Genome dynamics of hybrid *Saccharomyces cerevisiae* during vegetative and meiotic divisions


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ABSTRACT

Mutation and recombination are the major sources of genetic diversity in all organisms. In the baker's yeast, all mutation rate estimates are in homozygous background. We determined the extent of genetic change through mutation and loss of heterozygosity (LOH) in a heterozygous *Saccharomyces cerevisiae* genome during successive vegetative and meiotic divisions. We measured genome wide LOH and base mutation rates during vegetative and meiotic divisions in a hybrid (S288c/YJM789) *S. cerevisiae* strain. The S288c/YJM789 hybrid showed nearly complete reduction in heterozygosity within 31 generations of meioses and improved spore viability. LOH in the meiotic lines was driven primarily by the mating of spores within the tetrad. The S288c/YJM789 hybrid lines propagated vegetatively for the same duration as the meiotic lines, showed variable LOH (from 2-3% and up to 35%). Two of the vegetative lines with extensive LOH, showed frequent and large internal LOH tracts that suggest a high frequency of recombination repair. These results suggest significant LOH can occur in the S288c/YJM789 hybrid during vegetative propagation presumably due to return to growth events. The average base substitution rates for the vegetative lines (1.82 x 10^{-10} per base per division) and the meiotic lines (1.22 x 10^{-10} per base per division), are the first genome wide mutation rate estimates for a hybrid yeast. This study therefore provides a novel context for the analysis of mutation rates (especially in the context of detecting LOH during vegetative divisions), compared to previous mutation accumulation studies in yeast that used homozygous backgrounds.
INTRODUCTION

Most diploid organisms in nature possess heterozygous genomes. In budding yeast, homozygosity is thought to be the default state as seen from wild isolates that come from environments that are undisturbed by humans (MAGWENE 2014). Isolates of Saccharomyces cerevisiae from human associated (industrial, agricultural or clinical) environments are observed to have low (<1000 SNPs) to high (>30,000 SNPs) levels of heterozygosity (ARGUESO et al. 2009; BORNEMAN et al. 2011; MAGWENE et al. 2011; MAGWENE 2014).

These heterozygosities may be generated due to the accumulation of heterozygous mutations during vegetative divisions, by outcrossing during infrequent sexual cycles (once every 50,000 to 100,000 mitotic generations (RUDERFER et al. 2006), but see (KELLY et al. 2012)) or hybridization events mediated through human activities. The heterozygosity may be further maintained in natural environments by selection. On the other hand, mitotic recombination during vegetative divisions, intra-tetrad mating which is common during S. cerevisiae meiosis or selection on genetic variants may contribute to the loss of heterozygosity.

The baker’s yeast S. cerevisiae can undergo vegetative and meiotic divisions rapidly under laboratory conditions. The short generation time and small genome size of S. cerevisiae (12 Mb) facilitate analysis of genotypic changes through mutation and recombination processes over a large number of generations (LYNCH et al. 2008; HALLIGAN AND KEIGHTLEY 2009; NISHANT et al. 2009). Most mutation accumulation studies in yeast and other microbes involve propagation of isogenic asexual lineages (LYNCH et al. 2008; LEE et al. 2012), though sometimes the sexual cycle has also been incorporated (NISHANT et al. 2010). The effect of natural selection is minimized through the use of inbred lines and bottlenecks at each generation where a limited number of individuals are randomly selected to produce the next generation. However, the homozygosity of such isogenic lines can mask
the detection of genotypic changes induced by mitotic crossovers, gene conversions and other
types of DNA repair processes. Intra and inter-specific hybrids of yeast and other organisms
have been analyzed during experimental evolution. In addition, a recent study analyzed
mutation accumulation during asexual propagation in the microcrustacean *Daphnia* that is
naturally heterozygous (Flynn et al. 2017). Such hybrid genomes when propagated show
large scale aneuploidies, gross chromosomal rearrangements and LOH that create a more
homogeneous genome (Antunovics et al. 2005; Querol and Bond 2009; Burke et al.
2010; Morales and Dujon 2012; Dunn et al. 2013; Stelkens et al. 2014; Flynn et al.
2017). Much of this drive towards homogeneity is due to the selection on heterozygous
alleles and to purge out genetic incompatibilities (Greig et al. 2002; Dunn et al. 2013;
Wolfe 2015). Similar changes in copy number and genome rearrangements mediated by
mitotic recombination have been observed during experimental evolution of isogenic *S.
cerevisiae* (Hansche et al. 1978; Dunham et al. 2002). The mechanisms and distributions
of mitotic recombination events are well characterized in yeast (Lee et al. 2009; Rosen et al.
2013; St Charles and Petes 2013; Yin and Petes 2013; Yim et al. 2014). But it is not
clear to what extent genotypic changes occur in hybrid yeast over a large number of
generations through these somatic DSB repair processes especially when selection is
minimized. There are also no measures of mutation rates associated with mitotic and meiotic
divisions in hybrid yeast.

We experimentally measured genome wide LOH and base mutations in an artificial
*S. cerevisiae* hybrid strain (S288c x YJM789) propagated through successive vegetative and
meiotic divisions. External selection was minimal with the only constraint that the meiotic
lines sporulate efficiently and that the spores are viable. The S288c/YJM789 hybrid has
~60,000 heterozygous SNP markers distributed uniformly across the genome (Wei et al.
2007; Mancera et al. 2008). Since these SNPs are well characterized, we used them to track
genotype changes occurring in the S288c/YJM789 hybrid during vegetative and meiotic
divisions. We addressed the following questions: 1) What is the extent and pattern of LOH
during vegetative and meiotic divisions? 2) Is heterozygosity preserved on specific
chromosomal regions and are there potential fixation biases towards the parental S288c or
YJM789 alleles during LOH? 3) How does heterozygosity affect phenotypes such as spore
viability? 4) What is the mutation rate associated with vegetative and meiotic divisions in
the S288c/YJM789 hybrid?

We observed that LOH through intra-tetrad mating was rapid in the meiotic lines
(70% loss in three generations of meiosis) and associated with improved spore viability. A
few of the vegetative lines showed extensive LOH suggesting the occurrence of abortive
meiosis and return to growth events during vegetative propagation in laboratory conditions.
The base mutation rates in the *S. cerevisiae* S288c/YJM789 hybrid were similar to previous
estimates in other *S. cerevisiae* strains suggesting the S288c/YJM789 hybrid is not
mutagenic.

**MATERIALS and METHODS**

**Media and Strains:** The meiotic (M) and vegetative (V) lines were grown on
either YPD (yeast extract, peptone and dextrose) or synthetic complete (SC) media at 30°C
(ROSE et al. 1990). For inducing meiosis, diploids cells were patched on sporulation media
(ARGUESO et al. 2004). After 72 hours on sporulation media, tetrads were isolated on YPD
or SC using a Zeiss dissection microscope. To generate the parent diploid, cells from
overnight patches of YJM789 (*MATa, ho::hisG lys2, cyh*) and S288c (*MATa, ho, lys5*) were
crossed on SC plates to form diploids. They were then streaked on YPD and 12 single
colonies were picked and patched on sporulation plates. After three days on sporulation
media, one of the single colonies that sporulated was stocked and labelled as the parent

diploid hybrid strain (KTY162). The KTY162 strain was used to generate the V and M lines.

**DNA extraction and sequencing:** Diploid colonies or spore colonies from tetrads
were independently cultured overnight at 30°C in YPD liquid medium. Genomic DNA was
extracted from each culture using the PrepEase DNA isolation kit from Affymetrix following
the manufacturer’s protocol. Whole genome sequencing was performed on Illumina HiSeq
2500 machines at Fasteris SA, Switzerland.

**Read mapping, genotyping of whole genome sequencing data:** The sequence
reads were mapped to the S288c genome (version 64-1-1, 2011) using bowtie2 (version
2.1.0) (Langmead and Salzberg 2012). Uniquely mapped reads were only considered for
the SNP calling (duplicate reads were removed using picardtools). SNPs defined in
Mancera et al. 2008 were used for all analysis. In order to reduce misalignment due to
indels, we performed a local indel realignment after mapping the reads to the reference
genome using GATK IndelRealigner. SNPs were called with multiple samples for M line and
V lines using GATK unified genotype caller. R package was used for data visualization and
downstream statistical analysis. To detect conserved fixed SNPs in the M lines with high
spore viability, we subsetted the genotype matrix of all lines from M5 onwards and collapsed
consecutive markers that have no change in genotype across all samples. Markers with same
genotype across all samples were identified as the boundaries of conserved regions.

**Analysis of LOH tracts:** LOH tracts from sequencing of V1_57, V3_57, V4_57,
V5_57 diploids were compared with the sequence data from the four haploid spores from
these lines. None of the diploid LOH tracts supported by 10 or more SNPs were invalidated
when compared with the haploid sequence data from the V1_57, V3_57, V4_57, V5_57
lines. But for LOH tracts supported by fewer than 10 SNPs (and especially those supported
by only 2 or 3 SNPs), some of the SNPs were sometimes called differently in the diploid and
haploid data sets (Figure S1). This problem may be due to genotyping issues in the diploid sequence data. Therefore LOH tracts were called in all the V_57 diploid lines only if supported by 10 or more SNPs.

**Analysis of new mutations:** To detect new mutations from the M and V lines sequence data sets, we sequenced the parent diploid as well as the S288c and YJM789 strains used to generate the parent diploid. We recalibrated the base qualities in these bam files (generated using alignment with the S288c reference genome) using GATK. SNP positions where the coverage deviates from the median coverage of the sample (due to copy number variation or mapping issues) were excluded. More specifically, at a position, if coverage / median coverage > 1.65 || < 0.35, the SNP is filtered away. We also filtered away SNPs where QD (Quality by depth) <= 10. For each sample at each potential SNP, we consider the SNP as a mutation if it differs from the genotype in the parent diploid. We checked that it follows a non-mendelian inheritance pattern from the parents (YJM789 and S288c), and this corrects for potential genotyping error in the parent diploid. Next we filtered away sites if the genotype call is not optimal (Genotype quality < 30) in either the sample itself, or any of the parent diploid, S288c or YJM789 strains. All new mutations were verified by Sanger sequencing.

**Data availability:** All vegetative and meiotic lines listed in Table S1 are available upon request. Sequence data are available from National Centre for Biotechnology Information Sequence Read Archive under accession number: SRP098673. The Data S1, SNP segregation files and the custom R scripts are available online at the Dryad digital repository (http://datadryad.org/review?doi=doi:10.5061/dryad.s14m0).
RESULTS

Whole genome sequencing analysis of the S288c/YJM789 hybrid vegetative and meiotic lines

Vegetative and meiotic lines of the S288c/YJM789 parent diploid strain were set up as described in Materials and Methods (Figure 1A and (NISHANT et al. 2010)). For the meiotic lines, twenty diploid colonies derived from the parent diploid were patched on sporulation media for three days and a single complete tetrad from each colony was isolated. Each tetrad was placed on rich media and the germinated spores of opposite mating type mated to form diploids. The resulting colony was then sporulated and the bottleneck repeated. These lines were labelled M1_N to M20_N where N indicates the number of meioses. Most of the M lines showed reduced sporulation after successive rounds of meiosis and could not be propagated with a three day sporulation schedule. After seven generations of meiosis and intervening 140 vegetative divisions, only two lines could be maintained as the rest could not sporulate in three days (Figure 1A). The two lines were further continued until M_31 (corresponding to 31 generations of meiosis). Whole genome sequence data was obtained from the two lines after the 3rd (M1_3, M2_3), 5th (M1_5, M2_5), 7th (M1_7, M2_7), 10th (M1_10, M2_10), 15th(M1_15, M2_15) and 31st (M1_31, M2_31) generation of meiosis. Six additional lines after five generations of meiosis (M3_5, M5_5, M6_5, M7_5, M8_5, M9_5) were also sequenced to increase the sample size. To accurately genotype heterozygous sites, sequencing was performed on the diploid colonies as well as on the haploid spores obtained by sporulating the diploids (File S1). In parallel, twelve vegetative (V) lines, were bottlenecked to single cells from a colony, every 2 days (20 generations). These were initially propagated for a total of 19 bottlenecks (380 generations) that correspond to the amount of time taken for the M_7 lines. These lines were labeled V1_N to V12_N, where N indicates the number of bottlenecks. Five of these lines were propagated
further for a total of 57 bottlenecks (1140 mitotic generations) that correlate with the same length of time as the M_31 lines. But the number of mitotic generations in the M_31 lines (620) is fewer due to the intervening meiotic divisions. The V_57 lines (V1_57, V3_57, V4_57 and V5_57) were also sequenced as diploids as well as haploid spores. Due to a recessive lethal mutation in V2_57 (49% spore viability, Table 1), sequencing of haploid spores was not performed for this line. Sequencing details for all M and V lines are in Table S1. The average sequencing depth was ~70X (Table S1).

Meiotic lines show almost complete loss of heterozygosity and improved viability

Intra-tetrad mating of spores is expected to reduce heterozygosity in the M lines (File S2, (KNOP 2006; NISHANT et al. 2010)). Sequence information from diploid colonies of the two M lines, line 1 (M1_3, M1_5, M1_7, M1_10, M1_15, M1_31) and line 2 (M2_3, M2_5, M2_7, M2_10, M2_15, M2_31) were analyzed for heterozygous SNP markers (Table S2). We focused on 46281 SNPs that could be called out from all the M lines. The number of heterozygous SNPs rapidly declined with increasing number of meiotic generations (Figure 1B, Table 1). We traced the lineage of each of the 46281 SNPs for two M lines (1 and 2). By the 3rd round of meiosis, around 30% of the SNPs were heterozygous and by the 7th round it was around 10% in these two lines (Figure 2, Table 1). M1_7 and M2_7 contained 4688 and 5800 heterozygous SNPs respectively.

We were curious to know whether all heterozygous segregating sites would eventually be lost in the M lines (except for the MAT locus, which is under selection). To address this question we propagated the two lines until M_31. Sequence analysis of M1_31 and M2_31 showed the presence of 146 and 135 heterozygous sites respectively (Table 1, Table S2). Most of the heterozygous SNPs in M1_31 (85 out of 146) and M2_31 (102 out of 135) were in proximity to the MAT locus on chromosome III, suggesting that they were
retained in the heterozygous state because of passive linkage to the \textit{MAT} locus. The rate of
LOH was similar for the two lines and maximal in the initial rounds of meiosis when
heterozygous markers were maximum. The rate of LOH gradually declined in subsequent
generations of meiosis and intra tetrad mating. Similar results were observed from the
sequencing data of haploid spores from the M lines (File S1, Table S3).

Since meiotic recombination parameters of the S288c/YJM789 hybrid are well
categorized, we mathematically analyzed the loss of heterozygosity in the two M lines (1 and 2) by incorporating high resolution genome wide meiotic crossover data of the
S288c/YJM789 hybrid (File S2). The M lines are expected to retain on average 98 SNPs by
the 31st meiotic generation. This number is close to the experimentally observed number of
heterozygous sites in the two M lines. The excess of heterozygous alleles in the M lines
compared to the expectation, may be because of altered recombination (enhanced) beyond
M_5 as the lines become extremely homozygous or due to factors other than recombination
(e.g chromosomal structural features or genomic loci besides the \textit{MAT} locus that affect
mating) that contribute to the maintenance of residual heterozygosity in the M lines. Besides,
the \textit{MAT} locus, the distribution of the residual heterozygous sites between the two lines were
unique (Figure 2).

The M lines showed rapid fixation of alleles towards the S288c or YJM789 state. In
order to statistically test biased fixation towards S288c or YJM789 alleles, we used the
sequence data from the eight M5 lines (M1_5, M2_5, M3_5, M5_5, M6_5, M7_5, M8_5,
M9_5). Five of the eight M_5 lines showed excess fixation towards the YJM789 alleles.
Across these eight lines, an average of 18276 SNPs were fixed towards YJM789 and 16897
SNPs were fixed towards S288c (Figure S2A, Table S2). We simulated five successive
rounds of meiosis with intra spore mating, using crossover locations from (MANCERA \textit{et al.}
2008) and (LIU \textit{et al.} 2014) to test if the fixations towards S288c and YJM789 SNPs are
significant. We tabulated the number of fixed YJM789 SNPs in the eight M_5 lines, over 5000 simulations. The average number of YJM789 fixed SNPs in the eight M_5 lines (18276 SNPs) was within the expected range based on simulation data and suggest these biased fixations are not statistically significant (Figure S2B). Asymmetric fixation of alleles derived from either parent in a hybrid has been observed previously (Tang et al. 2010).

The S288c x YJM789 hybrid has a spore viability of 85% unlike the S288c diploid that has spore viability > 97% (Table 1, (McCusker et al. 1994)). The presence of ~60,000 SNPs along with indels, can create incompatibilities in the S288c/YJM789 hybrid causing reduced spore viability (Wei et al. 2007). Meiotic lines at M_3 and beyond showed significantly improved spore viability (>92%) compared to the parent diploid (Table 1). This result is interesting because it suggests that the significant loss in heterozygosity observed by the third generation of meiosis or fixation of certain S288c and YJM789 alleles may contribute to the improved spore viability in the M lines (see below).

**Variable LOH in hybrid vegetative lines**

Hybrid vegetative lines can be used to detect LOH during mitotic divisions. Hybrid S. cerevisiae strains have been previously used to map LOH events involving mitotic crossovers and break induced replication (BIR) events on specific chromosomes III, IV, V and genome wide (Lee et al. 2009; Rosen et al. 2013; St Charles and Petes 2013; Yin and Petes 2013; Yim et al. 2014). These studies have generated considerable insights into the mechanisms and distributions of mitotic recombination. The mitotic recombination events are thought to occur with ~10^5 fold less frequency than meiotic recombination and therefore require a selection system for their detection (Lee et al. 2009). But it is possible that over a large number of divisions they can cause significant genotypic changes that can be detected and is relevant to measure, given the ratio of mitotic to meiotic cycles in yeast.
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Diploid colonies from five vegetative lines (V1 to V5) were whole genome sequenced after 57 bottlenecks (corresponding to the 31st generation of meiosis for the M lines) (Table S4). We focused on the 47954 SNPs that are common to all the five V lines. In the lines V1_57 and V2_57 only 2-3% of the SNPs showed LOH compared to >99% of the heterozygous sites that became homozygous in the meiotic lines propagated for the same length of time (M_31) (Table 1). In V4_57, 8% of the SNPs showed LOH which was primarily due to a single large terminal LOH tract on chromosome XII (Figure 3). In the most extreme cases in V3_57 and V5_57, up to 35% and 22% of the heterozygous sites became homozygous. LOH in the V lines likely results from the repair of DNA lesions using the homologous chromosome followed by segregation of sister chromatids. Mitotic crossovers, local gene conversions, BIR and chromosome loss are implicated in LOH events during mitosis (Paques and Haber 1999, Barbera and Petes 2006).

We analyzed the SNP distribution data in the V_57 lines to map the accumulated LOH tracts genome wide. Sequence data from both the diploid V_57 lines as well as their haploid derivatives (Table S5) were used to call LOH tracts accurately (Materials and Methods). Genome wide distribution of 141 LOH tracts in the V lines is shown in Figure 3, Figure S3A along with information on chromosomal location and tract sizes (Table S6). Since the LOH tracts in the vegetative lines are summed over 1140 mitotic generations, these tracts may have been generated through multiple DSB repair mechanisms that are difficult to distinguish. The tract sizes showed a broad distribution from less than 100 bp to over 100 kb (Figure S3B,C, Table S6). The tracts were on average closer to telomeres than centromeres. The average distance of the LOH tracts to the nearest telomere was 126.2 kb compared to 244.7 kb for the centromere (Table S6). This is consistent with the increase in mitotic recombination rates away from the centromere in S. cerevisiae (Manegar and Otto 2007).
Centromere distal regions have also been shown to be more prone to spontaneous LOH
events in *S. cerevisiae* (Andersen et al. 2008). V1_57, V2_57 and V4_57 showed fewer
LOH tracts than V3_57 and V5_57. The long LOH tracts in V1_57, V2_57, V4_57 lines
were primarily terminal as expected to arise during mitotic COs or BIR (Figure 3 and Figure
S3A). In contrast V3_57 and V5_57 have frequent long internal LOH tracts that might arise
due to high levels of DNA damage and repeated rounds of mitotic recombination events
creating chimeric chromosomes that are fixed alternatively towards S288c or YJM789
(Moraes and Dujon 2012). The high frequency of long internal LOH tracts also suggest
the possibility that V3_57 and V5_57 may have been through a cycle of abortive meiosis and
return to growth involving DSB repair (Dayani et al. 2011; Laureau et al. 2016). Twenty
eight 2:0/0:2 LOH events, larger than 100 kb were observed (Table S6) with the largest one
around 827 kb on chromosome XII in V4_57. Chromosome XII has been previously also
observed to have large LOH tracts (Magwene et al. 2011). The short LOH tracts (< 1 kb)
are likely to have arisen though local gene conversions not associated with crossovers
(Palmer et al. 2003; Laureau et al. 2016). Chromosomal aneuploidies contributing to
LOH were not observed in the V lines (Figure S4) which suggests the S288c/YJM789 hybrid
does not show chromosomal instability during mitotic divisions.

During experimental evolution of interspecific hybrids, biased elimination of one of
the parent genomes as well as specific genome changes driven by selection are often
observed (Antunovics et al. 2005; Dunn et al. 2013). When the V line’s SNP data was
analyzed for biased fixation, 81 LOH tracts were fixed towards S288c compared to 60 tracts
towards YJM789 (*P* = 0.092, binomial test) (Table S6). Despite random fixation of the LOH
tracts towards S288c or YJM789, we observed more fixed YJM789 SNPs as most of the
longer LOH tracts are fixed towards YJM789 alleles (Figure S2A, Table S6).
Unlike the significant improvement in spore viability observed in all meiotic lines, the spore viability of two of the vegetative lines (V1_57 and V3_57) were not significantly different from the parent diploid strain (Table 1). V2_57 showed the presence of a recessive lethal mutation. V4_57 and V5_57 showed improvement in spore viability compared to the parent diploid (0.05 <p >0.01), but the differences were less significant when compared to the meiotic lines (p<0.0001). These results suggest LOH did not affect the spore viability of the hybrid vegetative lines.

**Base mutations in the hybrid vegetative and meiotic lines**

While LOH events and intra-tetrad mating can reduce heterozygosity, new mutations can add to the number of heterozygous sites in the genome. We estimated the number of base substitutions of the S288c/YJM789 hybrid vegetative (V1_57, V2_57, V3_57, V4_57, V5_57) and meiotic lines (M1_31, M2_31) with reference to the parent diploid genome. We detected 1 mutation in the meiotic line (M1_31) and 15 mutations across 3 vegetative lines (8 in V3_57, 5 in V4_57 and 2 in V5_57) (Table 2). No base substitution mutations were observed in M2_31 and V1_57, V2_57. It is possible that the recessive lethal mutation in V2_57 is caused by an in-del or other structural variation. All the 16 mutations were validated by Sanger sequencing. All the new mutations were heterozygous and did not occur in the coding region of the genome. Only 1 mutation was shared between two of the vegetative lines V3_57 and V4_57 (C>A on Chr IV). The vegetative lines were propagated for a total of 1140 mitotic divisions (57 bottlenecks) and the 24.04 Mb genome was sequenced at 99.9% sequence coverage. The mutation rates are 2.92 x 10^{-10} for V3_57; 1.82 x 10^{-10} for V4_57 and 0.73 x 10^{-10} for V5_57. The average mutation rate for the three vegetative lines is therefore 1.82 x 10^{-10} per base per division. The average mutation rates in the S288c/YJM789 hybrid are similar to mutation rate estimates from a
large set of 145 diploid *S. cerevisiae* vegetative mutation accumulation lines from (ZHU et al. 2014) \((1.67 \times 10^{-10} \text{ per base per generation})\). They are also comparable to the homozygous *S. cerevisiae* SK1 diploid mutation rates \((2.9 \times 10^{-10})\) in vegetative lines (NISHANT et al. 2010).

To calculate mutation rates for the S288c/YJM789 hybrid meiotic lines, we multiplied the number of mutations observed in the meiotic line by two, as half of the mutations in the meiotic lines are expected to be lost during intra-tetrad mating (NISHANT et al. 2010). The base substitution rate for M_31 is estimated to be \(1.33 \times 10^{-10} \text{ per base per division}\) (31 meiotic divisions + intervening 620 mitotic divisions and 99.9% sequence coverage of the 24.04 Mb diploid genome). For comparison, the base substitution rate in *S. cerevisiae* SK1 meiotic lines was \(3.9 \times 10^{-10}\) after 50 meiotic divisions and 1000 intervening mitotic divisions (NISHANT et al. 2010).

Higher mutation rates have been observed in heterozygotes compared to homozygotes based on sequence analysis of parent–progeny in *Arabidopsis* and rice (YANG et al. 2015). Since we do not have mutation rate data for *S. cerevisiae* meiotic lines that are homozygous for S288c or YJM789, we cannot infer whether the same is true in *S. cerevisiae* as well. It is also important to note that S288c/YJM789 is an artificial hybrid with uniform distribution of heterozygous markers and these heterozygosities were progressively reduced during the meiotic divisions. In the meiotic lines, heterozygosity is lost by 70% within three generations of meiosis. In addition, the meiotic lines undergo both vegetative and meiotic divisions and there is 50% loss of mutations during intra-tetrad mating of spores (NISHANT et al. 2010). It is therefore likely that more mutations were not observed in the S288c/YJM789 hybrid meiotic lines due to the study design ((NISHANT et al. 2010), see discussion). In the vegetative lines, no base mutations were observed in V1_57 and V2_57 that have 2-3% loss of heterozygous SNPs. All of the base mutations were observed in the V3_57, V4_57 and V5_57 lines, which have a significantly higher percentage of LOH events (Figure 3). This is
consistent with previous observations in yeast, where the repair of HO endonuclease induced
DSBs causes high mutation frequency proximal to the break site (STRATHERN et al. 1995).
But only two of the 16 mutations observed in the vegetative lines were located within the
LOH tracts (Figure 3). Since all the 16 mutations are heterozygous, it is possible that the
other mutations happened in locations where the mitotic recombination events were repaired
using the sister chromatid; the DSB repair through other mechanisms did not leave an LOH
signature or the SNP density was not sufficient to detect an LOH. It is also possible that
some of the mutations in the LOH tracts may have been fixed in favour of the parent allele,
and so the mutation event is not detected. It is also possible that at least for V3_57 and
V5_57, mutations may be associated with recombination repair of DSBs during partial entry
into meiosis followed by return to growth.

Heterozygosity determines spore viability in the hybrid meiotic lines
The meiotic lines showed enhanced spore viability. We tested if the improved spore
viability is due to the biased fixation towards S288c/YJM789 alleles or due to a general
reduction in heterozygosity. We observed specific conserved chromosomal regions that were
fixed either towards S288c or YJM789 alleles in the meiotic lines. We identified 20 such
regions (Figure S2C). The largest region was observed on chromosome XV, and it showed
fixation towards the S288c alleles. We back crossed M2_15 spores (1104 SNPs and 100%
SV) with the parent S288c and YJM789 strains. The backcross increases the heterozygosity
and reintroduces the incompatibilities that might have existed in the parent hybrid. The
heterozygous SNPs in the M2_15 x S288c cross increased to 22923 SNPs and in the YJM789
cross to ~19838 SNPs. In addition, the backcross with the parent S288c, creates an
S288c/YJM789 hybrid set for the YJM789 fixed candidate regions, while the backcross with
YJM789 created an S288c/YJM789 hybrid set for the S288c fixed candidate regions. In both
the crosses, we observed a spore viability of ~95% (n = 60 tetrads) which is significantly higher than wild type hybrid parent (85%, Fischer exact test, p< 0.0001). Similarly spores from M6_5 (12383 SNPs, 92% spore viability) crossed with S288c (26693 SNPs) and YJM789 (17004 SNPs) showed similar spore viability of 90%. These results suggest that the regions fixed either in favour of S288c or YJM789 may have no role in the improved spore viability of the M lines. Instead, the spore viability of the M lines is affected by the heterozygosity of the lines. Previous studies have observed a negative correlation of heterozygosity with sporulation efficiency as well as spore viability in *Saccharomyces* wild isolates (MORTIMER et al. 1994; LITI et al. 2006; CUBILLOS et al. 2011; MAGWENE et al. 2011).

We further tested this hypothesis using different *S. cerevisiae* artificial hybrid combinations of S288c, SK1, YJM789 and RM11-1a as well as data from the M lines (Figure 4). The heterozygosity in these hybrids and the M lines is well defined facilitating a calibrated comparison of spore viability with the level of heterozygosity. Among the different *S. cerevisiae* artificial hybrid combinations, the hybrid with the least heterozygosity i.e. RM11-1a x YJM789 (~30,000 SNPs,(GRESHAM et al. 2006)) showed the maximum spore viability (90%). Hybrids with higher heterozygosity, e.g. S288c x RM11-1a (~46,000 SNPs, (Qi et al. 2009)); S288c x SK1 (~62000 SNPs, (MARTINI et al. 2011)); SK1 x YJM789 (~65000 SNPs); SK1 X RM11-a (~69000 SNPs) showed lower spore viability (85%, 73%, 77% & 76% respectively) (Figure 4). Overall a strong negative correlation was observed between heterozygosity and spore viability (r = -0.94, P = 2.5 x 10^{-13}). These observations suggest the high spore viability phenotype of the M-lines is due to the reduction in the heterozygous load. Reduced heterozygosity can result in less heteroduplex rejection during recombination repair of DSBs (CHAKRABORTY AND ALANI 2016). The improved repair
outcomes and fewer genetic incompatibilities (e.g. between S288c-Mlh1 and SK1-Pms1, (HECK et al. 2006)) may increase spore viability in homozygous backgrounds.

Generation of a panel of heterogeneous inbred families (HIFs) from the hybrid meiotic lines

Crosses between inbred lines can facilitate fine mapping of QTLs (FLINT and MOTT 2001; BERGELSON and ROUX 2010). We experimentally determined the mating type of all spores derived from the M lines. These spores were mated in silico and the genome sequence information was used to generate a total of 1369 in silico diploid genomes (Table S7). These in silico diploid genomes show varying numbers of SNP counts and retain heterozygosity at specific regions on the genome (Data S1, available online at the Dryad digital repository (http://datadryad.org; http://dx.doi.org/10.5061/dryad.s14m0)). The rest of the genome remains homozygous for S288c or YJM789. This collection of 1369 in silico diploid genomes represents a panel of Heterogeneous inbred families (HIFs) that can be experimentally generated by crossing the haploid spores from the M lines. The distribution of SNPs for two representative HIF strains that were also experimentally analysed for spore viability is shown in Figure 5. The two diploid strains, M2BD_3 and M2DA_10 were generated by crossing two spores from M2_3 and M2_10 each. They have spore viability ≥ 90% and different numbers of heterozygous markers. M2BD_3 has 8765 heterozygous SNPs while M2DA_10 has 1108 heterozygous SNPs. Further, M2DA_10 has nine chromosomes (XIII, XI, X, IX, VIII, VI, V, IV and I) that are completely homozygous while the rest of the chromosomes have heterozygous regions. Such HIF strains can be used to study the effects of varying levels of heterozygosity on different biological processes including spore viability (Figure 5) and meiotic recombination. Since SNP markers are lost as the lines become homozygous, cytological or physical or biochemical methods can be used to test if genome
wide meiotic recombination is enhanced. The HIF lines are also useful for mapping QTLs for traits that differ between S288c and YJM789. The S288c x YJM789 hybrid displays heterosis across many phenotypes including high temperature growth and sporulation (Steinmetz et al. 2002). If a trait of interest segregates in such a diploid, it is relatively easy to identify the causative SNPs as the regions of heterozygosity are limited. If candidate regions for the QTLs are already known, one can use the sequence information of the spores from the M lines, to generate hybrid diploids that are heterozygous specifically in the candidate regions and thus fine map the QTLs.

DISCUSSION

An artificial hybrid such as S288c x YJM789, provides a unique opportunity to study the interaction between two independent genomes that have never existed together, in successive vegetative and meiotic generations. Unlike isogenic strains that can be propagated for successive meiotic divisions for large numbers of generations (Nishant et al. 2010), we observe that hybrid strains like the S288c/YJM789 pose unique problems during meiotic propagation. Only two of the twenty M lines could be propagated beyond the 7th generation of meiosis due to poor sporulation of the other lines. The most likely explanation is that both S288c and YJM145 (the diploid progenitor of YJM789) are poor sporulators (McCusker et al. 1994). In comparison, the S288c x YJM789 hybrid efficiently sporulates within three days. Reduction in sporulation efficiency of many of the meiotic lines suggest that alleles from both S288c and YJM789 contribute to the good sporulation phenotype of the S288c/YJM789 hybrid. During the meiotic propagation of the lines, some of the alleles contributing to the high sporulation efficiency may be lost through fixation of the opposite allele. Consistent with this, even after five generations of meiosis, the sporulation efficiency of the M_5 lines, were variable (Figure S5).
Whole genome sequence analysis of meiotic lines showed a significant decline in heterozygosity as expected from intra-tetrad mating. A significant decrease in heterozygosity was also observed in some of the vegetative lines (V3_57, V4_57, V5_57). These results are relevant for interpreting heterozygosity in *S. cerevisiae* populations, since meiosis occurs infrequently in *S. cerevisiae* and most diploids that arise are produced by intra-tetrad mating (MAGWENE et al. 2011). Previous studies have suggested a very low outcrossing frequency of 0.5 per $10^5$ mitotic divisions (RUDERFER et al. 2006). However, a recent study (KELLY et al. 2012) shows mating and outcrossing frequency in *S. cerevisiae* is much higher (1 in 100 mitotic divisions). These new estimates of outcrossing can better explain heterozygosity in wild *S. cerevisiae*, given the extent of LOH in a few of the vegetative lines and during intra-tetrad mating that we observed in this study. Residual heterozygosity observed in these two M lines were conserved only at the *MAT* locus since we were selecting for hybrids that can sporulate. Selection acting on standing genetic variation can cause fixation as well as LOH of linked regions (BURKE et al. 2010). We show that almost all heterozygous sites in the S288c/YJM789 hybrid yeast genome can be fixed even with minimal external selection. The fixation of SNPs occurred in large blocks after each meiosis consistent with fixation driven primarily by mating comprising recombinant spores.

The fixation of the heterozygous sites in the meiotic lines can occur randomly towards S288c or YJM789 alleles or may show biases, either local or genome wide, especially if selection favouring either of the alleles is involved (e.g high temperature where the YJM789 genotype grows better (WEI et al. 2007)). Even without apparent external selection, the presence of two different genomes (S288c and YJM789) in the hybrid could create fixation of either genotype through biased selection. Such regions were observed in the M lines (Figure S2C). The fixation of these specific regions towards S288c and YJM789 alleles may arise due to differences in expression from the S288c and YJM789 alleles of the
same gene; epigenetic reasons; preferential initiation of meiotic DSBs from chromosomal regions containing S288c or YJM789 SNPs followed by biased MMR; or due to selection for good sporulators.

Heterozygous SNPs in the S288c/YJM789 hybrid facilitated the analysis of genome dynamics as cells cycle through vegetative divisions. Genetic changes like LOH can uncover mutations in tumour suppressor genes commonly associated with cancer development (CAVENEE et al. 1983). LOH is shown to create extensive genome variation in Cryptococcus hybrids enabling pathogenesis (Li et al. 2012). Similarly LOH events have been associated with the adaptation of S. cerevisiae and Candida albicans to stressful conditions, such as the exposure to antifungals (SELMECKI et al. 2010; GERSTEIN et al. 2014).

Given the role of LOH in evolution and disease, it is useful to understand the extent of LOH over a large number of mitotic divisions. Previous analysis of mitotic LOH events in S. cerevisiae that allowed for selection of daughter cells containing the products of mitotic recombination have provided mechanistic insights into mitotic crossovers and gene conversion (LEE et al. 2009; ROSEN et al. 2013; ST CHARLES AND PETES 2013; YIM et al. 2014). Our study design does not facilitate mechanistic analysis, as the outcome of the DSB repair processes are analysed after many generations. Instead our study design provides information on the scale of genotypic changes brought about by the accumulated effects of various DSB repair processes over 1140 vegetative divisions (Figure 3). In our study, we have observed; 1) Long LOH events extending towards the ends of chromosomes, probably as a result of BIR or mitotic crossovers; 2) Small LOH tracts (less than 1 kb) as a result of local gene conversions; 3) Internal large LOH events that are probably a consequence of repeated mitotic recombination events in response to DNA damage during the propagation of the vegetative lines (PRYSZCZ et al. 2014). Alternatively, they may arise during DSB repair in response to Spo11 mediated DSBs during abortive meiosis. This is supported by the
observation that the number and distribution of LOH tracts in V3_57 and V5_57 are distinct from V1_57, V2_57 and V4_57 (Figure 3, Figure S3B,C). Extrapolating the spontaneous mitotic crossover rate of $6 \times 10^{-5}$ per division for the right arm of chromosome IV (ST CHARLES AND PETES 2013) to the whole genome, only 5-6 crossover/conversion events are expected after 1000 mitotic divisions. The number of long LOH tracts observed in V1_57, V2_57 and V4_57 are consistent with these numbers (Figure 3). But V3_57 and V5_57 have many more long LOH tracts that are also frequently internal. The pattern of LOH in V3_57 and V5_57 would either require a high frequency of DNA damage during vegetative growth or can also be explained for example by a double crossover during return to growth. The latter possibility suggests that yeast cells may enter the meiotic program and return to growth during vegetative propagation perhaps in response to nutrient stress. The extensive LOH observed in some of the vegetative lines (presumably due to return to growth) support the idea that LOH is an important tool for the evolution of vegetatively propagating cells by facilitating the fixation of beneficial alleles (MANDEGAR AND OTTO 2007). Given the applications of hybrid yeast strains in the industry, knowledge of the scale of genome dynamics in hybrid yeast will be also useful for the design of hybrid yeast genomes.

**Mutation rates in the hybrid yeast genome**

Mutation accumulation experiments have been previously used in a number of model organisms like *S. cerevisiae*, Drosophila and *C. elegans* to estimate spontaneous mutation rates (DENVER *et al.* 2000; VASSILIEVA *et al.* 2000; WLOCH *et al.* 2001; DENVER *et al.* 2004; JOSEPH AND HALL 2004; HAAG-LIAUTARD *et al.* 2007; LYNCH *et al.* 2008; KEIGHTLEY *et al.* 2009; NISHANT *et al.* 2010). Mutation accumulation studies in diploid yeast have been carried out in homozygous backgrounds (NISHANT *et al.* 2010; ZHU *et al.* 2014). The use of homozygous lines can affect the mutation process in the cells. For example,
fixation of recessive mutator alleles during generation of the homozygous lines can enhance mutation rates, while on the other hand genome rearrangements via ectopic recombination is significantly reduced in homozygous background compared to heterozygous ones (MONTGOMERY et al. 1991; SCHRIDER et al. 2013). Given that most organisms have heterozygous genomes, there is a need for accurate estimate of mutation rates in a hybrid context. Our estimates of the average mitotic base mutation rate observed in the S288c/YJM789 hybrid is similar to other homozygous S. cerevisiae strains (LYNCH et al. 2008; NISHANT et al. 2010; ZHU et al. 2014). These observations suggest that the S288c/YJM789 diploid hybrid is not mutagenic. This is relevant, since the S288c/YJM789 hybrid is used extensively for genome wide mapping of meiotic recombination events (CHEN et al. 2008; MANCERA et al. 2008; OKE et al. 2014; KRISHNAPRASAD et al. 2015). Previous studies by Yang et al., 2015 have shown a 3.4 fold increase in base mutations rates in the F2 progeny of heterozygous F1 Arabidopsis plants compared to the homozygous progeny of the selfed plants. A similar 3.4 fold increase in mutation rates was observed in rice heterozygotes over homozygotes (YANG et al. 2015). The increased mutagenesis in heterozygous genomes is due to the cis effects of heterozygosity, since homozygous regions within these genomes do not show higher mutation rates. The enhanced mutagenesis is thought to be due to the potential for new mutations during gene conversions associated with meiotic recombination in heterozygous regions (AMOS 2010; YANG et al. 2015). It may also reflect the enhanced mutagenicity of ssDNA tracts that are generated during meiotic recombination (YANG et al. 2008). In order to determine if the heterozygosity of the S288c/YJM789 hybrid contributes to enhanced mutations in meiosis, we need to compare several meiotic lines of the S288c/YJM789 hybrid with isogenic diploid S288c and YJM789 strains, preferably after a single division to avoid the effects of loss of heterozygosity as well as the loss of mutations during intra tetrad mating (NISHANT et al. 2010).
Reduced mutation rates were observed in the F3 and F4 selfed Arabidopsis plants due to a reduction in heterozygosity (Yang et al. 2015). In artificial yeast hybrids, interactions between DNA repair proteins may also play a stronger role in determining the mutation rate. It is possible *S. cerevisiae* hybrid meiotic lines may have elevated mutation rates compared to their homozygous reference strains, due to incompatibility in mismatch repair genes or heterozygosity ( Heck et al. 2006; Amos 2010; Yang et al. 2015).

DNA synthesis at specific loci undergoing mitotic DSB repair in *S. cerevisiae* show more mutations (Strathern et al. 1995). In heterozygous genomes, these effects may be enhanced due to mismatch triggered gene conversions that require additional DNA synthesis similar to the mechanisms proposed for enhanced mutagenesis in heterozygous regions during meiosis (Amos 2010; Yang et al. 2015). All the base mutations were observed in the vegetative lines V3_57, V4_57 and V5_57 that showed significant LOH. But a causal link between mutation rate and frequency of LOH in these lines is hindered by the small sample size and lack of tight correlation between the frequency of LOH and the number of mutations (Figure 3). It is also possible that LOH in V3_57 and V5_57 may have been caused by DSBs induced and repaired to different extent during abortive meiosis followed by completion of the repair during return to growth. In such a scenario, the results would suggest mutagenicity of the meiotic/mitotic recombination process in a heterozygous genome. Mutation rate estimates in other *S. cerevisiae* hybrids will be useful to further consolidate these observations on the mutagenicity of the DSB repair process in a heterozygous context.
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**FIGURE LEGENDS**

**Figure 1.** A) Experimental set up of the vegetative (V) and meiotic (M) lines from the parent diploid. The numbers in brackets indicate the number of V and M lines available at the vegetative bottleneck (V_19, V_57) or meiotic generations (M_3, M_5, M_7, M_10, M_15, M_31). LOH events are observed in both V and M lines. B) Loss of heterozygosity in the M lines following each generation of meiosis and intra tetrad mating. Continuous lines (Red and Green) show the
observed number of heterozygous SNPs in two M lines (Line 1 and 2). Black dashes show the number of heterozygous SNPs expected assuming wild-type \textit{S. cerevisiae} recombination rates.

Figure 2. Genome wide distribution of SNPs in the two M lines (1 and 2) after 3\textsuperscript{rd}, 7\textsuperscript{th}, 15\textsuperscript{th} and 31\textsuperscript{st} generation of meiosis. Homozygous SNPs are shown in orange while heterozygous SNPs are shown in cyan. More than 99% of the heterozygous sites are fixed by M_31.

Figure 3. Genome wide plots of LOH and new base mutations in the five V_57 lines. Regions showing loss of heterozygosity are in orange (2:0 or 0:2). Regions in cyan are heterozygous (1:1). Asterisk (*) symbols show the position of the new base mutations in the V_57 lines.

Figure 4. Heterozygosity is negatively correlated with spore viability. The number of heterozygous SNPs and spore viability are plotted against each other for the S288c x YJM789 diploid M lines and for hybrid crosses involving other \textit{S. cerevisiae} strains. Data for the M lines is from Table 1. Data for other hybrid crosses are shown in the manuscript text.

Figure 5. Representative \textit{in silico} genomes generated by crossing spores from M_3 and M_10 lines. White regions on the chromosome are homozygous for S288c or YJM789. The diploids generated from the cross have good viability (>90\%) and are heterozygous at specific chromosomal regions. The green and grey regions indicate centromere positions and chromosome boundaries respectively.
Table 1. Heterozygous SNP counts from sequencing of diploid M and V lines

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<th>p value</th>
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S.V(%) indicates percentage spore viability for M and V lines. Statistical significance of differences in spore viability between parent diploid (85% spore viability from 180 tetrads) and the M and V lines were determined using the p values from Fisher’s test. N: number of tetrads analysed for spore viability.
Table 2. Genomic locations of the sixteen base mutations identified in the M and V lines

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*Shared mutation
Figure 2
Figure 3
Figure 5

M2BD_3 (M2B_3 x M2D_3); Viability 90%; SNPs: 8765

M2DA_10 (M2D_10 x M2A_10); Viability 96%; SNPs: 1108