination, and/or fitness of *S. cerevisiae* in human host environments. Second, we found *TCB2*, *BIO5*, and *PDR5* genotype-clinical origin associations (Supplemental Table S7). Finally, clinical origin strains have significantly higher numbers of the copper metallothionein-encoding gene *CUP1* (median 4.0 vs. 2.0; Mann-Whitney  $U$  test,  $P = 0.00054$ ).

Consistent with the importance of *S. cerevisiae* phenotype for human colonization/infection, environmental and clinical *S. cerevisiae* are also phenotypically distinct. First, there are significant differences in the virulence phenotypes of *S. cerevisiae* clinical and nonclinical isolates (Clemons et al. 1994). Second, in three of six tested conditions, the sporulation phenotypes of clinical origin strains are significantly reduced (Table 1), consistent with increased *S. cerevisiae* fitness in human host environments having an unexpected cost. Finally, the copper resistance of clinical origin strains is significantly higher (Table 1). In addition to clinical strains having higher *CUP1* copy numbers than nonclinical strains, there is also a significant *CUP1* copy number-independent, clinical origin-dependent contribution to copper resistance (ANOVA,  $P=0.0023$ ) (Fig. 6). Interestingly, copper resistance is necessary for the virulence of multiple species of bacteria (Hodgkinson and Petris 2012; Samanovic et al. 2012; Neyrolles et al. 2013) and the opportunistic pathogenic yeast *Cryptococcus neoformans* (Ding et al. 2013), likely due to macrophages using copper to kill pathogens. Our finding that *S. cerevisiae* copper resistance associates with clinical origin suggests that copper resistance is similarly relevant to *S. cerevisiae* survival in human hosts. *S. cerevisiae* copper resistance has likely been selected by the common and historically long-term use of copper salts as agricultural fungicides (Borkow and Gabbay 2005; Holb 2009; Mackie et al. 2012) in natural *S. cerevisiae* environments. Thus, environmentally selected copper resistance likely contributes to the emergence of *S. cerevisiae* as an opportunistic pathogen.

## Conclusion

Combined with seven previously sequenced *S. cerevisiae* genomes, our 93 highly accurate, de novo assembled, extensively manually edited/annotated, and near-reference genome quality sequences constitute the 100-genomes strains. We placed the 100-genomes strains into five populations and a mosaic group, with most of the clinical origin strains being in the mosaic group. We identified abundant genetic variation in the 100-genomes strains. For many genetic variants, we identified significant GO, population, and clinical origin associations. Our phenotypic characterization of the 100-genomes strains identified significant genotype-, population-, and clinical origin-phenotype associations. In particular, the higher copper resistance of clinical origin strains provides novel insights into the emergence of *S. cerevisiae* as an opportunistic pathogen. In addition to population, genotype, phenotype, and clinical origin association studies, the 100-genomes strains can be used for classical genetic studies, which have previously been limited to laboratory strains, and for quantitative genetic studies. Thus, the 100-genomes strains are a novel, valuable, multipurpose genetic resource.

## Methods

### Strains

Supplemental Table S1 lists the 93 *S. cerevisiae* strains sequenced in this study and seven strains that are isogenic with strains sequenced in other studies (Goffeau et al. 1996; RM11 2004; Wei et al. 2007; Doniger et al. 2008; Dowell et al. 2010; Nishant et al. 2010). All sequence-based analyses in this study are on the genomes of the 93 sequenced strains, as well as the reference S288c genome; all phenotypic and association analyses include all 100 strains, unless otherwise noted. The rationales for the 93 homozy-

gous diploid strains (instead of their parental isolates), strain choices, the methods used to generate the 100 homozygous diploid *S. cerevisiae* strains, and the diverse geographic and environmental origins of the 100-genomes strains are described in detail in the Supplemental Material. The 100-genomes and other strains listed in Supplemental Table S1 are available from the Fungal Genetics Stock Center (McCluskey et al. 2010).

### Genome sequencing and analysis

Genome sequencing, assembly, editing, and annotation, as well as identification of novel genes, introgression events, aneuploidy, genomic rearrangements, and genetic assessment of chromosome colinearity were carried out as described in the Supplemental Material. Briefly, DNA from the 93 strains was sequenced (Illumina HiSeq 2000, paired-end reads of  $101 \times 101$  bases, average insert size of 300 bases). ABySS (v1.3.4) was used to assemble read pairs. BLAST (Altschul et al. 1990) was used to assemble contigs into chromosomes using synteny with S288c. The assembled chromosomes were aligned to S288c chromosomes using LAGAN (Brudno et al. 2003) to extrapolate the coordinates of the annotated sequences, and a table file was created using a Perl script. The table file was then utilized by the NCBI tool tbl2asn (<http://www.ncbi.nlm.nih.gov/genbank/tbl2asn2/>) to annotate each chromosome of each strain.

Highly conserved regions from each chromosome, totaling to 218 kb and including 124 protein-coding genes and intergenic regions, were extracted. SNP and indel polymorphisms relative to S288c were identified using LAGAN (Brudno et al. 2003) and Perl (see Supplemental Table S16). Phylogenetic analysis of the 218-kb regions was carried out using ClustalW v2.1 (Larkin et al. 2007), and principal component analysis was carried out using the pairwise sequence identity across all strains employing the prcomp function in the R package (R Core Team 2014). Introgression analysis is based on homology (75%–96% identity with S288c; 98%–99% identity with one of the sibling species) using the program ssearch36 (Smith and Waterman 1981) and BLAST (Altschul et al. 1990).

### Phenotyping of the 100-genomes strains

We determined multiple phenotypes of the 100-genomes strains (Supplemental Table S17). Low-throughput phenotypes included sporulation (six conditions), flocculation, and biofilm formation. High-throughput phenotypes (i.e., robotic handling of strains, quantitative image analysis) included growth at different temperatures, requirements for individual vitamins, and resistance to multiple inhibitors. For high-throughput phenotypes, liquid-grown yeast strains were transferred (BM5 robot, S&P Robotics Inc.) onto rectangular agar plates (Greiner Bio-One, Cat. No 781186) at a density of 1536 dots per plate. To minimize colony position effects (i.e., plate edge vs. internal area; colony neighborhood), for each tested strain, 24 replicates were plated as  $6 \times 4$  blocks, and only the eight colonies internal to each block were scored. Plates were incubated for 1–3 d and imaged every 24 h using the BM5 robot digital camera. Colony areas (i.e., phenotypes) were quantified using ImageJ 1.47v (<http://imagej.nih.gov/ij/index.html>) and Patch Detector Plus plug-in (University of Graz Microscopy Facility website: <http://microscopy.uni-graz.at/index.php?item=new1>). Thus, for each strain, we calculated the ratio of the median colony area (obtained in eight internal colony replicates) observed on experimental phenotype-tester plates to the median colony area (obtained in eight internal colony replicates) observed on control plates. These ratios were used to quantify and compare the phenotypes among individual strains. For

phenotype rationales and detailed phenotyping methods descriptions, see Supplemental Material.

### Population structure analysis and association testing

To examine population structure and conduct association tests in the 100 genomes, we called genetic variants using short reads and a common reference genome (S288c, SGD release 64). Briefly, short reads were aligned to the yeast reference genome using BWA version 0.7.4 (Li and Durbin 2009). For previously sequenced genomes, we performed *in silico* shredding of the genome sequence to simulate short reads (Supplemental Material). FreeBayes 0.9.9 (Garrison and Marth 2012) was used to obtain SNP and indel calls. *structure* version 2.3.4 (Pritchard et al. 2000) was used to infer population structure and assign individual strains to populations, with a burn-in period of at least 200,000 iterations and a minimum of 1,000,000 iterations of MCMC post-burn-in (Supplemental Material).

Genome-wide association mapping is feasible in *S. cerevisiae* but requires population structure corrections (Connelly and Akey 2012; Diao and Chen 2012). To conduct marker-trait association tests, we tested for association at all variation with a minor allele frequency of at least 5%, using the program GEMMA version 0.94beta, which is a mixed model approach that corrects for sample relatedness and population stratification (Zhou and Stephens 2012). To examine the power and false positive rate for tests of genotype-phenotype association, we used our actual genotype data and simulated phenotype data for causal alleles with a range of effect sizes (Supplemental Material). Simulations revealed that despite our analysis having the power to detect loci of large effect, false positives could arise even at very low significance thresholds (Supplemental Material; Supplemental Fig. S11).

### Data access

The genome sequence data from this study have been submitted to GenBank (<http://www.ncbi.nlm.nih.gov/genbank>). The accession numbers are listed in Supplemental Table S19. All strains used in this study have been deposited to the Fungal Genetic Stock Center (<http://www.fgsc.net>). The strain names are listed in Supplemental Table S1.

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**Author contributions:** P.K.S.: genome assembly/annotation/analyses, principal component analysis, writing; D.A.S.: population structure, associations, writing; S.G.K.: DNA preparation, genotyping, all nonsporulation phenotypes; G.M.: sporulation phenotypes; E.A.S.: population structure, associations; P.M.M.: population structure, associations; F.S.D.: genome assembly/annotation/analyses; J.H.M.: strain construction, genetic assessment of chromosome colinearity, writing.

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