Heterogeneous Mutation Rates and Spectra in Yeast Hybrids

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Abstract

Mutation rates and spectra vary between species and among populations. Hybridization can contribute to this variation, but its role remains poorly understood. Estimating mutation rates requires controlled conditions where the effect of natural selection can be minimized. One way to achieve this is through mutation accumulation experiments coupled with genome sequencing. Here, we investigate 400 mutation accumulation lines initiated from 11 genotypes spanning intralineage, interlineage, and interspecific crosses of the yeasts *Saccharomyces paradoxus* and *S. cerevisiae* and propagated for 770 generations. We find significant differences in mutation rates and spectra among crosses, which are not related to the level of divergence of parental strains but are specific to some genotype combinations. Differences in number of generations and departures from neutrality play a minor role, whereas polyploidy and loss of heterozygosity impact mutation rates in some of the hybrid crosses in an opposite way.

Key words: mutation accumulation, *Saccharomyces paradoxus*, hybridization, mutation rate, mutation spectrum.

Significance

Despite the mounting evidence on heterogeneity in mutation rates and spectra within species, still little is known about the role of hybridization on these parameters for species with diverse genomic backgrounds and different levels of divergence. By experimentally accumulating mutations in hybrids of wild yeast *Saccharomyces paradoxus* lineages and hybrids of *S. paradoxus* and *S. cerevisiae*, we find variation in mutation rates and spectra, which is not well predicted by the level of heterozygosity. Our study suggests that genotype effects may be more important than the level of divergence between the parental species in shaping mutational landscapes of hybridizing species.

Introduction

Mutations generate genome variation, which in turn fuels evolution. They can be a source of adaptations (Venkataram et al. 2016), though more often they contribute to increased genetic load and play an important role in disease development (Deciphering Developmental Disorders Study 2017), in particular the evolution of cancer (ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium 2020). Mutations result from DNA replication errors and DNA damage, from the activity of transposable elements and other

physical alterations of DNA molecules. The balance between mechanisms generating and diminishing mutations results in largely conserved mutation rates within large taxonomic units (Drake 1991; Lynch 2007), but to what extent rates of mutations and different mutation types (mutation spectra) differ among populations and between closely related species is only beginning to be understood.

Whole-genome population data can provide estimates of mutation rates; however, whether novel mutations become observable variants in natural populations depends on environmental variation, selection, recombination, and demographic history. Largely unbiased mutation rates can be measured in microorganisms using fluctuation assays (Luria and Delbrück 1943) or mutation accumulation (MA) experiments followed by reporter assays or genome sequencing. In MA experiments, the strains are first propagated through sequential bottlenecks for many generations and then sequenced to detect mutations. Both approaches have been used in different contexts with model organisms. In yeast, mutation rates and spectra have been shown to differ within and between species (Nguyen et al. 2020; Jiang et al. 2021), between environmental factors such as mild (Liu and Zhang 2019) and severe stressors (Shor et al. 2013) and vary depending on ploidy (Sharp et al. 2018) or sequence context (Ma et al. 2012). A wide array of genes, notably involved in DNA repair, have been shown to influence the mutation landscape (Demogines et al. 2008; Lang et al. 2013; Serero et al. 2014; Stirling et al. 2014; Gou et al. 2019; Loeillet et al. 2020).

Hybridization could play a role in shaping mutation rates as it could compound the effects of different mutator alleles (Demogines et al. 2008), introduce mutations through mutagenic crossing-over in high diversity regions (Yang et al. 2015) or disrupt mechanisms repressing transposable elements (Petrov et al. 1995; Serrato-Capuchina and Matute 2018). Alternatively, parental effects can dominate in shaping mutation rates in hybrids (Bashir et al. 2014). MA experiments by Tattini et al. (2019) showed that an interspecific hybrid between one *Saccharomyces cerevisiae* and *S. paradoxus* strain has mutation rates similar to *S. cerevisiae* diploid strain. However, to better understand the role of hybridization and genotype effects on mutation rate, different genetic backgrounds need to be considered.

Here, we analyzed whole-genome sequences from our previous MA experiments (Charron et al. 2019; Hénault et al. 2020; Marsit et al. 2021) to estimate and compare mutation rates and spectra in nine intraspecific crosses of *S. paradoxus* and two interspecific crosses between *S. paradoxus* and *S. cerevisiae* yeast. We find that parental sequence divergence does not explain differences in mutation rate, whereas polyploidy and loss of heterozygosity (LOH) correlate negatively or positively with mutation rate. Our results suggest that combinations of individual genotypes best determine mutation rates and spectra among crosses.

Results and Discussion

Mutation Rate and Spectrum

We analyzed 400 lines from 11 crosses, including five intralineage crosses of *S. paradoxus SpC* (CC) and *SpB* (BB), four interlineage crosses between *S. paradoxus SpB* and *SpC* (BC) and between *SpB* and *SpA* (BA) and two interspecific crosses between *S. paradoxus SpB* and *S. cerevisiae* (BSc) (fig. 1*A* and *B*; supplementary tables S1–S4, Supplementary Material online). After ~770 generations of MA, lines accumulated a total of 1,040 single and multiple nucleotide substitutions and 34 indels (supplementary tables S5 and S6, Supplementary Material online). Mean per-cross mutation rates range from 7.88×10^{-11} in the interlineage cross BA2 (4% parental nucleotide divergence) to 2.64×10^{-10} in the intralineage cross BB2 (0.3% parental nucleotide divergence) and they are generally consistent with mutation rates found previously in both laboratory and natural strains of *S. cerevisiae*, *S. paradoxus* and their hybrids (fig. 1*C* and supplementary table S7, Supplementary Material online). Mutation rates vary significantly among crosses (negative binomial regression, $P = 1.11 \times 10^{-6}$, supplementary table S8, Supplementary Material online). Mutation rates also vary between crosses which share one of their parental strains (Kruskal–Wallis test, P values 0.025 and 7.24×10^{-5} for B1 and B2 groups respectively), and the trends are not monotonic with sequence divergence of parental strains (fig. 1*C*). Differences in mutation rates between pairs of crosses of the same lineage are generally lower, but of the same order of magnitude than those between pairs of crosses from different lineages, with the highest differences for pairs including BB crosses (supplementary fig. S1, Supplementary Material online).

There are small and nonsignificant differences (chi-square contingency test, FDR-corrected $P > 0.98$) in frequency spectra of six mutation types (fig. 1*D*, after excluding two lines, see Materials and Methods). Mutations of type C > T (G > A) are most frequent in all crosses except BB2, which also has the lowest transition to transversion (Ts/Tv) ratio close to 0.5 (fig. 1*D*). Unlike *S. cerevisiae*, most *S. paradoxus* crosses show in general a high frequency of A > G (T > C) mutations, which results in a smaller fraction of G/C positions being hit by a mutation (fig. 1*E*). Similar to *S. cerevisiae*, we observe a bias of G/C to T/A mutations in hybrid crosses (fig. 1*E*). Again, we find no differences between intra- and interlineage crosses, and BB2 stands out in terms of both mutation rate and spectrum.

We investigated several biological factors which could explain differences in mutation rate and spectra among crosses and lines including differences in number of generations,

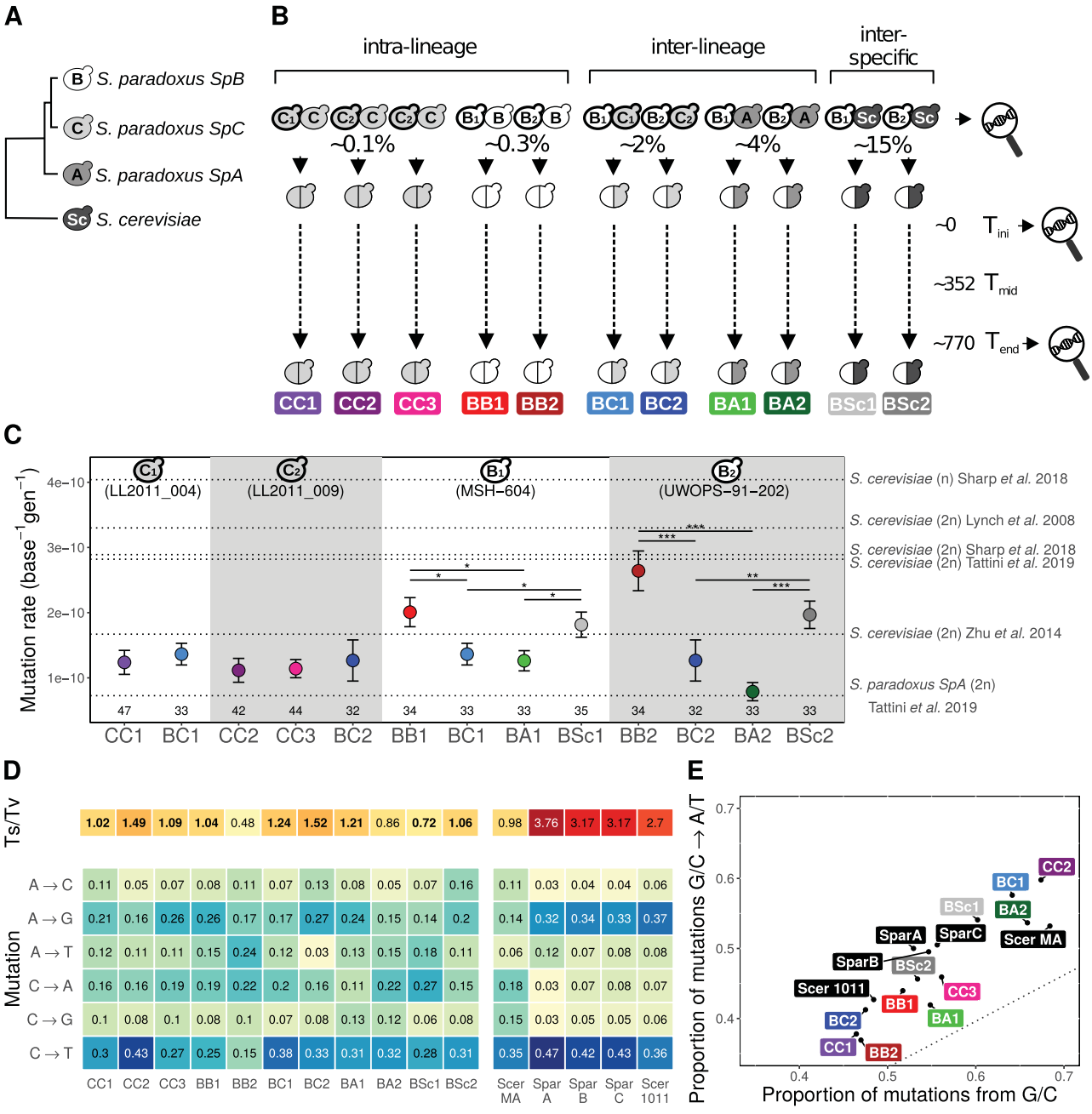

FIG. 1.—Mutation rates differ among crosses. (*A*) Schematic phylogenetic relationships among the lineages. (*B*) Analyzed crosses. Two *Saccharomyces paradoxus SpB* and two *S. paradoxus SpC* parental strains that were used in multiple crosses are marked with numbers and bold contour (shown in panel *C* and detailed in supplementary table S1, Supplementary Material online). Parental strains without numbers correspond to distinct strains. Percentages under the crosses indicate % of sequence divergence between parental genomes. Whole-genome sequencing was done in parental strains, at T_{ini} and T_{end} and growth was measured at three timepoints (T_{ini}, T_{mid}, T_{end}). (*C*) Mean mutation rates per haploid position per generation with SEs estimated from replicate lines over 770 generations of MA experiment shown in four groups sharing the same parental strain. Asterisks show FDR-corrected differences at $*P<0.1$,$**P<0.01$, $***P<0.001$, Wilcoxon rank sum test. Number of lines is depicted at the bottom. (*D*) Transition to transversion ratio (Ts/Tv, upper red heatmap) and frequencies of six nucleotide changes including their complementary changes (lower blue heatmap). Scer MA stands for mutation spectrum of *S. cerevisiae* from the MA experiment from Zhu et al. (2014). The three Spar columns correspond to a population data set of three lineages of *S. paradoxus*: *SpA*, *SpB*, and *SpC*. Scer 1011 corresponds to the population data set from Peter et al. (2018). Bold Ts/Tv estimates indicate crosses with Ts/Tv significantly different from 0.5 (chi-square contingency test, FDR-corrected $P<0.01$). (*E*) All crosses show higher frequency of G/C to A/T than expected from the proportion of mutated G/C positions. The dotted line represents the expected proportion of mutations G/C to A/T if mutations were random.

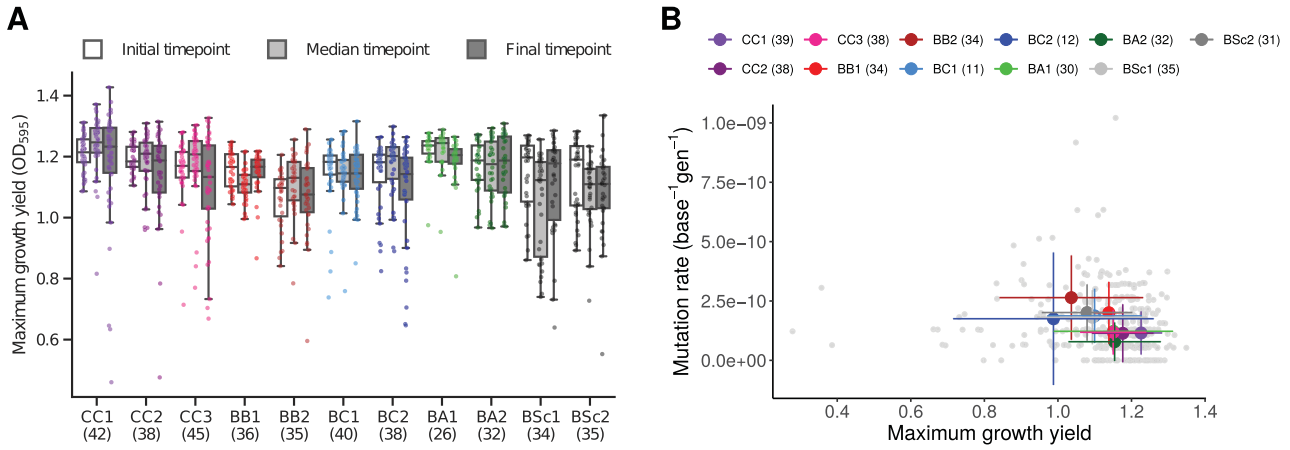

Fig. 2.—Number of generations does not explain mutation rate variation. (*A*) Growth yields differ significantly between crosses. The maximum growth yield of individual lines at the initial, median, and final timepoints of the MA experiment is shown with sample sizes in parentheses. (*B*) Mean growth rate (across three timepoints per cross) versus mean mutation rates per cross with errors depicting SD across lines (diploids only) with sample sizes in parentheses.

deviations from neutral evolution, whole genome or chromosome ploidy changes, the presence of LOH events, and disruption of DNA repair genes.

Number of Generations

Mutation rate estimates were calculated with an average estimation of the number of mitotic divisions within a colony during the MA experiments (Charron et al. 2019). However, these estimates can be biased by variation in actual generation time. We sought to systematically correct for this by measuring the growth of all individual lines at three timepoints of the MA experiments by extracting the maximal yield from growth curves in liquid medium (fig. 2*A*) and using these values as a proxy of generation time (supplementary fig. S2, Supplementary Material online). Overall, growth yields showed no significant trend with timepoints (mixed linear model with cross-specific intercepts and slopes, P=0.93), which is consistent with the absence of selection for growth efficiency. There are significant differences between crosses and timepoints (fig. 2*A*, two-way ANOVA, $P=5.7\times10^{-23}$ and 8×10^{-5} respectively), but no significant interaction between crosses and timepoints (P=0.56). We find that growth yield does not explain differences in mutation rates between crosses. Neither mean yield (across three timepoints) nor mean growth yield by cross interaction have a significant effect on mutation rate (fig. 2*B* and supplementary figs. S3 and S4 and table S9, Supplementary Material online, negative binomial regression, $P>0.718$ for both growth and interaction).

Functional Properties of De Novo Mutations

The demographic regimen of MA experiments is designed to minimize the efficiency of natural selection on the fixation of spontaneous mutations, which should result in fixed mutations that are randomly distributed in genomes, assuming a uniform mutation rate. We tested this hypothesis by classifying the de novo mutations according to three functional properties expected to impact their average fitness effect: whether they occur in protein-coding sequences, and if so, whether they lead to nonsynonymous substitutions or fall into essential genes. Assuming that spontaneous mutations are deleterious on average, efficient purifying selection would leave fewer mutations than expected by chance in coding regions and essential genes, and nonsynonymous mutations should be rare. We examined these predictions while accounting for the genomic composition and substitution spectrum of each cross (supplementary figs. S5–S8 and table S10, Supplementary Material online). Only BA1 exhibits a pattern consistent with purifying selection, with more mutations in nonessential genes than expected. In the BA1 lines, only three mutations were identified in essential genes. Despite all three being nonsynonymous, they are not associated with significant growth yield decrease (supplementary fig. S9, Supplementary Material online). Although growth differences may fall below our method's detection limit, this suggests that the scarcity of mutations in essential genes is not linked to a higher fitness for lines with no mutations in essential genes. On the other hand, four crosses exhibit opposite patterns for at least one property, with fewer noncoding mutations (BB1, BSc1), fewer mutations in nonessential genes (BB2), and more nonsynonymous mutations (BB2, BA1). Nevertheless, no pattern was consistent with increased growth yield (supplementary fig. S9, Supplementary Material online). Overall, these results indicate that accumulated mutations are largely consistent with neutral evolution.

Polyploidy and LOH

It was shown that *S. cerevisiae* diploids have lower mutation rates than haploids (Sharp et al. 2018). One type of change

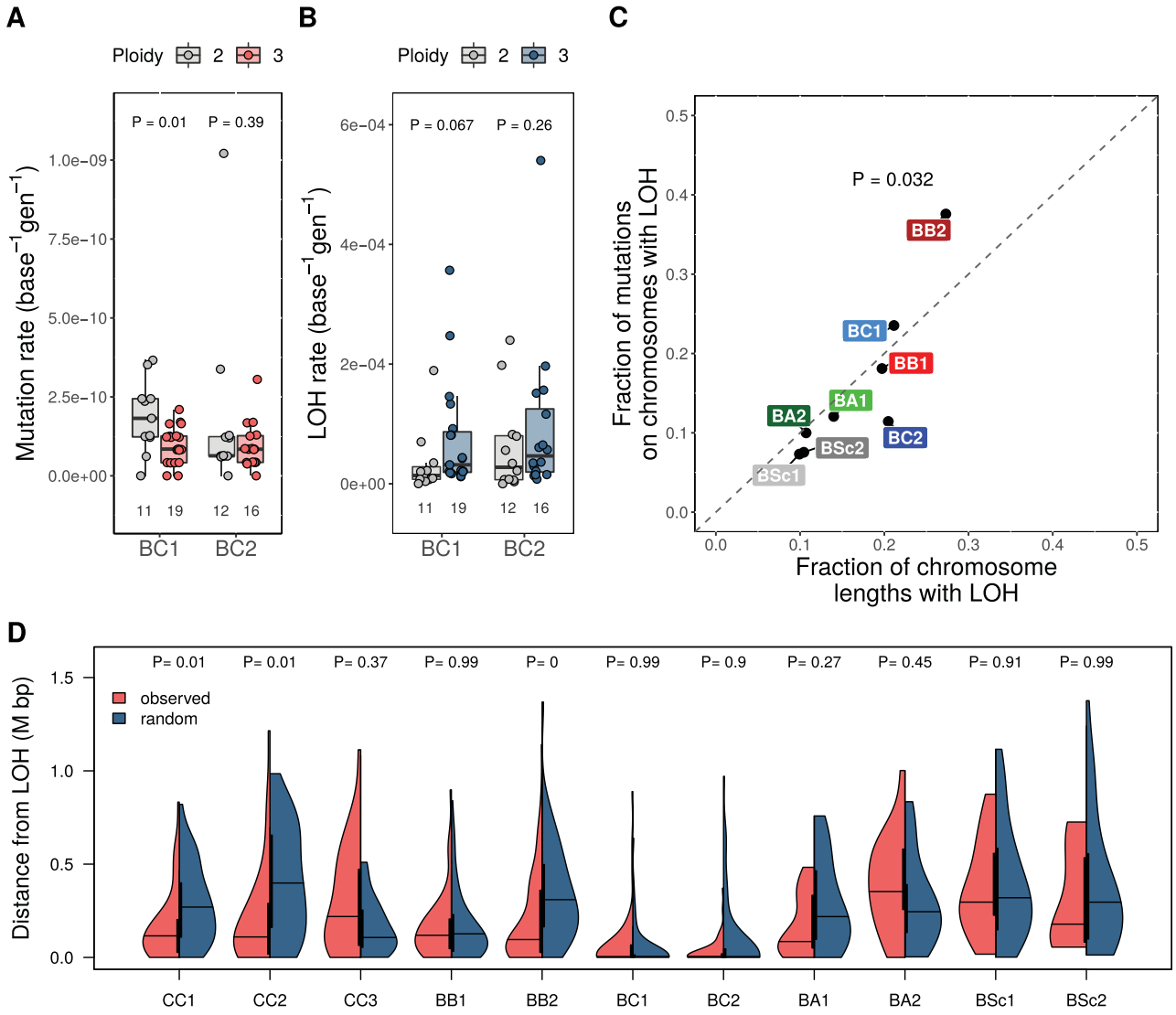

Fig. 3.—Polyploidy and LOH impact mutation rates. (*A*) Differences in mutation rates in BC crosses between diploid and triploid lines tested with Wilcoxon rank sum test. (*B*) Differences in LOH rates in BC crosses between diploid and triploid lines tested with Wilcoxon rank sum test. (*C*) Fraction of mutations on chromosomes with LOH compared with expected (diagonal), estimated as the fraction of the genome comprising the sum of chromosome lengths with LOH. BB2 has significantly more mutations on LOH-carrying chromosomes than expected from the length of LOH-carrying chromosomes. (*D*) Distribution of distances of de novo mutations to the closest LOH variants compared with the same number of mutations randomly drawn from the genome. Differences compared with Wilcoxon rank sum test.

that can occur in diploids is LOH. LOH can eliminate arising de novo mutations, but on the other hand, it is an error-prone process (Hicks et al. 2010; Deem et al. 2011), which can lead to higher mutation rates. We obtained LOH tracts from Marsit et al. (2021) for diploid and triploid lines from crosses BB, BC, BA, and BSc. To include CC crosses and LOH events smaller than the minimum LOH tract length (1 kb) we additionally calculated LOH rate using the proportion of heterozygous positions in which genotype changed between T_{ini} and T_{end} (supplementary figs. S10 and S11, Supplementary Material online). We find a significant negative effect of polyploidy and a significant positive effect of LOH on mutation rate (supplementary table S11, Supplementary Material online). The effect of interaction between polyploidy and LOH is negative but nonsignificant (negative binomial regression, P=0.08). In BC crosses, which carry most triploid lines, BC1 triploids have significantly lower mutation rates than BC1 diploids, whereas in BC2 there is no difference (fig. 3*A*). We confirmed that differences are not caused by uncalled mutations in low covered regions (supplementary fig. S12, Supplementary Material online). LOH rates are slightly higher for triploids than diploids, though the difference is nonsignificant (fig. 3*B*). Large scale LOH events (>300 bp) are less frequent in BC triploids than diploids (Marsit et al. 2021,

supplementary fig. 13, Supplementary Material online), suggesting that total observed LOH rates in triploids are driven mostly by very short LOH events, which do not disappear after excluding positions below read depth of 70x (supplementary fig. 12, Supplementary Material online). This implies that LOH events do not have a detectable impact on lowering mutation rates in polyploids at this stage of experiment. Contrary to triploidy, gains or losses of chromosomes, and whole-genome duplications occurring during the experiment do not have a measurable effect on mutation rate (supplementary fig. S14, Supplementary Material online).

We then investigated whether LOH can induce mutations in diploid lines. Chromosomes carrying LOH are not enriched for de novo mutations, except in BB2 (binomial test, fig. 3*C*). We find no difference in the number of de novo mutations around the breakpoints of LOH segments (±2 kb) compared with the remainder of chromosomes carrying LOH (binomial test, two out of 109 mutations, expected frequency = 0.012, P=0.395), which is not unexpected due to ambiguous LOH breakpoint positions for low heterozygosity crosses. However, de novo mutations in BB2, CC1, and CC2, are significantly closer to LOH positions than random mutations, suggesting the causal effect of LOH on mutation rate (fig. 3*D*). Increased mutation rates near LOH breakpoints were observed before (e.g., in pathogenic *Candida* yeast; Ene et al. 2018), which can be linked to mutagenic effects of homologous recombination. Even though the duration of our experiments is relatively short to observe strong LOH effects, our results suggest that some genotype combinations may experience stronger mutagenic effects of homologous recombination.

DNA Repair Genes

Observed differences in mutation rates and spectra are mainly driven by BB2, which has the highest mutation rate and the lowest Ts/Tv ratio. Malfunctioning DNA repair mechanisms can influence both mutation rates and spectra. One of the patterns observed in yeast with a disrupted DNA mismatch repair pathway is the tendency to accumulate spontaneous mutations in regions with repeated sequence motifs (Lang et al. 2013). BB crosses have indeed the largest (although nonsignificant) departure from the expected sequence complexity around de novo mutations toward lower values (supplementary fig. S15, Supplementary Material online). Disruption of DNA repair genes with an impact on all lines from the cross could occur before the formation of the cross or before sequencing at T_{ini}. We found no common nonsynonymous or nonsense variants at the beginning of MA in DNA repair genes in BB2. In addition, none of the DNA repair genes lost a copy (supplementary fig. S16, Supplementary Material online), and lines with lost, gained, or unbalanced number of copies of DNA repair genes do not have an increased mutation rate (supplementary fig. S15, Supplementary Material online). However, outlier lines with the highest mutation rates in five crosses have a nonsynonymous mutation in at least one DNA repair gene (supplementary fig. S17, Supplementary Material online). Presence of de novo nonsynonymous or mutations causing stop codon gain/loss in DNA repair genes is a strong predictor of mutation count (negative binomial regression, P=8.79e-8, incidence rate ratio = 2.6, CI: 1.8–3.7). Acquired mutations, therefore, explain part of the variation in the mutational landscape. Skewed mutation rate and spectrum in BB2 are not driven by a single line but elevated A > T and C > A mutations across multiple lines, most often surrounded by A and T nucleotides (supplementary fig. 18 and table S5, Supplementary Material online). This indicates that parent-specific factors or between parent interactions must account for this change. The list of 22 DNA repair genes with nonsynonymous private variants in the parental strain specific to BB2 (LL2012_021, supplementary table S12, Supplementary Material online), includes a gene encoding DNA mismatch protein MSH2, which binds to DNA mismatches upon heterodimerization with MSH6 (Marsischky et al. 1996). Mutation Lys957Glu is in a conserved position (ConSurf conservation score=−0.697, CI: −1.022 to −0.501; Landau et al. 2005) in the region of dimerization with MSH6, pointing to a potential trigger of biased mutational landscape in BB2 cross.

In this study, we demonstrate significant differences in mutation rates between 11 types of hybrid crosses of yeast. Including genetically diverse backgrounds is therefore essential for uncovering species diversity in mutation rates. Low mutation rates in *S. paradoxus SpA* reported previously (Tattini et al. 2019) are closest to BA2 (4% parental divergence), which has the lowest mutation rate among all crosses. Out of several potential factors leading to such variation, differences in growth rate and natural selection play a negligible role in our experiment. Polyploidy and LOH have observable and opposite impacts on mutation rates of some crosses. LOH is generally more frequent in less heterozygous hybrids (Tattini et al. 2019; Marsit et al. 2021), therefore its effects should be more prominent in crosses involving closely related lineages (BB and CC). On the contrary, the level of sequence divergence between parental strains does not have a systematic impact on mutation rate. This is consistent with the results of Hénault et al. (2020) who found no relationship between the accumulation of transposable elements and the level of divergence between parental genomes in the same crosses. Even though we are not able to measure the impact of each parental genotype on mutation rate in this experimental setup, our results suggest that hybrid genotypes show variation of mutation rates and spectra either through inheritance of parent-specific rates or interaction between parental genotypes. In consequence we may expect the natural populations of *S. paradoxus*, which has experienced many hybridization events in the recent past (Leducq et al. 2016; Eberlein et al. 2019), to exhibit such variation.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Acknowledgments

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Data Availability

The data underlying this article are available in NCBI under project numbers: PRJNA515073 (short-sequence data) and PRJNA514804 (genome assemblies).

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