Review

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Review

Roles of Mutation, Ploidy, and Recombination in Adaptive Evolution in Divergent Model Yeasts *Saccharomyces cerevisiae* and the Human Pathogenic *Cryptococcus*

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Abstract

Genetic variation underlies the capacity of populations to adapt, yet what drives how this variation is generated and maintained in natural populations remains poorly understood. Fundamental processes such as mutation, ploidy, and recombination are known to shape genetic variation and adaptive potential but are typically studied in isolation and under controlled laboratory conditions. How these processes act together under varying environmental conditions to structure genetic variation across complex natural populations remains unresolved. In yeasts, these processes are dependent on reproductive mode, ploidy shifts, and environmental stressors which jointly shape genomic stability and adaptive potential. Here we review our current knowledge on the roles of mutation, ploidy, and recombination in adaptation in the model yeasts *Saccharomyces cerevisiae* and the human pathogenic *Cryptococcus*. We highlight heterogeneity in mutation rates, recombination, and ploidy states across strains, environments, and populations, challenging the assumption that these parameters are uniform. We argue that fluctuating environments, increasingly driven by climate change, are likely to intensify interactions among these processes in ways that remain difficult to predict. Integrating population genomics with ecologically realistic frameworks will be essential for understanding natural evolutionary dynamics and anticipating fungal adaptation and disease emergence.

Keywords: Baker's yeast; cryptococcosis; mutation accumulation; whole-genome sequencing; mitotic recombination; sexual reproduction; haploidy; diploidy

1. Introduction

Evolution is the change in genetic material over time and is a fundamental component of biology. Even before heredity was understood at the molecular level, Darwin introduced life as a dynamic system shaped by descent with modification, fundamentally altering how biological diversity was viewed[1]. Yet it still took until the mid-20th century for Avery, MacLeod, and McCarty to demonstrate that DNA is the hereditary material underlying biological variation which allows evolution to occur[2]. As genetics and molecular biology continue to advance, our appreciation for the complexity of this system as only increased. In 1968, Kimura, followed by King and Jukes, independently proposed the Neutral Theory of Evolution, which argues that most genetic changes are selectively neutral[3,4]. This theory highlights spontaneous mutation and genetic drift as playing central roles in evolution, shifting the framework away from selection as the dominant force. However, though stochastic processes are increasing recognized as key to understanding natural genetic variations, adaptation driven by selection continues to be a fundamental aspect of evolutionary change[5]. Adaptation is the process where a population increases its fitness relative to others in the immediate environment by acting on genetic variations among individuals[5]. Thus, a

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population's capacity for adaptation is directly related to the genetic variation present. This variation is shaped by many biological processes including mutation, recombination and ploidy level.

Yeasts are unicellular fungi. They are phylogenetically diverse and have been found to inhabit a wide range of ecological niches, including those associated with human activities[6–8]. For example, domesticated nearly 9,000 years ago for alcoholic fermentation, the budding or baker's yeast *Saccharomyces cerevisiae* has since become central to baking, brewing, cheese making, and many other applications[9–12]. However, the same adaptive potential also contributes to the evolution of pathogenicity which establishes many yeast species as human pathogens[13–15]. Although uncommon, isolates of *S. cerevisiae* are also observed in clinics [16,17]. Invasive fungal infections caused by yeasts are estimated to contribute to more than one million deaths worldwide annually[18]. Among these, the human pathogenic *Cryptococcus* (HPC), which comprises seven species, can cause cryptococcal meningitis, a leading cause of mortality among HIV/AIDS+ individuals [19,20]. More recently, the emergence of the multidrug-resistant yeast *Candidozyma auris (syn. Candida auris)*, a species first reported in 2009 and its global spread illustrates how yeasts can evolve into critical public health threats[21]. Climate change is expected to amplify these evolutionary pressures by shifting environmental conditions and altering selective pressures, such as temperature and humidity[22]. These shifts are predicted to influence both pathogenic and domesticated species, underscoring the need to understand how yeast populations respond to stress to predict future disease risk and antifungal resistance.

Increased genetic variation in natural pathogen populations can increase adaptive potential, enabling rapid evolutionary responses to host immune defenses and antifungal use. Elevated genetic variation increases the likelihood that drug-resistant or ecologically-adapted genotypes are present prior to infection-treatment intervention, allowing pathogens to quickly respond to treatments or the host immune response[23,24]. This genetic heterogeneity complicates treatment strategies and pathogen surveillance while cryptic and unsampled variation in natural reservoirs reduces the predictability of pathogen evolution. As a result, public health responses which must defend against ongoing and pre-existing adaptive mutations can lag pathogen adaptation. Thus, increased natural genetic variation creates a challenge for management of pathogenic yeast populations. Yet, how genetic variation is increased and maintained across natural reservoirs remain largely unresolved. Additionally, understanding how genetic variation is generated and contributes to adaptation in response to stress is fundamental for our understanding of evolution. Mutation, ploidy and recombination are not independent processes but rather interact with each other to influence genetic variation. The divergent yeasts *S. cerevisiae* and the HPC represent model yeasts from which to investigate the combined impact of these processes on evolution.

Both *S. cerevisiae* and HPC have well-annotated genomes, robust laboratory strains and are recognized as model systems used to study molecular genetics. Each yeast can reproduce both asexually and sexually, are observed to undergo ploidy variation and contain pathogenic and non-pathogenic strains. In addition, *S. cerevisiae* and HPC represent the two largest divergent fungal phyla Ascomycota and Basidiomycota, respectively. They differ in their natural ploidy state and are associated with distinct ecological and anthropological conditions, subjecting them to different selective pressures. These shared and divergent traits make *S. cerevisiae* and HPC strong candidates to compare conserved evolutionary processes while contrasting between key parameters such as natural ploidy state and environmental pressures. Laboratory experiments of *S. cerevisiae* are central for understanding fundamental principles of yeast genetics while HPC provides a system for evaluating these principles in the context of human pathogens.

This review aims to outline the life cycle of *S. cerevisiae* and HPC, discuss the process of mutation, the influence of ploidy levels and the role of recombination in shaping their adaptive potentials. Understanding these mechanisms is critical as we anticipate increased rate of evolution under changing environmental conditions.

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2. Life Cycle

To help understand the three processes to be reviewed in this study, we first outline the life cycles of *S. cerevisiae* and HPC, as these cycles provide the framework for describing when mutations arise and how recombination and ploidy shifts occur. Figure 1 provides a visualize summary of this section. Both yeasts possess two mating types, *MAT***a** and *MAT*α, specified at the mating-type locus (*MAT*)[25,26].

The life cycle of *S. cerevisiae* involves regular alternation between haploid and diploid states, with the diploid state considered the more stable one of the two[26]. In nutrient-rich conditions, both haploid and diploid cells can proliferate mitotically by budding. Under nutrient limitation, diploid mitotic growth will arrest and transition to meiosis producing haploid ascospores. When conducive conditions arise, mating between *MAT*α and *MAT***a** haploid cells will occur, fusing to restore diploidy[26]. Both heterothallic and homothallic strains of *S. cerevisiae* occur in nature. Heterothallic strains produce daughter cells of the same mating type, whereas homothallic strains switch mating type, enabling mating and self-fertilization between recently divided sister cells containing opposite mating types[26].

In contrast, HPC cells are predominantly haploid, with a heterothallic mating system, meaning there is no mating-type switching[25]. The canonical sexual cycle involves fusion between *MAT***a** and *MAT*α cells followed by meiosis, though accumulating evidence indicates that (α–α) unisexual reproduction also occurs in nature[25]. Initiation of the sexual cycle is generally associated with stressful conditions. Diploid cells of *Cryptococcus spp.* are rare and often associated with hybridization between divergent lineages where sequence divergence results in disrupted meiosis[25].

For both yeasts, most reproduction is thought to be clonal, through asexual budding or selfing, with outcrossing being rare[26,27]. However, the relative rates of clonal versus sexual reproduction in natural populations remain largely unknown**.** This mixture of reproductive modes and shift in ploidy creates a complex evolutionary system where sexual reproduction can generate genetically diverse progeny for selection to act, while clonal proliferation then amplifies genotypes with advantageous mutations or allelic combinations. The occasional return to sexual reproduction then allows for recombining genetic materials which can facilitate adaptation and reduce the accumulation of deleterious mutations[28,29]. Taken together, the reproductive strategies of *S. cerevisiae* and HPC shape the timing and frequency of genetic change. Because the life cycle is thought to be dependent on environmental conditions and changing conditions are predicted to increase the ecological range of lineage boundaries, increasing the likelihood of divergent strains interacting, climate change may alter natural rates of sexual reproduction, further contributing to increased genetic variation. To understand how these differences translate into evolutionary potential, the following sections explore the three key processes that generate genetic variation: mutation, ploidy, and recombination.

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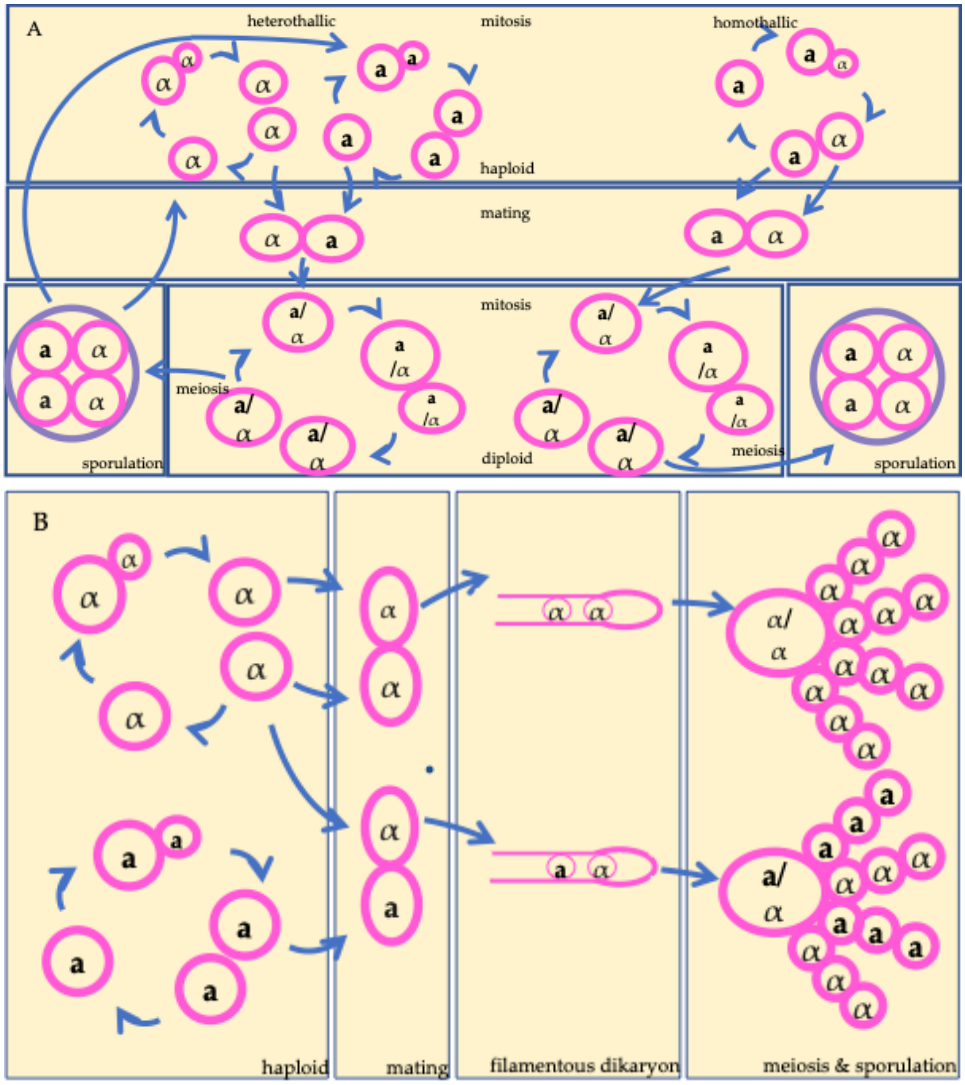

Figure 1. Schematic life cycles of the two model yeasts reviewed in this study. (A) The life cycle of *Saccharomyces cerevisiae* including both heterothallic and homothallic cycles. (B) The life cycle of the human pathogenic *Cryptococcus* representing both a-α and α-α mating.

3. Mutation

Mutations are the ultimate source of genetic variation that enable evolutionary change. Although most mutations are neutral or deleterious, rare beneficial mutations are produced and necessary for adaptation. Mutations include both structural changes in the genome such as deletions, insertions, inversions, translocations, and duplications as well as single nucleotide changes[30]. Mutations may arise spontaneously through imperfect DNA replication or repair, or by exposure to environmental mutagens[30].

Spontaneous mutations arise due to limitations in the efficiency of a cell's DNA replication and repair machinery. Replication fidelity depends on DNA polymerases correctly selecting base pairs and removing incorrect nucleotides during proofreading. In addition, the mismatch repair system detects and corrects helical distortions caused by replication errors, playing a critical role in maintaining genomic integrity[31–33]. Mutations can arise when these repair mechanisms are disrupted which can increase the overall mutation rate[34,35]. Mutated genes that increase mutation rates are referred to as mutator alleles and are often considered detrimental as the increased accumulation of deleterious mutations can have profound effects on the fitness of an organism. However, in large populations, mutator alleles are also thought to accelerate adaptation in a dynamic environment by increasing the rate of novel genetic diversity for selection to act on[36,37]. Mutations

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may also arise through stress-induced mutagenesis, in which exogenous stressors disrupt repair systems. Such stressors include proteotoxic and oxidative stress, chemical agents, temperature shock, and exposure to antifungal drugs[38–41]. However, it is difficult to distinguish whether mutations arise from intrinsic DNA replication and repair fidelity or from environmental stress. Thus, identifying the source can be challenging without comparative analyses across controlled environments that examine changes in mutation rate and mutational spectrum which link these patterns to specific DNA damage or repair pathways.

Mutation rate refers to the number of mutations per site per generation and reflects how frequently genetic changes arise. It is a key parameter in evolutionary biology because it directly influences the rate at which populations accumulate genetic variation [42]. Mutation rates are commonly estimated using laboratory-based experiments, such as Luria–Delbrück fluctuation assays, mutation accumulation experiments, as well as be inferred from patterns of genetic polymorphism in natural populations[33,35,43–45]. Luria–Delbrück fluctuation assays estimate mutation rates based on selectable phenotypes arising in clonally expanding populations subjected to artificial selective pressure at the end. This method captures only mutations that confer a detectable phenotype under selection and reflect a non-neutral subset of mutations[44]. In contrast, mutation accumulation experiments aim to estimate the baseline neutral mutation rate by imposing replicate lines through repeated single-cell bottlenecks, thereby minimizing the efficacy of selection. After many generations, accumulated mutations are identified by comparing evolved genomes to their ancestral state[46]. Although mutation accumulation experiments provide a more direct estimate of spontaneous mutation rates, they are time-intensive and may still underestimate mildly deleterious mutations that are purged over the course of the experiment.

Although informative, the above two laboratory-based estimates do not capture the ecological and demographic complexity of natural populations. As an alternative, mutation rates can be inferred from observed genetic variation in natural populations[45]. These estimates are sensitive to assumptions about selection, demographic history, and effective population size. Events such as population bottlenecks and expansions can strongly bias inferred rates because genetic diversity scales with effective population size. Thus, errors in estimating effective population size or variation in effective population size can further influence mutation rate inference[36]. Consequently, inferred mutation rates typically represent long-term averages and may not reflect short-term environmentally or context-dependent change. As growing empirical evidence challenges the assumption that mutation rate should be treated as a constant parameter, accurately inferring natural mutation rates and understanding the factors shaping their temporal and population-level variability has become important in population genetic studies. It is increasingly recognized that mutation rates vary substantially among species, populations, and even among strains within a species[35,37,47]. Gou et al. (2019) demonstrated variation across seven strains of *S. cerevisiae* ranging from 1.1×10^{-7} to 5.8×10^{-7} [48]. Similarly, Jiang et al. (2021) reported a 10-fold range across 16 haploid, unstressed *S. cerevisiae* strains[35]. In *Cryptococcus spp.* Xu et al. (2001) observed mutation rates ranging across 21 strains from 0.41×10^{-9} to $3{,}135.36 \times 10^{-9}$, with replicates of the same strain varying from 23.52×10^{-9} to 272.55×10^{-9} [49].

Two theoretical frameworks have been proposed to explain this variation: the drift barrier hypothesis and the model of stabilizing selection. The drift barrier hypothesis proposes that, because most mutations are neutral or deleterious, selection acts to minimize mutation rates until further increases in fidelity are outweighed by the energetic cost of maintaining highly accurate replication and repair[37]. Under this model, the lower bound of mutation rate is determined by the strength of genetic drift. Thus, mutation rates are primarily shaped by selection against the accumulation of deleterious mutations, whereas selection for beneficial mutations or the cost for fidelity is assumed to be inconsequential[36,37,50].

Alternatively, the model of stabilizing selection proposes that an optimal mutation rate is determined by balancing multiple selective pressures[51]. Here, mutation rate is jointly influenced by both genotype and environment, and reflects selection acting on deleterious mutations, beneficial

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mutations, and the energetic costs of replication fidelity[51]. Two forms of positive selection can modify mutation rate. First-order selection acts directly on alleles that modify the cost of fidelity through DNA replication and repair mechanisms. And Second-order selection increases mutation rate when mutator alleles hitchhike with linked beneficial mutations[3,51]. If selection favouring an increased mutation rate is strong, the optimal mutation rate may be maintained above the drift barrier [35,51]. Likewise, if the pressure to maintain a high mutation rate is removed and the accumulation of deleterious mutations outweighs the benefit the mutation rate will decrease[51]. The outcome of these opposing forces is highly context-dependent, with environmental variation altering both the costs and benefits associated with mutation rate. Under this framework, it is expected that a higher mutation rate will be maintained under fluctuating environmental conditions and selected for in the presence of stress[51].

Empirical evidence supports the stabilizing selection model in *S. cerevisiae*. Liu et al. (2021) accumulated mutations across replicate lines, observing 19 lines with a significantly higher and 13 lines with a significantly lower mutation rate than the progenitor with some lines reducing mutation rate by 40-50%[51]. Under a strict drift barrier hypothesis this level of reduction should not occur as it suggests the initial mutation rate was maintained above the drift barrier[37]. Researchers calculated *S. cerevisiae's* mutation rate to be more than 3000 times higher than expected under the drift barrier[51]. Liu et al. (2021) further identified *PSP2* as a mutator gene, its knockout nearly halved mutation rate, suggesting the function of *PSP2* contributes to an increased mutation rate[51].

Second-order selection is particularly relevant in organisms that reproduce asexually as mutator alleles can persist without reshuffling from meiosis[52]. Hypermutator strains, defined as strains with significantly increased mutation rates, commonly arise in laboratory evolution experiments[53–55]. These strains are often observed to emerge under stress where they happen to provide a selective advantage by increasing the supply of adaptive mutations. For example, exposure to mildly stressful conditions such as ferulic acid and lithium chloride significantly increase the mutation rate of *S. cerevisiae*[40]. However, it is unclear if this is due to a regulated increase in mutation rate or a result of an advantageous coincidence. Regardless, this pattern is repeatedly observed across laboratory experiments and recently in natural populations[35].

Hypermutators are highly relevant in human pathogenic yeasts. It is hypothesized that hypermutator phenotypes may facilitate colonization of a host by accelerating adaptation to the harsh host environment[32,56]. Laboratory-derived mutants of HPC lacking functional *MSH2*, *MLH1*, or *PMS1* genes exhibit mutation frequencies approximately 200-fold higher than those observed in wild-type strains[32]. Clinical isolates of HPC have been identified with mutations in *MSH2* that result in significantly elevated antifungal resistance rates, exceeding 120-fold increases in certain context[56]. Because stress may drive selection for hypermutator strains, environmental change and antifungal exposure may elevate rates among pathogenic populations, further increasing adaptative potential. Currently, our knowledge of what drives mutator alleles in nature or how long they persist is incomplete. Jiang et al. (2021) recently identified the first natural hypermutator strain of *S. cerevisiae* associated with mosaic beer fermentation revealing a mutation in the *OGG1* that drove the increased mutation rate[35]. Additionally, by analyzing 93 clinical isolates of *S. cerevisiae,* Strope et al. (2015) identified four strains to contain a *MLH1* and *PMS1* allele combination that is engineered in laboratory strains to artificially increase the mutation rate 40 fold above the baseline rate[57,58]. However, direct mutation-rate estimates obtained by Skelly et al. (2017) using fluctuation assays revealed only a 5.6-fold increase in mutation rate in these strains relative to non-mutator backgrounds, substantially lower than expected based on engineered laboratory mutators[59]. Although these clinical isolates did not exhibit mutator phenotypes in the diploid state, Raghavan et al. (2018) generated haploid spore clones from three strains and observed an approximately 340-fold range in mutation rates [60]. These results highlight the relationship between mutation and ploidy state. **Table 1** summarizes various estimates of mutation rates for *S. cerevisiae* and HPC.

Further understanding how hypermutators emerge and persist in natural populations could strengthen our ability to predict the emergence of drug resistance and disease outbreaks, as climate

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change alters temperature, nutrient availability, and host–pathogen interactions in ways that may favour rapid evolution. The evolutionary consequences of mutation greatly depend on ploidy state and rates of recombination reshuffling new variants. Thus, mutation must be considered in the context of these two processes to determine how genetic variation is generated and maintained.

Table 1. Summary of reported mutations rate for *Saccharomyces cerevisiae* and the human pathogenic *Cryptococcus*.

Species	Strain	Experimental design	Ploidy	Reported rate	Rate calculated based on	Meiosis	Growth conditions	Source
S. cerevisiae	FY10	Mutation accumulation	Haploid	3.3×10^{-10}	nucleotide site per generation	No	YPD, 30 °C	Lynch et al. 2008[61]
S. cerevisiae	EAY2531	Mutation accumulation	Diploid	2×10^{-10} - 3.8×10^{-10}	nucleotide site per generation	No	YPD, 30 °C	Nishant et al. 2010[62]
S. cerevisiae	Lab strain	Mutation accumulation	Diploid	1.67×10^{-10}	nucleotide site per generation	No	YPD	Zhu et al. 2014[45]
S. cerevisiae	SEY6211 derivatives	Mutation accumulation	Haploid	4.04×10^{-10}	nucleotide site per generation	No	YPD + 40 mg/L adenine sulfate,30 °C	Sharp et al. 2018[63]
S. cerevisiae	SEY6211 derivatives	Mutation accumulation	Diploid	2.89×10^{-10}	nucleotide site per generation	No	YPD + 40 mg/L adenine sulfate,30 °C	Sharp et al. 2018[63]
S. cerevisiae	S288C × YJM789	Mutation accumulation	Diploid	7.3×10^{-9} – 2.92×10^{-10}	nucleotide site per generation	No	YPD, 30 °C	Dutta et al. 2017[64]
S. cerevisiae	S288C × YJM789	Mutation accumulation	Diploid	9.8×10^{-9}	nucleotide site per generation	No	YPD, 30 °C	Pankajam et al. 2020[65]
S. cerevisiae	S288C x RM11-1a	Mutation accumulation	Diploid	1.7×10^{-9}	nucleotide site per generation	No	YPD, 30 °C	Pankajam et al. 2020[65]
S. cerevisiae	S288C	Mutation accumulation	Diploid	1.35×10^{-10}	nucleotide site per generation	No	YPD, 30 °C	Pankajam et al. 2020[65]
S. cerevisiae	RM11-1a	Mutation accumulation	Diploid	5.4×10^{-9}	nucleotide site per generation	No	YPD, 30 °C	Pankajam et al. 2020[65]
S. cerevisiae	YJM789	Mutation accumulation	Diploid	1.16×10^{-10}	nucleotide site per generation	No	YPD, 30 °C	Pankajam et al. 2020[65]

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S. cerevisiae	GIL104	Fluctuation assay (*URA3* & *CAN1*)	Haploid	3.07×10^{-6}	a-Factor phenotypic resistance	No	Synthetic complete medium, 30 °C	Lang & Murray 2008[43]
S. cerevisiae	GIL104	Fluctuation assay (*URA3* & *CAN1*)	Haploid	1.52×10^{-7}	10x canavanine resistance	No	Synthetic complete medium, 30 °C	Lang & Murray 2008[43]
S. cerevisiae	GIL104	Fluctuation assay (*URA3* & *CAN1*)	Haploid	5.43×10^{-8}	5-FOA phenotypic resistance	No	Synthetic complete medium, 30 °C	Lang & Murray 2008[43]
S. cerevisiae	Natural isolates	Fluctuation assay (*CAN1*)	NA	1.1×10^{-7} - 5.8×10^{-7}	Canavanine phenotypic resistance	No	Synthetic complete medium, 30 °C	Gou et al. 2019[48]
S. cerevisiae	YAS101, YAS106	Fluctuation assay (*CAN1*)	Haploid	9.08×10^{-7}	Canavanine phenotypic resistance	No	YPD, 30 °C	Ohnishi et al. 2004[66]
S. cerevisiae	YAS3001 (YAS101 x YAS106)	Fluctuation assay (*CAN1*)	Diploid	1.03×10^{-4}	Canavanine phenotypic resistance	No	YPD, 30 °C	Ohnishi et al. 2004[66]
S. cerevisiae	GRY2691	Fluctuation assay (*CAN1*)	Haploid	2.8×10^{-8}	Canavanine phenotypic resistance	No	YPD, 30 °C	Rattray et al. 2015[67]
S. cerevisiae	GRY3262	Fluctuation assay (*CAN1*)	Diploid	37×10^{-8}	Canavanine phenotypic resistance	Yes	YPD, 30 °C	Rattray et al. 2015[67]
S. cerevisiae	Natural isolates	Fluctuation assay (*CAN1*)	Haploid	2.1×10^{-7} - 2.1×10^{-6}	Canavanine phenotypic resistance	No	YPD, 30 °C	Jiang et al. 2021[35]
Cryptococcus spp. (*C. neoformans*)	Clinical isolates	Mutation accumulation	Haploid	0.41×10^{-9} to $3{,}135.36 \times 10^{-9}$	Fluconazole phenotypic resistance	No	YEPD+ fluconazole, 37 °C	Xu et al. 2001[49]
Cryptococcus spp. (*C. neoformans*)	JEC50, MCC3	Mutation accumulation	Haploid	3.6×10^{-3} - 2.32×10^{-2}	Filamentation phenotype	No	YEPD, 25 °C	Xu 2002[68]
Cryptococcus spp. (*C. neoformans*)	JEC50, MCC3	Mutation accumulation	Diploid	1.72×10^{-2} - 7.72×10^{-2}	Filamentation phenotype	Yes	YEPD, 25 °C	Xu 2002[68]
Cryptococcus spp. (*C. deneoformans*)	JEC21	Mutation accumulation	Haploid	5.662×10^{-3}	Vegetative growth	Yes	YEPD, 25 °C	Xu 2004[69]
Cryptococcus spp. (*C. deneoformans*)	JEC21	Mutation accumulation	Haploid	5.332×10^{-3}	Vegetative growth	Yes	YEPD, 37 °C	Xu 2004[69]
Cryptococcus spp. (*C. deneoformans*)	JEC20a	Fluctuation assay (*FRR1*)	Haploid	8.59×10^{-8}	Rapamycin+FK506 phenotypic resistance	No	YPD + rapamycin+FK506, 37 °C	Priest et al. 2021[70]
Cryptococcus spp. (*C. gattii*)	134 natural isolates	Polymorphic data	Haploid	1.59×10^{-8} – 2.70×10^{-8}	nucleotide site per generation	NA	Bayesian evolutionary analysis by sampling trees (BEAST)	Roe et al. 2018[71]

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4. Ploidy

Populations of haploid and diploid yeast frequently exhibit distinct evolutionary dynamics in experimental systems. However, factors underlying these differences remain unclear. It's likely that differences in repair pathways, replication fidelity, and selection pressures between ploidy states contribute to this pattern, but their relative contributions remain to be elucidated[63,72,73]. Comparative studies of spontaneous mutation rate across ploidy revealed that diploids do not simply accumulate twice as many mutations as haploids, indicating that ploidy influences the mechanisms of DNA replication and repair as well as the adaptive potential of cells[63]. For example, in one study spanning 51 strains across 33 environmental conditions, the relative fitness advantage of haploids versus diploids depended strongly on the type of stressor[74]. It is hypothesized that recessive deleterious mutations would be masked in diploids. However, accumulated single nucleotide mutations demonstrated a stronger negative effect on diploids than on haploids in mutation accumulation experiments, leading to a reduced average growth rate in diploid lines. An explanation for this pattern could be that selective pressure against mutations could be more uniformly applied or stronger in haploid than in diploids[63]. Haploid *S. cerevisiae* cells accumulate approximately 40% more spontaneous single nucleotide mutations per nucleotide site than genetically identical diploid cells, consistent with enhanced replication fidelity in diploids[63]. Sharp et al. (2018) further demonstrated that the genomic distribution of single nucleotide mutations differs significantly between haploid and diploid *S. cerevisiae* strains, suggesting that ploidy influences both the rate and the spatial pattern of mutations[63].

In contrast to point mutations, structural mutations are substantially more common in diploids than in haploids, with some experiments reporting nearly twice as many whole-chromosome changes per cell division in diploids[63]. Diploid *S. cerevisiae* cells are also expected to undergo approximately one loss-of-heterozygosity event per mitotic division, and in heterozygous diploids these events can rapidly generate homozygosity for advantageous alleles[75]. Similar patterns are observed in HPC, where disrupted meiosis during hybridization frequently results in diploid or aneuploid hybrids[76]. Zhu et al. (2016) identified a third of clinical *S. cerevisiae* isolates analyzed to be aneuploids (>2n) [77]. Aneuploidy in both *S. cerevisiae* and HPC contributes substantial genome plasticity and has been repeatedly associated with adaptive responses. In HPC, aneuploidy has been shown to enhance drug resistance through gene dosage effects mediated by increased chromosome copy number[78]. By shifting ecological ranges which bring previously isolated populations of HPC together, climate change has the potential to increase the likelihood of hybridization. Increased hybridization events can further drive genomic alterations and potentially accelerate evolutionary responses to host immune and antifungal treatments[79,80]. Understanding how ploidy and hybridization operate alongside mutation and recombination will be critical for predicting disease emergence from yeast populations.

5. Recombination

Recombination can occur during both mitotic and meiotic replications. During mitotic replication, recombination contributes to loss of heterozygosity events while during meiosis, sexual recombination can accelerate adaptation by reshuffling genetic material. Meiotic recombination creates novel allele combinations, facilitates the purge of deleterious mutations and increases the pool of genetic variation available for selection to act. Experimental evolution of *S. cerevisiae* has demonstrated that sexual recombination can increase the efficacy of natural selection in adapting populations. However, this advantage is strongly dependent on the environment[81]. If a clonal genotype has optimized fitness in relation to the environment, recombination is not only an energetically costly event, but it can risk decreasing the fitness level[81]. Meiosis alone can elevate the mutation rate by approximately 4–21 times relative to the mitotic rate, additionally expanding the mutational supply[67]. The initiation of sexual reproduction is frequently associated with stressful environmental conditions, such as nutrient depletion, high temperatures, and oxidative stress. These

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conditions likely favour the production of spores for long-term survival while simultaneously generating a pool of diverse genotypes with different fitness spectra across environments through recombination and assortment of genetic material [82].

Genome-wide analyses demonstrate that meiotic recombination hot- and cold-spots shift with temperature which highlight that recombination patterns can be sensitive to the environment[83]. The formation of interspecies hybrids within HPC enables divergent genomes to mix, producing offspring that can display hybrid vigour by producing novel phenotypes which enhance stress tolerance[76,84,85]. These traits can result in a fitness advantage in new or challenging environments, such as within a host or in the presence of antifungal treatments. The increasing prevalence of hybrids in clinical settings suggests that hybridization is an ongoing evolutionary force driven by the enhanced adaptive potential of hybrid genotypes [80,86,87]. For example, a whole-genome analysis of 144 *S. cerevisiae* strains identified three clinical isolates contained genomic regions of two closely related species *Saccharomyces paradoxus* and *Saccharomyces kudriavzevii*[77]. However, accurately estimating recombination rates in natural populations remains challenging. Recombination rates are often heterogeneous across genomes, varying among chromosomes, genomic regions, strains, life stages, and environmental conditions, which complicates efforts to define a single, representative population-level rate[88,89]. In natural populations, recombination is typically inferred indirectly from patterns of linkage disequilibrium or phylogenetic incompatibility, approaches that rely on assumptions such as the infinite sites model[90]. Violations to these assumptions and past demographic events can bias recombination signals[91]. As well, a comprehensive understanding of environmental triggers that initiate recombination is largely unresolved in most species, making it difficult to determine how frequently recombination occurs in natural populations and how it varies across environments.

Although meiotic recombination appears infrequent in natural populations of *S. cerevisiae* and HPC, genomic data provide evidence that it does occur[27,92–94]. Recent analyses have revealed signatures of recombination in a natural population of HPC composed almost exclusively of a single mating type (*MAT*α), indicating that sexual reproduction may occur more frequently than previously expected[95]. The most recent outbreak of cryptococcosis, which primarily impacted immunocompetent individuals, is thought to have resulted from clonal expansion of a genotype derived from sexual reproduction. It is hypothesized that recombination between two lineages produced a genotype able to expand into Vancouver Island as a new ecological niche [96,97]. These strains also demonstrated increased fertility and virulence, illustrating how meiotic recombination can drive the emergence of novel pathogenic strains[98–100]. Additionally, clinical isolates of *S. cerevisiae* demonstrate higher levels of heterozygosity compared to non-clinical isolates, consistent with the importance of outcrossing of the human pathogenic population of this model yeast[75].

In addition to meiotic processes, mitotic recombination which occurs during vegetative growth of diploid or aneuploid cells can contribute to loss of heterozygosity, gene conversion, or chromosomal rearrangements[101,102]. Under stress, these chromosomal changes can fix advantageous alleles and drop deleterious alleles in diploid cells without requiring meiosis, allowing for quick adaptation. This mechanism is often observed in the presence of antifungal and oxidative pressure [76,80,103,104]. Thus, recombination is a powerful evolutionary force generating diversity under stress and enabling rapid adaptation, yet the frequency and drivers of recombination in natural populations remain unresolved for these and other fungi, making it difficult to understand how recombination, mutation and ploidy interact to shape adaptive potential.

6. Future Directions

Even though ample work has been completed to characterize the role of mutation, ploidy and recombination in maintaining genetic variation, many unresolved questions remain. These processes are typically studied independently under controlled laboratory conditions which fail to reflect the true complexity of natural populations. As this review highlights, these processes are interdependent and additionally influenced by life cycle, environment and population history, and the interaction of

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these factors determines a population's ability to adapt. Thus, it is important that future research aims to address how these factors act beyond controlled, single parameter estimates to improve our capacity for predicting how genetic variation is maintained across changing environments in complex natural systems.

Although fundamental to our understanding, dependence on laboratory-derived parameter estimates alone restricts the extent to which current knowledge can be generalized to natural systems. Due to the nature of experimental design, often mutation, ploidy and recombination are tested under a constant environment with fixed environmental parameters. While such designs can provide a sense for how these processes work, they fail to capture the true complexity of the system. In addition, current designs often rely on single-colony transfers that impose relaxed selection through population bottlenecks and are conducted under relatively short timeframes. Even with artificially induced selection, most experiments often investigate a single adaptative trait and are restricted to clonal lineages, simplifying how adaptation is governed under natural conditions.

Considering that both *S. cerevisiae* and HPC only undergo sexual reproduction under specific conditions, which are not fully elucidated in nature, designing experiments which reflect natural life cycle variation remains difficult. If sexual reproduction does not occur within an experimental design, it is unclear if this reflects a biological constraint or if it is due to conditions which do not reflect what occurs in nature. In these species, outcrossing is expected to occur infrequently over a long period and depends on mating type compatibility which may not be fully captured in a typical laboratory experiment. In addition, research often restricts experiments to haploid, clonal lineages with manipulated genetic backgrounds that aim to supress the likelihood of meiosis. For example, most experiments of *S. cerevisiae* are done using haploid strains, although the diploid state is considered more frequent in nature. Ploidy changes can influence both reproductive mode and genetic diversity, yet most experiments aim to limit variation in ploidy state by starting with a defined ploidy background. Ploidy shifts which occur during an experiment are often excluded and treated as experimental noise, generating a biased representation of how variation in ploidy across a population may influence genetic variation.

Additionally, most research is completed using a sub-set of laboratory strains which reflect a similar genetic background. These strains are often optimized for controlled laboratory conditions which decreases pressure for adaptative processes. Long-term culturing of these strains can lead to an accumulation of laboratory-specific traits which do not reflect local adaptation and demographic history of natural strains. Therefore, strains used for experimental design do not represent the true diversity and population structure of natural populations and overlook divergent sub-populations. For example, a natural *S. cerevisiae* population was found to demonstrate a highly elevated rate of C -> A mutations that distinguishes them from traditional lab strains[35]. To address this limitation, environmental sampling of yeast populations across heterogenous ecological niches is required. By doing so, researchers can identify rare genotypes and begin to capture a more realistic representation of genetic variation across natural populations. Additionally, whole-genome sequencing of natural isolates is required to elucidate cryptic variation and detect rare signals of recombination and ploidy changes. Combining whole-genome polymorphic data analysis with comprehensive sampling data and in vitro examination of natural strains will greatly improve our overall understanding of how these processes shape genetic variation.

The role of lateral gene transfer in fungal evolution should also be critically examined further. Although traditionally considered rare in eukaryotes, increasing genomic evidence suggests that horizontal gene transfer may contribute to rapid adaptation in fungal pathogens[105]. Lateral gene transfer complicates inference of mutation, ploidy and recombination because it can lead to signals which bias the interpretation of these processes. Thus, understanding how lateral gene transfer interacts with mutation, recombination, and ploidy will be critical for fully elucidating how genetic variation is maintained across natural populations.

As climate change continues to increase the instability of environmental conditions, it is expected that altering temperature and humidity averages, shifting ecological boundaries and

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increasing overlap among previously isolated populations will continue to drive the adaptation of natural yeast populations, including human pathogens[22,106–109]. This makes it increasingly more important to recognize how mutation, ploidy and recombination work interdependently to maintain genetic variation and drive adaptation to heterogenous environmental conditions.

7. Conclusion

Mutation, ploidy, and recombination are not intrinsically beneficial but have the capacity to increase adaptive potential of organisms in specific environment. In a well-adapted population across a stable environment, increases in mutation rate, recombination, or ploidy shifts can decrease fitness by disrupting genome stability and locally - adapted genotypes. Here, it is expected that selection will favour mechanisms that reduce genetic change. Yet, under stressful conditions these same processes are essential for success by favouring increased genetic variation conducive for adaptation. Pathogen populations often experience dynamic and stressful environments such as host immunity and antimicrobial treatments which likely contribute to maintained genetic variation within hosts. Although controlled laboratory experiments contribute to our understanding of how mutation, ploidy changes and recombination occur, they are insufficient for understanding the complexity of these processes across natural populations. Increasing evidence supports that rates of mutation, recombination and ploidy shifts vary across environments, populations and strains. The variability of these processes increases the sophistication of pathogen adaptation, including traits that influence pathogenicity and antifungal resistance. A comprehensive understanding of how genetic variation is generated and maintained across complex and changing environments is central to combating disease emergence.

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