Yeast Spontaneous Mutation Rate and Spectrum Vary with Environment

Graphical Abstract

**Highlights**

- Yeast nuclear mutation rate decreases with cell growth rate across environments
- Yeast mitochondrial mutation rate rises with cell growth rate across environments
- Yeast nuclear mutation spectrum varies among environments

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**In Brief**
Using whole-genome sequencing following mutation accumulation in each of seven media, Liu and Zhang report that yeast mutation rate and spectrum vary substantially, even among different benign environments, which has important implications for both neutral and adaptive evolution.
Yeast Spontaneous Mutation Rate and Spectrum Vary with Environment

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SUMMARY

Mutation is the ultimate genetic source of evolution and biodiversity, but to what extent the environment impacts mutation rate and spectrum is poorly understood. Past studies discovered mutagenesis induced by antibiotic treatment or starvation, but its relevance and importance to long-term evolution is unclear because these severe stressors typically halt cell growth and/or cause substantial cell deaths. Here, we quantify the mutation rate and spectrum in Saccharomyces cerevisiae by whole-genome sequencing following mutation accumulation in each of seven environments with relatively rapid cell growths and minimal cell deaths. We find the point mutation rate per generation to differ by 3.6-fold among the seven environments, generally increasing in environments with slower cell growths. This trend renders the mutation rate per year more constant than that per generation across environments, which has implications for neutral evolution and the molecular clock. Additionally, we find substantial among-environment variations in mutation spectrum, such as the transition to transversion ratio and AT mutation bias. Other main mutation types, including small insertion or deletion, segmental duplication or deletion, and chromosome gain or loss also tend to occur more frequently in environments where yeast grows more slowly. In contrast to these findings from the nuclear genome, the yeast mitochondrial mutation rate rises with the growth rate, consistent with the metabolic rate hypothesis. Together, these observations indicate that environmental changes, which are ubiquitous in nature, influence not only natural selection, but also the amount and type of mutations available to selection, and suggest that ignoring the latter impact, as is currently practiced, may mislead evolutionary inferences.

INTRODUCTION

The classical evolutionary theory asserts that the rate of mutation occurring in an organism is independent of the environment of the organism [1, 2], a view that has been challenged by the observation of stress-induced mutagenesis (SIM) [3–5]. Specifically, it was discovered that the mutation rate in bacteria tends to rise when they are under antibiotic treatment, starvation, or other stresses [3]. A similar trend has also been reported from limited studies of eukaryotes [6–8]. Although the molecular mechanisms of SIM are not fully understood, bacterial studies showed that they often involve the SOS response, including the upregulation of error-prone DNA polymerases that leads to elevated mutagenesis [3, 9]. It is currently debated whether SIM is a consequence of relaxed selection for the accuracy of rarely used DNA polymerases, a pleiotropic byproduct of selection for increased stress tolerance, and/or a direct outcome of second-order selection for evolvability [10, 11]. Regardless, SIM may accelerate organismal adaptations to stressful environments [12–14] and hence has been of substantial interest to evolutionary biologists [15–18].

Notwithstanding, previous studies of SIM were typically conducted in environments that were so stressful that a substantial fraction of cells were killed and/or population growth was largely halted [3, 14, 19]. Conclusions drawn from such experiments have two caveats. First, mutation rates are likely overestimated in the presence of substantial cell death, because cell death increases the number of cell divisions needed to bring the population to the observed size, which renders the mutation rate per generation overestimated when this factor is ignored [20]. Indeed, a recent study showed that accounting for cell death either removes or greatly reduces the purported mutagenic effect of antibiotic treatment [20]. Second, the stress severity required to induce mutagenesis is unclear. If SIM occurs only under rare, severe stresses, its impact on long-term evolution would be limited. However, because the environment of virtually no species is constant for a long time, if the mutation rate of an organism varies to a sizeable extent even among different, benign environments, the evolutionary impact of the phenomenon would be much broader and a number of evolutionary theories or models that rely on the assumption of a constant mutation rate (e.g., the explanation of the molecular clock phenomenon) would require major revisions.

In addition to the impact on mutation rate, recent studies revealed varied impacts of different severe stresses on the molecular spectrum of mutations. For instance, in six different nutrient starvations that all reduced the Escherichia coli growth rate by over 20 times, the mutation spectrum was found to vary substantially [19]. Nonetheless, this result was based on one to four reporter genes and its genomic generality remains unknown. It is also unknown how stressful the environment needs to be to...
induce such mutation spectrum changes and how widespread this phenomenon is, especially among eukaryotes.

To assess the impact of mild environmental changes on genome-wide mutation rate and spectrum, we performed mutation accumulation (MA) experiments in the unicellular eukaryotic model organism *Saccharomyces cerevisiae* in each of seven different environments where cell death is minimal and the population growth rate is greater than 50% of that in the optimal condition. We then applied whole-genome sequencing (WGS) of MA lines to detect mutations. Our results show that the yeast mutation spectrum varies substantially among these environments and that the mutation rate is generally higher in environments where the growth is slower.

**RESULTS**

**Growth Rate Declines Faster during MA in Environments where the Initial Growth Is Slower**

We performed MA experiments in the diploid BY4743 strain of *S. cerevisiae* because diploids accumulate more mutations than haploids per generation [21] and because yeast is mostly in the diploid form in nature. Seven environments were used, including the nutrition-rich yeast-extract-peptone-dextrose (YPD) medium that supports optimal growth of the BY4743 strain in lab. To introduce mild stressors, we switched the sugar source, switched the nitrogen source, or added various inorganic salts in YPD, resulting in the following six additional media: yeast-extract-peptone-xylose (YPX); yeast-extract-peptone-lactose (YPL); yeast-nitrogen-base-dextrose (YNB); YPD with 6 mM CuSO4; YPD with 100 mM LiCl; and YPD with 1 M NaCl (Table S1). Yeast growth rate and cell viability were respectively quantified in these seven media. Population growth rate per hour, a fitness proxy, varies significantly in the seven media (p < 10^-4; ANOVA), although even the lowest rate is higher than 50% of the highest rate (blue bars in Figure 1A). Cell viability is uniformly high (p = 0.077; ANOVA) and is comparable to that in YPD (Figure 1B). There is no significant correlation between yeast growth rate and cell viability in these environments (Spearman’s r = −0.54; p = 0.24).

A total of 168 parallel MA lines were established from the same ancestor, with 24 lines in each medium. MA was achieved by regular single-cell bottlenecks, known to minimize the influence of selection on spontaneous mutations [22]. To keep the number of cell divisions between bottlenecks roughly the same across media, single-cell bottlenecks were enforced every 24, 36, or 48 h, depending on the medium, for a total of ~1,000 cell divisions in each medium (Table S1). Two lines died out in the MA experiment, probably due to lethal mutations, resulting in a total of 166 lines at the conclusion of the experiment. As expected, growth rate generally declined after MA (red bars in Figure 1A). The speed with which the growth rate declined per generation tends to be higher in the media where the initial growth rate before MA is lower (Figure 1C), hinting the possibility that the mutation rate per generation is higher in environments where cells grow more slowly.

**Rates of All Four Main Mutation Types Vary among Environments**

To identify the mutations that accrued in each MA line, we applied Illumina WGS on the ancestor (202 × sequence coverage) as well as all 166 MA lines (average 95 × coverage). In total, 1,415 single-nucleotide variations (SNVs), 259 small insertions or deletions (indels) shorter than 60 nt, 18 segmental duplications or deletions longer than 1 kb, and 33 whole-chromosome gains or losses were identified (Table S2; Data S1). Sanger sequencing confirmed all of the 18 randomly picked SNVs and small indel mutations tested (Table S3; see STAR Methods). Two SNV-based parameters are commonly used to confirm the ineffectiveness of selection in MA experiments. First, 74% of SNVs in the yeast genome are expected to occur in genomic
regions if mutations are not subject to selection [21]. Indeed, the observed fraction does not deviate significantly from the expected value in any of the seven environments (all $p > 0.2$; binomial test followed by multiple testing correction; Figure S1A).

Second, the expected fraction of nonsynonymous SNVs in yeast coding regions is 76% when coding mutations are not selected [21]. Again, no significant deviation from the above expectation is observed in any of the seven environments (all $p > 0.1$; Figure S1B). There is also no significant among-environment variation in the proportion of SNVs that are germline or the proportion of coding SNVs that are nonsynonymous (both $p > 0.05$; chi-square test). These observations confirm that all mutations are effectively neutral during MA in any of the seven environments.

We found that all four main mutation types exhibit significant rate variations among the seven environments ($p < 10^{-4}$ for each type; ANOVA). Specifically, the rate of SNV in YPD is $1.95 \times 10^{-10}$ (95% confidence interval $[1.60-2.34 \times 10^{-10}]$ per nucleotide per cell division, consistent with a previous estimate in yeast ($1.67 \times 10^{-10}$) [23] but significantly lower than another ($2.89 \times 10^{-10}$) [21], possibly due to the use of a different yeast strain in the latter study. The SNV rate varies by 3.57 times from $1.95 \times 10^{-10}$ in YPD to $6.97 \times 10^{-10}$ in LiCl (Figure 2A). In three (YNB, LiCl, and NaCl) of the six environments, the SNV rate is significantly greater than that in YPD ($p < 10^{-4}$; Wilcoxon rank-sum test followed by Bonferroni correction for multiple testing; Figure 2A).

The small indel rate ranges from $0.19 \times 10^{-10}$ (in YPD) to $1.58 \times 10^{-10}$ (in NaCl) per site per cell division in the seven environments, again showing the lowest value in YPD. Two environments (LiCl and NaCl) exhibited a significantly higher rate than that in YPD ($p < 10^{-4}$; Wilcoxon rank-sum test followed by Bonferroni correction; Figure 2B). Furthermore, the insertion to deletion ratio differs among the environments ($p < 0.0002$; chi-square test). This ratio is significantly higher than 1 in two environments ($p < 10^{-4}$ in NaCl and $p = 0.0018$ in LiCl; binomial test) but is not significantly different from 1 in the other five environments tested separately or together (all $p > 0.1$; binomial test).

In addition to small-scale mutations, we identified two types of large-scale mutations based on changes in sequence coverage. There were 18 segmental duplications or deletions longer than 1 kb, including 17 mutations in LiCl and 1 mutation in YPD (Figure 2C). These mutations affected more nucleotides per event in LiCl (48–562 kb) than in YPD (4 kb). Furthermore, 16 whole-chromosome gains and 17 whole-chromosome losses were found, mostly in LiCl (18) and NaCl (8; Figure 2D). There is no significant correlation between chromosome length and the rate of chromosome gain or loss (Pearson’s $r = -0.46$; $p = 0.073$). There is also no evidence that growth rate, SNV rate, or small indel rate differ between the MA lines with and without large-scale mutations in the same environment (all $p > 0.05$; Wilcoxon rank-sum test).

### Mutation Rates Are Higher in Environments with Lower Initial Growth Rates

We found significant, negative correlations between the SNV rate, small indel rate, and whole-chromosome gain or loss rate in an environment and the initial yeast growth rate in the environment (Spearman’s $p = -0.45$, $p < 10^{-4}$ for SNV; $p = -0.41$, $p < 10^{-4}$ for small indel; $p = -0.23$, $p = 2.8 \times 10^{-3}$ for whole-chromosome gain or loss; Figure 3; Table S4; see STAR Methods). The corresponding correlation is negative, but not significant, for the segmental duplication or deletion rate ($p = -0.085$; $p = 0.28$; Figure 3C). These results suggest that both small- and large-scale mutations occur more frequently in environments where yeast grows more slowly.

For the SNV data, which contained the highest number of mutational events, we also fitted a linear model in which the mutational rate of an MA line is the dependent variable, whereas the growth rate of the strain before MA is the independent variable. As expected, the effect of the growth rate on the mutation rate is significantly negative ($p < 10^{-4}$). When we extended the model by adding a fixed categorical effect from each environment and an effect of interaction between environment and growth rate, the additional effects were not found to be significant ($p > 0.3$).

The most pronounced mutation rate elevations are observed in the LiCl and NaCl media. Nevertheless, even after the removal of the data from these two environments, the negative correlation between SNV rate and initial growth rate holds ($p = -0.19$; $p = 0.035$). But the corresponding correlation is no longer
Previous studies revealed a universal guanine or cytosine (GC) to adenosine or thymine (AT) mutational bias across divergent evolutionary lineages [24–26]. Although this bias is evident in all seven environments, the extent of the bias varies significantly among the environments (p = 3.5 × 10^{-4}; chi-square test). The number of GC-to-AT mutations, relative to the number of AT-to-GC mutations, ranges from 1.34 in YPX to 3.22 in NaCl (Figure 4C). Compared with that in YPD, the GC-to-AT/AT-to-GC mutation ratio is significantly higher in NaCl (p = 2.2 × 10^{-4}; chi-square test followed by multiple testing correction; Figure 4C), but not significantly different in the other five environments.

**Expressions of Error-Prone DNA Polymerases**

As mentioned, SIM in *E. coli* has been shown to involve the up-regulation of three error-prone DNA polymerases [9, 27]. To examine whether the mutation rate variation revealed here in yeast is similarly attributable to the actions of error-prone DNA polymerases, we used qRT-PCR to quantify the mRNA concentrations of six DNA polymerases, including three essential polymerases (POL1, POL2, and POL3), two error-prone polymerases (RAD30 and REV3), and one polymerase involved in double-strand break (DSB) repair (POL4). Under the premise that the mutation rate should rise with the amount of DNA replication catalyzed by error-prone polymerases relative to that catalyzed by essential polymerases, we measured the expression level of each nonessential polymerase by its mRNA level relative to the total mRNA level of the three essential polymerases. We found that each of the three nonessential polymerases has a significant among-environment expression variation (p < 10^{-4}; ANOVA; Figure 5). Each of RAD30, REV3, and POL4 is significantly upregulated in one or two environments relative to the expression in YPD (Figure 5). But no significant Spearman’s or Pearson’s correlation was found between the expression level of any of these three nonessential polymerases and the mutation rate (SNV mutation rate, small indel mutation rate, or their sum; p > 0.3). Compared with the clear correlation between the expression levels of error-prone DNA polymerases and the mutation rate found in *E. coli* under various concentrations of an antibiotic [14], our results do not provide sufficient evidence for the involvement of error-prone polymerases in the among-environment mutation rate variation in yeast.

**Among-Environment Variation of the Mitochondrial Mutation Rate Supports the Metabolic Rate Hypothesis**

In many eukaryotes, the mutation rate in mtDNA is higher than that in nuclear DNA [28]. We identified 32 SNV and 70 indel mutations in the 166 MA lines (Table S2), yielding a mutation rate of 2.2 × 10^{-9} per site per cell division for indel mutations and 4.9 × 10^{-9} per site per cell division for indel mutations, respectively. Both of these values are higher than the corresponding values in nuclear DNA (both p < 0.001; t test). This elevated mitochondrial mutation rate is thought to be caused at least in part by the oxidative radicals generated in mitochondria [29], the amount of which presumably rises with the metabolic rate. Hence, it has been hypothesized that the mutation rate in mitochondria should correlate with the metabolic rate [30]. Using growth rate as a proxy for the metabolic rate, we here test this hypothesis. We found that both the

**The SNV Mutation Spectrum Variates among the Environments**

There are six types of SNV, and their proportions vary significantly among the seven environments (p < 10^{-4}; chi-square test; Figure 4A). When compared with YPD, four environments (YPX, LiCl, YPL, and NaCl) show significantly different SNV spectrums (all p < 0.05 after Bonferroni correction; Figure 4A).

The number of transitional mutations (i.e., between C and T and between G and A) relative to that of transversional mutations (i.e., all other mutations), or Ts/Tv, should be 0.5 under no mutational bias. But Ts/Tv is often greater than 0.5 or even 1 because transitions occur with a higher probability than transversions. Surprisingly, Ts/Tv varies among the seven environments examined (p < 10^{-4}; chi-square test; Figure 4B). Ts/Tv significantly exceeds 0.5 in three environments (CuSO4, YPX, and LiCl; all p < 0.001; binomial test followed by multiple testing correction) but is not significantly different from 0.5 in the other four environments (Figure 4B). Compared with that in YPD, Ts/Tv is significantly higher in LiCl (p < 10^{-4}; chi-square test followed by multiple testing correction; Figure 4B), but not significantly different in the other five environments.

**Figure 3.** Correlation between Initial Growth Rate and SNV, Small Indel, Segmental Duplication or Deletion, and Whole-Chromosome Gain or Loss Mutation Rates across the Seven Environments (A and B) The SNV rate (A) is measured by the number of mutations per nucleotide per cell division and the small indel rate (B) is measured by the number of mutations per nucleotide per cell division. (C and D) The segmental duplication or deletion rate (C) is measured by the number of mutations per cell division and the whole-chromosome gain or loss rate (D) is measured by the number of mutations per cell division. Each colored point represents the mean from all MA lines in the corresponding environment, whereas the horizontal and vertical error bars represent SDs of the mean and of the sample data, respectively. The dashed line represents linear fitting on all 166 MA lines, and the gray shaded region shows the SE predicted by the linear fitting. Spearman’s ρ and Pearson’s r and their associated p values are based on all 166 MA lines. See also Figure S2 and Tables S4 and S5.
mitochondrial SNV and indel mutation rates are positively correlated with the growth rate among the seven environments ($p = 0.31, p < 10^{-4}$ for SNV, Figure 6A; $p = 0.31, p < 10^{-4}$ for indel, Figure 6B). These results are consistent with the metabolic rate hypothesis on mitochondrial mutation rate and are in stark contrast to the observation on nuclear mutation rate (Figure 3). Mitochondrial SNV mutations accumulated in our experiment were too few (Table S2) to allow studying the potential environmental impact on their spectrum.

**DISCUSSION**

By mutation accumulation in seven relatively benign environments followed by whole-genome sequencing, we discovered that yeast spontaneous mutation rate varies with the environment. For the mtDNA, the mutation rate is higher in environments where yeast grows faster, supporting the metabolic rate hypothesis of mitochondrial mutagenesis. For the nuclear DNA, the opposite trend is true. Below, we focus on the nuclear DNA.

The among-environment fold-change in SNV mutation rate observed here (3.6-fold) is comparable to that in *E. coli* when the concentration of the antibiotic norfloxacin rises from 0 to 62.5 ng/mL, corresponding to cell viability decreasing from 100% to 5% [14] or that in *E. coli* upon nutrient (e.g., Fe, O, or N) starvation that slows the population growth to 5% of the normal rate [19]. The same can be said for small indels. Chromosome gains/losses do not exist in *E. coli*, and large duplications or deletions in *E. coli* are primarily caused by a different mechanism (transposon activities), so they are not compared with the present yeast data. Thus, our data suggest that even relatively benign environmental changes influence the yeast mutation rate substantially.

To explore the mechanism behind the yeast mutation rate variation among different benign environments, we measured the expression levels of various DNA polymerases, including error-prone polymerases. Although the expression levels of the error-prone polymerases RAD30 and REV3 and DSB-repair polymerase POL4 are found to vary significantly among the seven environments, we did not detect a significant correlation between their expression levels and mutation rates. Although this negative result does not establish a role of these DNA polymerases in the observed mutation rate variation among environments, it cannot exclude this role either due to the limited statistical power as a result of the relatively small number of environments examined here. Nevertheless, we note that the expression variations of error-prone DNA polymerases among the seven environments studied here are much smaller than that previously observed in *E. coli* under different norfloxacin concentrations [14] (<50% difference in our study versus ~400% difference in *E. coli*), despite that the mutation rate variations detected in the two studies are comparable. Future MA studies of more environments that are relatively benign, especially in mutants lacking error-prone DNA polymerases, may help clarify the role of these polymerases.

One potential explanation of the negative correlation between the yeast growth rate and mutation rate per generation is time-dependent (but replication-independent) mutagenesis. However, the correlation between the growth rate and mutation rate per year is still significantly negative ($r = -0.169, p = 0.029$ for SNVs; $r = -0.318, p < 10^{-4}$ for small indels; $r = -0.219, p = 0.0045$ for chromosome gains or losses; and $r = -0.159, p = 0.041$ for segmental duplications or deletions). Time-dependent mutations mainly originate from unpaired DNA lesions [31]. The number of time-dependent mutations per year should be higher in rapidly dividing cells, because slowly dividing cells have more time to repair lesions before cell division [31]. Our result is inconsistent with this prediction so is unlikely explained even partially by time-dependent mutations.

It was reported that reducing *E. coli* population density in liquid medium increases its mutation rate by three-fold [32]. The same trend was also found in yeast [33]. Although the underlying mechanism of the negative correlation between population density and mutation rate has not been fully worked out, one wonders whether this correlation explains our observation of the among-environment mutation rate variation. To this end, we reanalyzed Krasovec et al.’s [33] three yeast datasets that included...
the information of both population density and growth rate. We found that the correlation between growth rate and mutation rate is more negative than that between population density and mutation rate (Figure S2). Furthermore, the partial correlation between growth rate and mutation rate after the control of population density remains negative in all three datasets and is significant in two of the datasets (Table S5). By contrast, the partial correlation between population density and mutation rate upon the control of growth rate becomes positive in all three datasets (Table S5). Thus, it appears that the negative correlation between population density and mutation rate can be explained by the negative correlation between growth rate and mutation rate, not the other way around.

Our finding of the environment dependency of mutagenesis, especially the negative correlation between growth rate (i.e., fitness) and mutation rate, has several implications for evolution. First, increased mutagenesis in stressful environments can accelerate organismal adaptations due to a heightened rate of neutral substitution rate and spectrum. Second, our finding that, due to inevitable environmental changes in evolution, such inferences are likely less reliable than is currently thought. For instance, it becomes uncertain whether one can infer the action of natural selection on the basis of disparities between genomic features (e.g., nucleotide frequencies) observed and those expected from the mutation spectrum revealed by a laboratory MA experiment [26], because the condition used in an MA experiment cannot possibly represent fully the varying environment of a species. In addition, comparing mutation
...spectrum among species on the basis of MA followed by WGS becomes less meaningful if the environments used in the lab are different in the MA experiments of different species or do not represent their respective native environments.

In conclusion, our finding that yeast genome-wide mutation rate and spectrum vary among even relatively benign environments suggests that the environment not only affects natural selection but also the amount and type of genetic variations available to the selection. The natural environment of almost any species varies in many aspects, but the conditions examined here varied only in carbon source, nitrogen source, and inorganic salts. In the future, one should investigate additional common environmental variables, such as the ambient temperature, which has already been suggested to impact mutagenesis [42, 43]. It will also be important to confirm our findings in other species and study to what extent the environment dependency of mutagenesis impacts adaptations as well as neutral evolution.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2019.03.054.

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AUTHOR CONTRIBUTIONS

H.L. and J.Z. conceived and designed the project; H.L. performed the experiments and analyzed the data; and H.L. and J.Z. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES


STAR METHODS

KEY RESOURCES TABLE

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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jianzhi Zhang (jianzhi@umich.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The strain used in this study is S. cerevisiae BY4743. The cells were propagated at 30°C in seven different solid media (Table S1) with detailed methods described below.

METHOD DETAILS

Establishment and propagation of MA lines
The commonly used diploid S. cerevisiae strain BY4743 was chosen for MA. A total of 168 MA lines were established from the same ancestral colony. These lines were propagated at 30°C in seven different solid media (Table S1), with 24 parallel lines per medium. All
MA lines were passaged by single-cell colony transfers, where a randomly selected average-size colony was streaked onto a new plate. Selecting average-size colonies prevents the fixation of petite mutations. Plates from previous transfers were stored at 4°C to allow additional transfers if the first transfer was unsuccessful, which occurred 38 times (cells did not grow in 37 cases and fungal contamination in one case) in a total of ~10,000 transfers. Among the 38 incidents, two lines failed to recover from 4°C possibly due to the occurrence of cold sensitive mutations; these two lines were thus excluded from the study. Because the yeast growth rate varied in the seven media, we set different bottleneck intervals for different media to allow similar numbers of cell divisions between bottlenecks across media. This interval was 24 hours in YPD and CuSO₄, 36 hours in YNB and YPX, and 48 hours in LiCl, YPL, and NaCl. We kept the total number of cell divisions in all MA lines to ~1000, corresponding to ~60 bottlenecks in each medium (Table S1).

In the preparation of the seven media, pH was adjusted in YNB to ~6 by adding 2.5 mL 1 M NaOH in 1 L medium. Without the adjustment, this medium would have been too acidic and not solidified well. The pH was ~5 in CuSO₄ and YPX and ~6 in the other media. The mutation rate does not show significant elevations in the two media with relatively low pH when compared with that in YPD, suggesting that medium pH does not play an important role in our study.

**Growth rate and cell viability**

The conditions used in growth rate measurement were the same as those in the MA experiment. Specifically, to measure the yeast growth rate in a solid medium, we counted the number of cells in a single colony after growing for a certain amount of time (t = 24, 36, or 48 hours according to the media). In each estimate, one average-size colony was randomly chosen and the cells in the colony were collected in 1 mL of 1 M sorbitol under microscope. Cell number (N) was estimated using a hemocytometer and growth rate per hour (r) was calculated assuming exponential growth described by $N = e^{rt}$. It can be shown that the generation time $g = \ln 2 / r$ hours and the number of generations per day is $24/g = 24r/\ln 2$.

Cell viability is determined by counting colony-forming units (CFUs) as previously described [46]. Specifically, cells were first cultured in liquid YPD to control the number of cells to be plated onto each solid medium. Cells growing in liquid YPD at the mid-log phase were collected by centrifuge and washed with water. Cells were then resuspended in water and diluted to ~1000 cells per mL. We then plated 100 mL of the cell population (~100 cells) on each solid medium, followed by incubation at 30°C for 48 hours before CFU counting. Relative cell viability is the number of CFUs divided by average number of CFUs in YPD, averaged among six replicates per medium type.

**DNA extraction, library construction, and genome sequencing**

Genomic DNAs of the ancestor and all MA lines were extracted using MasterPure Yeast DNA Purification Kit (Lucigen; Cat. No. MPY80200). Sequencing libraries were constructed using Nextera DNA Flex Library Prep (Illumina; Cat. No. 20018705). Paired-end 2 × 150 sequencing reads were generated on Illumina HiSeq 4000 platform by Admera Health (http://www.admerahealth.com/). The genome coverage was 202 × for the ancestor and ranged from 66 × to 155 × for the 166 MA lines.

**Identification of mutations**

For each sample, the sequencing reads were mapped to the *S. cerevisiae* reference genome (version R64-2-1) by Burrows-Wheeler Aligner [44]: marking of duplicates and realignment around indels were carried out by Genome Analysis Toolkit (GATK) [45]. SNVs and small indels were called by GATK HaplotypeCaller. Variants were screened based on the following criteria after initial calling: (i) each variant must be supported by at least five reads, (ii) each variant must be supported by both forward and reverse reads, (iii) quality score is at least 50, and (iv) manual inspection confirms mis-alignments.

Segmental duplications/deletions and whole-chromosome gains/losses were determined based on the sequencing coverage following previous published methods [23]. In particular, an event is called if the sequencing depth of a chromosome or a segment of a chromosome differs by more than 35% from other chromosomes in the same sample.

To verify Illumina sequencing and variant calling, we randomly chose 11 SNVs and 7 small indels for confirmation by Sanger sequencing, and all of the Sanger sequencing results were consistent with the whole-genome resequencing data (Table S3).

**RT-qPCR**

Cells were collected after growing on each solid medium for ~16 generations. Total RNA was extracted using RNeasy Mini Kit (QIAGEN; LOT: 160019171). Reverse transcription was performed using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen; LOT: 1903730) with random hexamers. Quantitative PCR reactions were run on QuantStudio 3 (Applied Biosystems) using Power SYBR Green PCR Master Mix (Applied Biosystems; PIN: 4367659). Expression levels relative to the total expression level of the three essential DNA polymerase genes were computed.
QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses in this work were carried out in R [47]. In the analysis of the correlation between growth rate and mutation rate in Figures 3 and 6, both Pearson’s and Spearman’s correlations were computed with $N = 166$ lines, as recommended [48]. This is because different MA lines from the same environment provide independent mutation rate estimates. The same approach was previously used in MA studies [39]. Generalized linear models were fitted using R.

DATA AND SOFTWARE AVAILABILITY

The accession number for the raw sequencing reads reported in this paper is NCBI BioProject: PRJNA510430.