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Ploidy Variation in Fungi – Polyploidy, Aneuploidy, and Genome Evolution

Robert T. Todd¹, Anja Forche², and Anna Selmecki¹

¹Creighton University, Department of Medical Microbiology and Immunology, 2500 California Plaza, Omaha, NE 68178

²Bowdoin College, 6000 College Station, Brunswick, ME 04011-8451

Abstract

The ability of an organism to replicate and segregate its genome with high fidelity is vital to its survival and for the production of future generations. Errors in either of these steps (replication or segregation) can lead to a change in ploidy or chromosome number. While these drastic genome changes can be detrimental to the organism, resulting in decreased fitness, they can also provide increased fitness during periods of stress. A change in ploidy or chromosome number can fundamentally change how a cell senses and responds to its environment. Here, we discuss current ideas within fungal biology that illuminate how eukaryotic genome size variation can impact the organism at a cellular and evolutionary level. One of the most fascinating observations from the last two decades of research is that some fungi have evolved the ability to tolerate large genome size changes and generate vast genomic heterogeneity without undergoing canonical meiosis.

1. Introduction

Cellular ploidy is the number of complete sets of chromosomes in a cell. Many eukaryotic species have two (diploid) or more than two (polyploid) sets of chromosomes (1). These diploid and polyploid states are often the result of ancient whole genome duplication (WGD) or hybridization events that occurred throughout the evolution of plants, animals, and fungi (2–4). Ploidy changes also occur during the development of many organisms, and can vary within different tissues of the same organism and between individuals of the same species. For example, ploidy changes occur during the sexual cycle of eukaryotes, from haploid gametes to diploid somatic cells. Additionally, some cells continue to increase in ploidy during development, resulting in somatic tissues that have a mixture of diploid and polyploid cells, including human hepatocytes and megakaryocytes (5, 6, 7 F. (1967) *Exp. Cell Res.*). These ongoing, developmentally programmed changes in ploidy are important for viability and provide a beneficial role to many organisms (8), however the mechanisms controlling ploidy and the physiological significance of each ploidy level are not well characterized.

Many clinically relevant fungi undergo ploidy changes during adaptation, especially to adverse or novel environments. Some fungi exist as stable haploid, diploid, or polyploid (e.g. triploid, tetraploid) cells while others change ploidy under certain conditions and revert back to the original ploidy level in other conditions. Aneuploidy, an abnormal chromosome number is also observed in novel environments, during periods of cellular stress, and during

ploidy level changes. An increase in ploidy can occur through mating, endoreduplication, or failure to undergo cytokinesis after replication (described in detail below). Here we refer to events that increase cellular ploidy as WGD, diploidization (e.g. haploid to diploid), or polyploidization (e.g. diploid to tetraploid). A decrease in fungal ploidy can occur through meiosis, reductional division, and random chromosome loss events (9). Here we will discuss only non-meiotic ploidy decreases, and refer to these events as whole genome reduction events or haploidization events (e.g. diploid to haploid). The examples we provide here challenge the textbook definition of somatic ploidy as a consistent or defining trait of a fungal species. Instead, fungal ploidy is often context dependent and can rapidly change from one environment to the next.

Ploidy values currently assigned to fungal species may be influenced by laboratory growth conditions and selection for traits that prove to be beneficial for conducting genetic manipulations. However, as whole genome sequencing and molecular assays for ploidy detection become standard in fungal research labs, the identification of ploidy variants is increasing. For example, polyploid strains of the typically haploid and/or diploid species *Saccharomyces cerevisiae*, *Candida albicans*, and *Cryptococcus neoformans* exist in diverse environments including desert canyons, fermentation and industrial cultures, and from human patients before and after therapeutic treatment with antifungal drugs (10–16). This suggests that many different ploidy-environment interactions may select cells with increased or decreased ploidy that may provide these cells with an adaptive advantage (1, 17, 18). A mechanistic understanding of what drives environment-dependent ploidy changes remains to be discovered. Genome sequencing and phenotypic characterization of mutations in these comparatively small eukaryotic genomes (e.g. compared to human genomes) will likely place fungi at the forefront of ploidy research (19–21).

Molecular Detection of Ploidy and Aneuploidy

Ploidy is most commonly measured by flow cytometry of fluorescently labeled cells (e.g. propidium iodide) where the relative fluorescence of an unknown isolate is compared to strains of known ploidy (22). More than 30,000 single cells can be analyzed within seconds and linear increases in ploidy are detected with great accuracy (Figure 1A). Aneuploid isolates are detectable by flow-cytometry with the caveat that isolates with a single aneuploid chromosome may not be significantly different in the fluorescent signal compared to the known ploidy control. In addition, the fluorescent signal of isolates with multiple aneuploidies (e.g. gain of one and loss of another chromosome) may not show different DNA fluorescence by flow cytometry because these specific aneuploidies cancel each other out. Instead, more quantitative methods must be used to identify the specific aneuploid chromosomes. Flow cytometry coupled with additional molecular methods such as comparative genome hybridization (aCGH), quantitative PCR (qPCR), and double-digest restriction site-associated DNA sequencing (ddRADseq) is the most comprehensive approach to detect ploidy changes and identify specific aneuploidies (23–25).

The ploidy level and chromosome copy number of isolates can also be determined using whole genome sequencing (WGS). The copy number of each chromosome is determined relative to the entire genome based on the number (depth) of aligned sequence reads.

Aneuploidy is detected as an increase or decrease in read depth relative to the entire genome (Figure 1B). Segmental chromosome aneuploidies and small gene amplifications/deletions are also detected. Additionally, WGS bioinformatics tools use allele frequencies to determine the baseline ploidy of the sequenced genome (16, 26). For example, a haploid (1N) genome will have allele frequencies at 1, a diploid (2N) genome will have allele frequencies at 0.5 and 1, while a triploid (3N) genome has allele frequencies at 0.33, 0.67, and 1, and a tetraploid (4N) genome has allele frequencies at 0.25, 0.5, 0.75, and 1 (Figure 1C and D). While limited to strains with significant heterozygosity, WGS simultaneously detects cellular ploidy level, chromosome copy number, and sequence polymorphisms. Further studies are needed to truly understand the extent of ploidy variation in natural populations within and between species.

Ploidy variation in natural isolates

We first highlight three examples of ploidy variation found in isolates of *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, and *Candida albicans*. These three species have different genome sizes, haploid chromosome numbers, sexual cycles, and preferred base ploidy levels. Despite these differences, all three fungal species have been isolated from natural ecosystems and/or human and animal hosts with ploidies that range from haploid to polyploid. Furthermore, all three species can undergo somatic ploidy changes under laboratory conditions.

S. cerevisiae—The budding yeast *S. cerevisiae* contains 16 haploid chromosomes and reproduces by mating and meiosis or asexually via budding. Environmental isolates of *S. cerevisiae* include haploids, diploids, and polyploids (12, 27–30). Clinical and industrial isolates also show a wide range of ploidies and aneuploidy (16, 29). For example, whole genome sequencing of 145 *S. cerevisiae* clinical isolates found that 34% were triploid or tetraploid and 36% of them were aneuploid (16). Many of these ploidy changes are thought to be adaptive, however the correlation between environmental selection and ploidy changes is not well characterized (17).

C. neoformans—Naturally found in diverse environmental niches, *Cryptococcus neoformans* is also responsible for pulmonary infections as well as cryptococcal meningoencephalitis in immunocompromised individuals (31, 32). Basidiospores are thought to be the infectious propagules, which are inhaled into the lungs followed by dissemination into other organs (33). *C. neoformans* is normally found in the haploid state with 14 chromosomes, reproduces both sexually and asexually, and these haploid cells can vary in chromosome copy number due to non-disjunction events (34) or due to unisexual or bisexual reproduction (35). Dramatic ploidy changes have been observed during infection. Polyploid ‘titan’ cells that range from 4N to > 64N, make up ~ 20% of the infectious population within the host tissue (36, 37). In conjunction with the ploidy increase, cell size, capsule structure, and cell wall structure are also modified. A haploid *C. neoformans* cell is generally 5 – 10 µm in diameter; ‘titan’ cells can be much bigger with some reaching upwards of 50 – 100 µm in diameter (38, 39). Very little is known about the mechanism of titan cell formation; it may involve endoreplication due to alteration of cyclin proteins as is observed in *Drosophila melanogaster* and human hepatocytes (40, 41). Aneuploidy, in

particular the amplification of chromosome 1, has also been observed in response to antifungal drug stress (42, 43).

C. albicans—The most common human fungal pathogen, *C. albicans*, contains 8 homologous chromosome pairs. Previously considered an obligate diploid organism (44, 45), alternative ploidy states have been described including haploid, triploid, and tetraploid cells (10, 15, 46–48). No meiosis has been observed in *C. albicans*. Rather, it undergoes a parasexual cycle in which cells of opposite mating type fuse to form tetraploids (49–53). Under nutrient starvation, tetraploid cells show an increase in genome instability that leads to the loss of individual chromosomes over time returning progeny cells to either diploidy or (more often) near-diploidy (9, 50, 52, 54). Ploidy changes also occur in response to specific environmental conditions. For example, growth on alternative carbon sources (e.g. L-sorbose), exposure to antifungals (e.g. fluconazole), high temperature, and interactions with the host all result in ploidy changes and aneuploidy within a few cell divisions (15, 55–62). The molecular mechanisms driving these ploidy changes are currently unknown.

Other fungi with altered ploidy levels and evidence of aneuploidy

Aneuploidy has been found in many other fungi. *Ashbya gossypii*, a filamentous fungus, has a single syncytia with multiple nuclei, and each nucleus can have a different ploidy (63). In *A. gossypii*, ploidy increases with age while stress exposure can shift a population with high ploidy variation towards a more homogenous, haploid population (63). Ploidy level variation and aneuploidy are also common in isolates of the amphibian chytrid pathogen, *Batrachochytrium dendrobatidis* (*B.d.*) (64), and in plant pathogens such as the fungal pathogen *Fusarium oxysporum* (65, 66). In fact, karyotype variability in *B.d.* seems to be the norm rather than the exception; out of 22 isolates analyzed by whole genome sequencing, 18 were aneuploid with base ploidies ranging from diploid to tetraploid (64). While these are only a few examples of ploidy changes that are known to occur in the fungal kingdom, they highlight that ploidy changes play a central role in adaptive evolution and genomic diversity.

2. Ploidy changes in the context of laboratory mutants: towards a mechanism of asexual ploidy change

The underlying mechanisms that drive ploidy changes are not completely understood (12). Some genes encoding ploidy regulators have been identified in yeast deletion mutant screens and gene overexpression studies (67). However, in many mutants the ploidy-altering phenotype is not 100% penetrant (see below), indicating that there are redundant mechanisms that regulate genome copy number. Alternatively, the mutant genotype may stochastically acquire fitness-associated ploidy changes in which case the ploidy change would be secondary to the initial gene mutation.

Here we will discuss mutations that affect cell cycle, spindle pole body, kinetochore attachment, cohesion, chromatin formation, and cytokinesis, focusing on their impact on ploidy changes. Many mutations that affect chromosomal instability (CIN) can also cause ploidy increases or decreases. Importantly, because chromosome aneuploidy is frequently observed in mutants that undergo ploidy change, it is difficult to determine if aneuploidy

itself is driving whole genome ploidy changes. For example, a mutation may cause aneuploidy, which then gives rise to a whole genome ploidy change. Alternatively, this mutation may first induce a whole genome ploidy change, and subsequent aneuploidy results from increased genome instability. The examples provided below support that the mechanisms driving changes in whole genome ploidy and chromosome copy number are extremely complex and often involve the essential machinery of the cell.

Mutations underlying ploidy amplification

Alterations in cell cycle control can cause ploidy amplification. Endoreplication, the process in which DNA replication is not followed by cytokinesis, but instead by another round of DNA replication, has been described in multiple organisms (68–70). In the fission yeast, *Schizosaccharomyces pombe*, Cyclin B (p53^{cdc-13}) regulates the temporal order of DNA replication and mitosis. Control of Cyclin B levels and the timing of this cell cycle regulator is important; formation of the p34^{cdc-2}-p53^{cdc-13} complex specifies that the cell is in the G2 phase of the cell cycle. Loss of this complex will re-define a cell in the G2 phase to a G1 phase cell, and the cells can then reenter S-phase and re-replicate their genomes causing unscheduled whole genome duplication. Reentry into S-phase can occur multiple times and can lead to ploidy shifts from 1N to 32N (71). Interestingly, 42 uncharacterized genes were recently identified in human cells for their role in preventing endoreplication (72), supporting that DNA replication controls are still being discovered.

Under certain circumstances endoreplication is an environment-induced or programmed cell event. For example, environment-induced endoreplication likely leads to ‘titan’ cell formation in *C. neoformans* (36, 38, 39). The exact mechanism of cell cycle alteration is not known for ‘titan’ cell production, but like in *S. pombe*, the control of cyclin B level is a potential candidate. In the human liver, hepatocytes undergo genome replication followed by programmed cytokinesis failure to produce a bi-nucleate daughter cell that is 4N (73). These bi-nucleate tetraploids can then undergo another round of DNA replication followed by cytokinesis to generate mono-nucleate tetraploid cells. This cycle can continue to produce octaploid cells and so on. In addition, these polyploid hepatocytes can undergo mitosis with multipolar spindles, resulting in ploidy reduction and aneuploidy (8). It has been hypothesized that these aneuploid daughter cells may provide adaptive benefits during periods of cellular stress and allow for repopulation and restoration of the liver (73–75).

Accurate spindle pole body (SPB) function and attachment of the spindle microtubules to the chromosomes is required for proper chromosome segregation, and ploidy changes can occur when different components of the SPB complex are altered. During normal cell division, sister chromatids are attached to opposite SPBs and are pulled apart, thereby segregating the sister chromatids. *NDC1* (nuclear division cycle 1) encodes a subunit of the nuclear pore complex in *S. cerevisiae*, and is required for SPB duplication and insertion into the nuclear membrane (76). Mutant *ndc1* cells have only a single functioning SPB to which all chromosomes attach and are then segregated into a single daughter cell, resulting in whole genome duplication.

Proper attachment of the SPB microtubules to the kinetochore is a major point of regulation during cell cycle progression. In *S. cerevisiae*, mutations in the essential gene *IPL1*

(increased ploidy level 1) can result in aneuploidy and/or elevated ploidy level. *IPL1* encodes Aurora kinase, which is involved in the attachment of the spindle microtubules to the kinetochores, chromosome segregation, and checkpoints including mitotic spindle dis/assembly and DNA damage (77–79). Ipl1 is also responsible for sensing mitotic spindle attachment at the kinetochore and preventing segregation in cases where the chromosome is attached to only one SPB. Using a temperature sensitive mutant of *IPL1*, Chan & Botstein (1993) observed that haploid cells rapidly acquire multiple aneuploid chromosomes when grown at the restrictive temperature (67), and some of these mutants gain enough chromosomes to result in a ploidy increase.

In addition to SPB subunits, ploidy is affected by defects in chromosome cohesion. After replication, sister chromatids are packaged together by cohesion, a protein complex that holds the sister chromatids together until they are separated during anaphase (80). Improper loading or disassembly of the cohesion complex during the cell cycle leads to aberrant chromosome segregation that can lead to ploidy shifts and aneuploidy (81). Defects in the cohesion complex, as well as regulators of the complex, lead to release of sister chromatids before proper attachment to the SPB. This increases the chance that sister chromatids will be inherited together because the sensing of microtubule attachment does not occur and segregation of sister chromatids is no longer inter-dependent (81).

Chromatin structure and regulation are important regulators of ploidy. The dynamics of chromatin structure is determined by histone conformation and modifications. Mutations within the globular domain of histone 4 (H4) result in heterogeneous colony sizes and an increased frequency of whole genome duplication and aneuploidy in *S. cerevisiae* (82). Further analysis of these colonies shows that small colonies consist of a mixed population of haploid and diploid cells, while large colonies are completely diploid, suggesting extensive autodiploidization. Alterations of the amino acids in the globular domain of H4 (L97, Y98, or G99 to alanine) do not alter the ability of H4 to interact with other histones. Instead, these mutations alter the H4 interaction with the histone chaperones Rtt106 and Caf-I. Disruption of these interactions prevents the nucleosome from being loaded onto the DNA. This decrease in histone occupancy could disrupt kinetochore architecture and assembly, leading to the increase in aberrant chromosome segregation and autodiploidization (82).

Just as histone modification can affect genome stability, nucleosome stability at centromeres is necessary for kinetochore assembly and proper chromosome segregation. Nucleosome stability is achieved through proper distribution of histone variants. The histone variant H2A.Z is conserved across all fungi and higher eukaryotes and is enriched at pericentric DNA, but excluded from CENP-A (Cse4) nucleosome binding sites (83). Ies6 is an essential subunit of the INO80 chromatin-remodeling complex and loss of Ies6 results in polyploidization (84). Loss of Ies6 function leads to increased pericentric H2A.Z localization, resulting in changes to chromatin structure that inhibits centromere/kinetochore function. Furthermore, overexpressing H2A.Z in an *ies6* mutant strain further increases chromosome instability leading to more rapid polyploidization (84).

After genome replication and chromosome segregation, the cell undergoes a cleavage event controlled by the contraction of an actin ring found between the mother and daughter cell.

Myo1, the sole myosin II motor in *S. cerevisiae*, associates with the actin ring and promotes cleavage between the mother and daughter cell (85). Haploid strains with a *myo1* deletion often undergo whole genome duplication events in order to survive, causing ploidy level increases from 1N to 4N (86). Many of the polyploid *myo1* evolved clones are mononucleate and contain multiple aneuploid chromosomes. These polyploid *myo1* evolved clones are not only viable, but have restored cytokinesis through a variety of mechanisms, unlike most of the *myo1* cells that remained haploid. Therefore, the mutations that disrupt cell division and alter ploidy level can provide increased adaptability (86).

Mutations underlying ploidy reduction

Several *S. cerevisiae* mutantions have been identified that lead to genome reduction from diploid to haploid. For example, diploid cells with a null mutation in *RAD52* (involved in strand exchange during recombination and DNA damage repair), undergo chromosome loss via sequential aneuploid transitions (87). Loss of Rad52 leads to a failure of the repair mechanism that keeps the chromosome homologs together after a double strand break (DSB). Functional loss of this pathway gradually leads to genome reduction towards haploidy over ~500 generations. Interestingly, cells with higher ploidy show increased Rad52 dependency because *RAD52* is essential for growth of tetraploid cells but not isogenic diploid cells (88). This supports that increasing ploidy results in an increased frequency of DSBs that must be repaired by Rad52, and diploids, but not tetraploids, can resolve some of this damage by sequential chromosome loss and eventual ploidy reduction.

In addition to mutations in *RAD52*, null mutations in *CTF18* (involved in sister chromatid cohesion) also lead to rapid genome reduction from diploidy to haploidy (89). This reduction in ploidy occurs over a relatively short amount of time (~50 generations), and suggests that the haploid cells have a fitness advantage over the diploid progenitor. Surprisingly, genome duplications are also observed within the diploid *ctf18* population, resulting in a heterogeneous population (haploid, diploid, aneuploid, and polyploid cells). This suggests that specific mechanisms of genome instability may simultaneously induce aneuploidy, ploidy loss, and ploidy gain.

3. Impact of ploidy level on DNA damage repair

At all ploidy levels, DSBs are repaired by two main pathways, non-homologous end-joining (NHEJ) and homologous recombination (HR). NHEJ repairs DSBs via ligation of the broken DNA ends with little or no processing of the DNA ends. NHEJ is considered error-prone because this process can introduce novel mutations. Alternatively, HR uses a homologous DNA sequence to serve as a donor for recombination and DSB repair (90, 91). The availability of homologous DNA sequences (sister chromatid, homologous chromosome, or an ectopic sequence) is influenced by the ploidy of the cell and phase of the cell cycle: haploid cells have homologous DNA sequences available during S/G2 only, while diploid and polyploid cells have homologous DNA sequences available throughout the cell cycle. Therefore diploid and polyploid cells have an increased preference for HR to repair DSBs resulting in increased frequencies of recombination, gene conversion, cross over events, and gross chromosomal rearrangements (92–94).

Isogenic yeast strains with different ploidies exhibit different mutation rates and sensitivity to DSBs. The forward mutation rate at either the *CAN1* or *URA3* locus is two orders of magnitude higher in diploids than in haploids, and diploids are less sensitive to DSBs than haploids as well (88, 89, 95). Interestingly, the forward mutation rate at *CAN1* is lower in tetraploids compared to diploids (88). Ploidy also affects sensitivity to DSBs: both haploids and tetraploids are more sensitive to DSBs than diploids. One reason for these observations may be the different requirements for DSB repair at different ploidy levels (94).

Ploidy-specific genome maintenance mechanisms may exist, but many questions still remain (94). For example, in *S. cerevisiae* there are ploidy-specific stress responses to gross chromosomal rearrangements (96), and tetraploid yeast also have a greater requirement for genes involved in recombination and mitosis than haploids or diploids (88). This phenomenon, called “ploidy-specific lethality”, was used to identify the physiological alterations that accompany tetraploidy. Of the 3740 deletion mutants screened, only 39 genes exhibited ploidy-specific lethality. Almost all of these mutations affect genomic stability by impairing homologous recombination, sister chromatid cohesion, or mitotic spindle function (88). Why tetraploid cells have an increased requirement for these genes remains unknown (97). The rate and spectrum of mutations available to each ploidy level is remarkably different, therefore genome maintenance mechanisms could play an important role during adaptation.

4. Genome Instability in Polyploid Cells

One of the most striking features of polyploid cells is their increased genome instability relative to diploid cells. This phenomenon is seen across many eukaryotic species including many plant species (98–101). In fungi, polyploid cells frequently give rise to aneuploid progeny (both whole chromosome and segmental), chromosome rearrangements, and translocations. For example, tetraploid *S. cerevisiae* cells have a 200–1000 fold increase in the rate of chromosome loss compared to isogenic diploid cells (88, 102, 103). Similarly, tetraploid *C. albicans* have a 50-fold higher rate of loss of heterozygosity (LOH) compared to isogenic diploid cells (54). Increased genome instability often results in rapid ploidy reduction during *in vitro* growth of most polyploid populations (9, 19, 20, 43, 54, 104), yielding progeny with high karyotypic diversity. Here we highlight examples for *C. neoformans*, *C. albicans*, and *S. cerevisiae*.

The dramatic ploidy reduction of *C. neoformans* ‘titan’ cells was recently analyzed by microdissection of sequential daughter cells from a polyploid titan cell followed by colony formation assays and WGS (43). In a replete environment, polyploid ‘titan’ cells produced true haploid daughter cells. In contrast, when exposed to fluconazole, ‘titan’ cells frequently generated aneuploid haploid and aneuploid diploid daughter cells (43). Furthermore, daughter cells from the same titan cell parent had diverse aneuploid karyotypes, while point mutations were rarely observed. It remains to be tested whether this aneuploidy is due to an effect of fluconazole on mitotic fidelity (as was seen in *C. albicans* (62)) or is the result of a general stress response.

A foundational study of *C. albicans* tetraploid cells found that they undergo a rapid, non-meiotic genome reduction in response to nutrient starvation, termed “concerted chromosome loss” (9). Genetic analyses of many progeny by microarray (single nucleotide polymorphism (SNP) and comparative genome hybridization (aCGH) arrays) found that only a very small fraction returned to a true euploid diploid state, and the majority of the progeny was aneuploid for one or more chromosomes. In addition, recombination, albeit limited to a few progeny, occurred between heterozygous loci on homologous chromosomes during tetraploid genome reduction (52). The appearance of multiple gene conversion tracts within several strains, and the general absence of gene conversion tracts in other strains, suggests that some cells become generally competent for recombination at more than one locus, while other strains do not undergo such recombination events at all. Importantly, these recombination events were dependent on *SPO11*, a conserved protein required for the introduction of double strand breaks (52). These findings suggest that at least one meiosis-specific gene has been re-programmed to mediate genetic recombination during the alternative parasexual life cycle of *C. albicans*.

In a more recent study, populations of tetraploid, triploid and diploid *C. albicans* strains were grown for 28 days in YPD and chromosome loss dynamics were analyzed at different time points using flow cytometry and ddRADseq. Initially, the tetraploid-evolved clones were highly aneuploid, and once the cells became aneuploid further ploidy reduction accelerated with aneuploid strains changing ploidy faster than tetraploid strains (54). Eventually, the tetraploid and highly aneuploid evolved cells converged to stable euploid levels: diploid, triploid and (rarely) tetraploid. In addition, this study showed that chromosome loss was random; ploidy reduction in biological replicates of the same polyploid strain rarely followed the same chromosome loss trajectory.

Similar non-meiotic ploidy reductions have been observed in tetraploid populations of *S. cerevisiae* during *in vitro* evolution (19). In this study, tetraploid *S. cerevisiae* strains were passaged for ~1800 generations in rich or high salt medium, and genome reduction at the population level was detected with flow cytometry after ~186 generations. Results from this study support that ploidy loss occurs as if selection is acting on multiple chromosome loss events simultaneously rather than on individual, sequential chromosome loss events (105). In addition, these *in vitro* evolution experiments found that ploidy loss in a tetraploid population often paused at near-euploid levels, from tetraploid to near-triploid to near-diploid (105). Furthermore, aneuploidies (detected by aCGH) present in the tetraploid progenitor were often found to be the only aneuploidy that remained in the near-diploid evolved population (19). Thus, while the mechanism of tetraploid genome reduction is unknown, the rapid ploidy loss observed in these environments supports that multiple chromosomes can be lost simultaneously.

The ploidy reduction process of *S. cerevisiae* tetraploid cells was further analyzed with whole genome sequencing of strains from many parallel *in vitro* evolution experiments (20). The ploidy of tetraploid-evolved clones was determined by flow cytometry and the copy number of every chromosome was determined by whole genome sequencing. It was found that aneuploidy is more extensive during the tetraploid to diploid transition than previously appreciated. In raffinose medium, ploidy reduction did not occur by loss of a full set of

chromosomes; rather, many of the tetraploid evolved cells were highly aneuploid, including individual chromosome copy numbers that ranged from 4C to 2C in the same cell (Figure 2). This indicates that while some chromosomes are lost multiple times, others are maintained at the original tetrasomic level (e.g. 4C, 3C, and 2C). Interestingly, specific chromosome pairs were more frequently lost together and underwent multiple loss events independent of other chromosome pairs (20). These copy number differences are likely a result of the environmental selection for particular karyotypes with increased fitness; indeed the most common aneuploid chromosome in this study, amplification of chromosome XIII, was found to provide a significant fitness benefit to tetraploid cells in raffinose medium (20).

These studies in *C. albicans* and *S. cerevisiae* show that tetraploid-evolved clones can be highly aneuploid with many evolved isolates reaching a near-euploid state (near-triploid or near-diploid). The rate of chromosome loss is largely dependent on the growth environment and generation time, as well as the frequency of sampling throughout the experiment. In general, the random chromosome loss that occurs during *in vitro* culturing of tetraploid *C. albicans* and *S. cerevisiae* populations appears mechanistically distinct from the haploid bud produced from *C. neoformans* ‘titan’ cells. Future studies in these and other fungi may identify unique mechanisms of chromosome and genome stability and aneuploid tolerance in polyploid cells.

Given that ploidy reductions can occur over short evolutionary timescales, it is puzzling that polyploid cells are observed at all. One hypothesis is that polyploidy is simply an intermediate state to which a cell shifts for survival, for example after cytokinesis failure due to cell wall stress (86, 106). However, another hypothesis is that the polyploid state is beneficial in specific environments and is selected upon due to increased fitness. Indeed, tetraploid *S. cerevisiae* strains passaged *in vitro* for ~1000 generations at 23°C maintain stable euploid tetraploid genomes (107). These evolved tetraploid cells are resistant to benomyl, a microtubule depolymerizing drug that tetraploid cells are more sensitive to than diploids (88). The stable tetraploids have increased levels of Sch9, a kinase involved in protein homeostasis, G1 cell cycle progression, nutrient signaling and stress response, suggesting that newly formed tetraploid cells can acquire genome stability through improved cell growth processes (107).

5. Impact of ploidy on fungal physiology

Alterations in genome architecture have wide reaching effects on many cellular processes. How ploidy shifts change cellular physiology is a long-standing question. A ploidy increase is associated with enlarged cell size in all eukaryotes (38, 39, 108–111). However, we lack a mechanistic understanding of how genome size controls cell size (112). Research that has compared growth rate, gene expression patterns, and cell surface area to volume ratios between haploid and diploid, or diploid and polyploid cells has provided insight into the complex, sometimes subtle, differences between isogenic fungal strains of different ploidy. Here we highlight how ploidy affects the physiological properties of cell size and gene expression.

The reduction in cell surface area to volume ratio of polyploid cells likely impacts nutrient transport, signaling pathway components, and ultimately growth rate in some environments. Upon genome duplication, polyploid cells undergo a 2-fold increase in cell volume, but only a 1.57-fold increase in cell surface area relative to their diploid progenitors (88, 113, 114). This reduced surface area in polyploid cells is expected to cause growth disadvantages in environments where nutrients are absorbed across the membrane. However, there is little evidence to support this cell surface area-to-volume ratio theory in polyploid cells (115). A robust analysis of this theory, performed with 51 different haploid and diploid *S. cerevisiae* and *S. paradoxus* strains in 33 different growth conditions, found that ploidy accounts for a majority (70%) of the differences in growth rate between strains (17). However, the results were inconsistent with the surface area-to-volume model. Rather, it appears that the preference for the haploid versus diploid state is due to condition-specific effects in the strains tested, not due to differences in surface area (17). Thus, more research is needed to understand the physiological changes that occur after polyploidization and what effect the environment has on fitness and genome stability. Furthermore, identifying transcriptional changes that occur in other environments, including nutrient-limitation, will provide important clues to the growth differences polyploids experience in these environments.

Gene expression studies have been used to identify molecular signatures that are unique to polyploid cells. The first gene expression microarray study on polyploid *S. cerevisiae* cells identified only a few genes with significantly different levels of expression in isogenic haploid and tetraploid cells when grown in rich medium (114). For example, tetraploid cells had reduced expression of genes encoding G₁ cyclins (*CLN1* and *PCL1*) and proteins involved in cytoskeletal organization (e.g. *GIC2*). In another study, a different *S. cerevisiae* strain (S288C instead of Σ 1278b) was analyzed under the same growth conditions and no genes had significantly different levels of expression in isogenic diploid and tetraploid cells (88). This suggests that either there are more transcriptional differences between haploids and tetraploids compared to diploids and tetraploids, or there are strain-specific ploidy differences (116).

Using RNAseq analysis, Wu *et al.* identified 65 transcripts with differential expression in isogenic haploid and tetraploid *S. cerevisiae* cells, again in rich medium (109). A majority of these genes encode cell surface proteins, which suggests that the enlarged cell size of tetraploids causes the differential regulation of these genes, and not ploidy *per se*. Indeed, it was found that haploid mutants with increased cell size show differential expression for many of the same genes as tetraploids, and the degree of changes in expression correlated with the degree of size increase (109). Importantly, gene expression of most of these cell surface proteins is repressed in tetraploid cells (and enlarged haploids) likely due to the reduced surface area relative to cell volume (88, 113, 114). These observations are consistent with proteomic studies that find reduced amounts of cell surface proteins in diploids compared to haploids (117).

6. Ploidy changes and aneuploidy in the context of experimental evolution

Ploidy changes and aneuploidy occur during *in vitro* evolution and provide a fitness benefit

In vitro evolution experiments have provided the strongest evidence that fungi can undergo asexual ploidy changes during adaptive evolution. The environmental cues driving these genome changes are not known and a comprehensive study of environments that induce ploidy changes (whole genome duplication and ploidy reduction) is lacking (Table 1). In this section, we will examine the different environments in which ploidy changes and acquisition of aneuploidy are documented in fungi, and how these genome size changes affect organismal fitness and adaptation.

We start by describing the studies in which whole genome ploidy changes are observed and end with several studies in which chromosome copy number changes are observed *in vitro*. Many of these examples describe changes in ploidy that occur at the population level. If beneficial (e.g. increasing fitness), ploidy or chromosome copy number changes are selected for within the population. The exact order of events or mechanisms that drive the initial ploidy change is unknown.

Results from multiple studies show that in *S. cerevisiae*, whole ploidy level changes causing diploidization are advantageous in multiple environments. For example, isogenic haploid, diploid, and tetraploid *S. cerevisiae* populations passaged in rich medium, medium containing high salt, or increased concentrations of ethanol all converge towards diploidy (19, 105, 118). The diploidized cells have significantly higher fitness than the isogenic progenitor strains in competition experiments (118). Furthermore, the rate of ploidy change is dependent upon the specific growth conditions. For example, in high salt medium, the rate of diploidization of haploids was faster than the rate of diploidization of isogenic tetraploids (19). Therefore, becoming diploid under these growth conditions is one of the most common and accessible routes to increase fitness.

Frequent diploidization of haploid cells also occurs during *in vitro* evolution experiments under glucose depletion (21, 119). In this unique study, the evolving populations consisted of ~500,000 uniquely barcoded haploid *S. cerevisiae* lineages. The autodiploidized clones had a strong fitness benefit relative to their haploid progenitor and WGS revealed that in most cases diploidy was the only adaptive mutation (21). The autodiploid lineages were traced back to their respective founding populations, suggesting that the transformation conditions (LiAc & heat shock) used for barcoding led to the formation of autodiploids (21). Interestingly, transformation conditions also induce ploidy loss, aneuploidy, and LOH in *C. albicans* (55, 120).

Convergence towards diploidy is also observed in *C. albicans*. Tetraploid mating products and rare haploid isolates return to the diploid state after *in vitro* passage (9, 47, 54). The convergence towards diploidy occurs under different growth conditions, but ultimately depends upon the environment and initial genotype, not necessarily the fitness benefit of the acquired ploidy change (121). For example during phosphorus depletion, the autodiploidization rate of haploids was reduced, while the diploidization rate of tetraploids

was increased. Further studies are needed to uncover the factors that drive organisms to a 'baseline' ploidy.

In contrast to the convergence towards diploidy observed in many environments, a wide array of genome changes can occur under a single growth condition. For example, growth in the fungistatic drug fluconazole often results in a wide variety of ploidies and aneuploid changes in *C. albicans* and *C. neoformans* (35, 43, 46, 59, 61, 62, 122–124), and just 8–12 hours of drug exposure results in both ploidy reduction and ploidy amplification in *C. albicans* (62, 123). Fluconazole is known to alter membrane fluidity, which causes abnormal cytokinesis and cell cycle defects leading to polyploid and aneuploid formation (62). The immediate fitness effect of these ploidy level mutations is not known, but it can be inferred by measuring how rapidly mutant cells accumulate within a population. For example, during *in vitro* evolution of *C. albicans* in the presence of fluconazole, acquisition of multiple aneuploid chromosomes is detected after just ~3.3 generations in the majority of cells in the population (61). One of these aneuploidies, isochromosome 5L (i(5L)), confers fluconazole resistance due to the amplification of two genes, *ERG11* and *TAC1* (13, 59). Due to the high fitness benefit, i(5L) accumulates rapidly in the population, and in many cases is maintained over ~330 generations in the presence of fluconazole (61).

Many additional examples support that acquisition of aneuploid chromosomes can lead to rapid adaptation to specific environments, and like ploidy changes these aneuploid chromosomes are reversible. For example, growth on alternative carbon sources leads to loss of specific chromosomes in multiple fungal species and the addition of glucose leads to reduplication of the remaining chromosome (56, 104). Additionally, during adaptation to heat stress, *S. cerevisiae* rapidly duplicates chromosome III, however after continued heat stress the chromosome aneuploidy is lost while elevated expression of genes on this chromosome are maintained, suggesting that aneuploidy provides time for cells to search for optimal adaptive solutions (125).

While acquisition of aneuploidy is not always beneficial (126), aneuploidy can lead to a wide variety of fitness effects in different growth environments. Studies on the heat shock protein Hsp90 in *S. cerevisiae* showed that during periods of heat stress Hsp90 was titrated away from its regular clients, such as kinetochore proteins (127, 128). Importantly, exposure to heat stress or inhibition of Hsp90 led to a marked increase in chromosome instability and tolerance to other stressors including hydrogen peroxide, cyclohexamide, tunicamycin, benomyl, and radicicol (128). More recently, Dunham and colleagues performed a comprehensive analysis of aneuploid genotypes in *S. cerevisiae* and found that each karyotype had large, but condition-dependent changes in fitness, supporting that aneuploidy can be an important driver of adaptation (129, 130). Many more *in vitro* evolution experiments show that aneuploidy and ploidy change can be beneficial during adaptation, especially during adaptation to nutrient limitation and heat stress (55, 120, 131).

While we have discussed how changes in ploidy and chromosome copy number can be adaptive in certain environments, some species of pathogenic fungi carry multiple, non-essential chromosomes called accessory, supernumerary, B, or dispensable chromosomes (132). Accessory chromosomes are characterized by highly variable regions, high mutation

rates, and genes acquired via horizontal gene transfer (133–136). Many of these harbor virulence genes that allow the fungus to infect its host. The plant pathogen, *Nectria haematococca*, carries an array of genes on accessory chromosome 14 that allows the fungus to detoxify phytoalexin used by the pea plants as a defense mechanism (136, 137). In *Fusarium oxysporum*, a striking 40% of the genome was found exclusively in strains that infect tomato plants. Moreover, most of the genetic material in these accessory chromosomes lacks sequence homology to any close relatives of *F. oxysporum* (138). Further research is needed to investigate how these accessory chromosomes evolved, are maintained, and what roles they play in virulence.

Many filamentous fungi, including the cotton pathogen *A. gossypii*, have evolved alternative strategies to generate genotypic diversity. This fungus has multiple nuclei in the same cell (syncytia) that undergo asynchronous nuclear division (139). Originally thought to be a predominantly haploid fungus, Gladfelter and colleagues recently demonstrated frequent ploidy variations for different nuclei within the same syncytium including aneuploidy (63). To identify ploidy changes, they used lac operator arrays to track the copy number of individual chromosomes within the nuclei of the same syncytia and found nuclear ploidies ranging from 1N to >4N (63). Interestingly, in response to cellular stress (cell wall stress, osmotic stress, excess zinc, the antifungal fluconazole, and increased temperature), polyploid nuclei diminished and haploid nuclei predominated. These results suggest that nuclei with different ploidies are tolerated within single syncytia, and that there may be costs associated with ploidy level variation, as stress homogenizes the genome content of nuclei (63). Remarkably, ploidy reduction and homogenization of ploidy under stress is the opposite of what has been observed for the human pathogens *C. albicans* and *C. neoformans*, where stress led to genomic diversification within populations and increased aneuploidy (43, 59, 61).

In summary, the above examples establish that fungal organisms can adapt rapidly to their environment by undergoing ploidy and chromosome copy number changes. It also highlights the challenge of determining the causative interactions between environment and ploidy levels (Table 1). While it is unclear what types of ploidy changes will arise in any given environment, it is a common strategy of pathogenic fungi to generate genomic variation in response to environmental perturbations.

It is important to note that in any given fungal population, one or more subpopulations may exist that have acquired viable aneuploidies or undergone ploidy shifts. This is due to background rates of chromosome missegregation and failed mitosis. It is of great interest to understand how entire populations of cells, when subjected to stress, can become polyploid or aneuploid. Are the vast majority of cells dying and selection acts upon the survivors resulting in cells with ploidy changes beneficial for survival? Or, could there be a mechanism permissive or instructive in the generation of aneuploid or polyploid organisms? Both of these questions remain open to further investigation.

Ploidy and aneuploidy changes arise during *in vivo* evolution

Ploidy level heterogeneity and aneuploidy is frequently observed in clinical isolates of fungal species including *C. albicans*, *C. neoformans*, and *C. glabrata* (10, 11, 15, 140, 141).

However, very little is known about the dynamics of ploidy changes during *in vivo* evolution and the effects on host-fungus interactions.

To assess the types of genome alterations that *C. albicans* undergoes upon host encounter, single passage *in vivo* experiments were conducted in a murine model of systemic infection. Ploidy changes compared to the diploid progenitor were detected in ~3% percent of recovered strains and included segmental and whole chromosome aneuploidy. While this frequency is relatively low, no aneuploids were detected in strains from an *in vitro* control population. Results from this study suggest that conditions within the animal host affect chromosome disjunction more strongly and that this may reflect the very different population growth responses *in vivo* (60). More recent studies on the adaptive potential of *C. albicans* in response to the oral and systemic host niche show that the acquisition of aneuploidy is much more frequent than previously reported. Excitingly, the first haploids ever reported for *C. albicans* were found among strains recovered from an oral model of infection (47). Haploid isolates arose as early as 3 days post infection with a frequency of $\sim 2 \times 10^{-5}$ and not only differed in their genotypes (e.g. *MTL* status) but also showed phenotypic differences compared to their diploid progenitor. These phenotypes include decreased fitness in YPD at 30°C and 37°C and defects in filamentation ((47), and A. Forche, unpublished). Importantly, haploids arose independently in 6 different mouse hosts and multiple haploids with different genotypes were found in the same host (A. Forche, unpublished). Overall, the frequency of phenotypic changes, such as fitness at different temperatures, filamentation, or the production of hydrolytic enzymes that accompanied aneuploidy in general was much higher compared to cells that remained diploid. This suggests that some of these aneuploid states may be adaptive. Interestingly, the dramatic changes in ploidy may be specific to pathogenic host niches: passage of *C. albicans* using a murine commensal model found no ploidy or chromosome copy number changes by flow cytometry and WGS (142), supporting that host microenvironment impacts genome architecture.

Upon infection with *C. neoformans*, the host's immune system responds by trying to combat the fungus via oxidative or nitrosative attacks by macrophages and neutrophils. The formation of polyploid 'titan' cells inside the host (as previously discussed) is accompanied by a ploidy shift from a 1N euploid state to one up to >64N (143). Current evidence points to the importance of 'titan' cells and their ability to withstand various stresses that may be encountered within a host (38, 43). For example, among cells harvested from infected lungs, 'titan' cells exhibited resistance to both sodium nitrate and tert-butyl hydroperoxide, and survive under conditions lethal to non-titan cells (38, 43).

In summary, single passage of diploid *C. albicans* cells in an oropharyngeal model of infection leads to ploidy reduction to a haploid or near-haploid state (47). In addition, aneuploidy arises frequently upon encounter with the host in different models of infection (60). In contrast, haploid *C. neoformans* undergoes polyploidization upon host encounter (144). It is currently unknown what specific factors influence ploidy shifts in these fungi but it is likely a combination of many variables including temperature, pH, host immune cells, and interactions/competition with other microbes (145, 146).

7. The Beneficial Effects of Ploidy Changes

Adaptation of an organism to a novel environment is a function of the rate in which beneficial, growth-promoting mutations are acquired and spread throughout the population. The rate of adaptation is affected by multiple, inter-related, properties that determine the appearance, persistence, and fixation of beneficial mutations in a population. These properties include the rate of beneficial mutations, the fitness effect of these mutations, the dominance of mutant alleles, and effective population size (147–153). While aspects of this process are still poorly understood, theoretical studies indicate that ploidy will affect the rate of adaptation (2, 154, 155).

Ploidy change can impact the evolutionary trajectory of a cell by altering the rate and spectrum of beneficial mutations. For example a ploidy increase (whole genome duplication) increases the mutational target size. A ploidy increase will also provide the cell with the ability to buffer deleterious mutations due to redundant gene copy numbers (1, 156). Furthermore, some mutations and/or genome changes are only accessible to cells with specific ploidies, and this can have a profound impact on adaptation. For example, diploid and polyploid cells can undergo LOH, while haploid cells can acquire recessive mutations upon which selection can act (1, 157, 158). Similarly, polyploid cells have high rates of whole chromosome aneuploidy, which can provide fitness benefits during adaptation (20, 86, 88), yet haploid cells rarely acquire aneuploidy during adaptation likely due to the increased fitness cost of these mutations in most environments (126). Lastly, the fitness effect of a given mutation is assumed to be equal across all ploidy levels, however recent experimental evidence suggest that this is not the case for all mutations (20, 159–161).

8. Impact of ploidy and aneuploidy on cancer biology

Ploidy changes and acquisition of aneuploid chromosome copy numbers are hallmarks of cancer cells. Tetraploid cells are frequently observed in precancerous lesions of Barrett's Esophagus (162, 163) and in pre-neoplastic cervical cells (164). Recently, a systematic comparison of over 5000 human tumors found that 37% had undergone genome duplication during tumorigenesis (165). Evidence for ploidy changes in these tumors came from gene copy number and allelic ratio data obtained from single nucleotide polymorphism (SNP) microarrays. Compared to diploid tumors, tumors that underwent WGD had higher rates of gene copy number alterations, including chromosome loss, resulting in ploidy levels ranging from triploid to tetraploid. WGD often preceded other gene copy number alterations in these tumors, supporting that WGD can be a driver of tumorigenesis. The idea that WGD can promote tumor development was previously shown in a mouse breast cancer model (p53^{-/-} mammary epithelial cells (166)). Isogenic diploid and tetraploid cells were generated *in vitro*, mimicking a recent whole genome duplication event. When transplanted into mouse mammary epithelia, only the tetraploid cells generated tumors (166). Additional studies support that WGD resulting in tetraploidy is an intermediate step towards aneuploidy and tumorigenesis (106, 164) while other studies support that in some cancer types aneuploidy may precede the tetraploidization event (167).

Whole genome reduction is also observed during development of rare leukemia subtypes, including acute lymphoblastic leukemia (168). These haploid leukemias are considered near-haploid because the lymphoblastic cells are often aneuploid for one or more chromosomes (1N+1), frequently involving chromosome 21 (168). Haploidization and LOH nearly across the entire genome may underlie the aggressiveness of these cancers, resulting in very poor prognosis (168). However, this haploid population appears to be unstable and undergoes WGD (autodiploidization) with some frequency. Evidence for autodiploidization was found in patients with mixed lymphoblast populations of both a near-haploid karyotype (1N+1) and a near-diploid population that was exactly twice that of the aneuploid karyotype (2N+2) (168). These data support that ploidy variation is directly linked to cancer evolution, yet the effects of ploidy change *vs* effects of aneuploidy *per se* are difficult to distinguish.

Concluding remarks and outlook

There is a common theme in the examples discussed in this review – somatic ploidy changes increase the genetic heterogeneity of a population of cells (169–171). This genetic heterogeneity includes increased frequency of aneuploidy, but importantly, both ploidy and aneuploidy are reversible genomic changes. For example, reduplication of a lost aneuploid chromosome or chromosome loss after a whole genome duplication event, frequently occurs in fungi.

The initial ploidy-changing event may occur very rarely in some environments, but if there is a fitness benefit that accompanies the ploidy change then it will spread throughout the population over time. What is surprising then, is how often these ploidy-changing events occur within short-term *in vitro* evolution experiments and over eons of evolution. Perhaps the adaptable genome is the most successful genome, and cells with the ability to undergo rapid genome expansion and/or contraction will be able to survive the most environmental insults. Additionally, it's possible that there is a sub-population of persister cells that are primed and/or programmed to undergo ploidy changes during the response to stress. More population-level and single-cell approaches are needed to define the mechanisms and environmental conditions that cause ploidy changes in all eukaryotes (especially during fungal infections and tumorigenesis). The impressive examples of genomic heterogeneity observed in fungal pathogens support using these simple model systems to determine the mechanisms of ploidy change that occur during the somatic evolution of human cancers.

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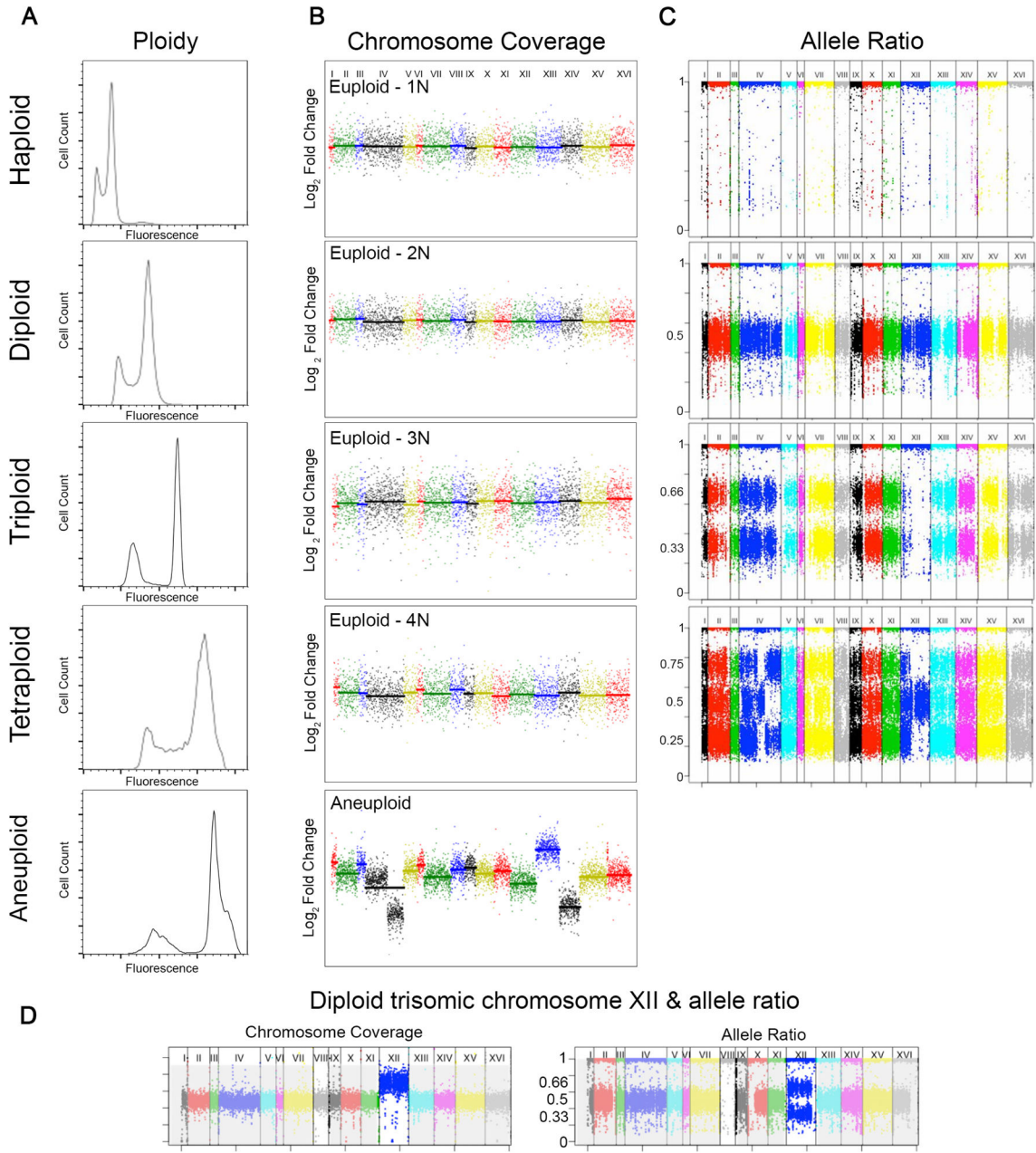


Figure 1. Methods for detection of ploidy and aneuploidy

A) Ploidy is determined with flow cytometry. Total genome fluorescence, measured using a fluorescent nucleotide label (e.g. propidium iodide or Sytox Green). Cells are first fixed (in ethanol) and RNA is removed with RNase, then gDNA is fluorescently labeled and analyzed on a flow cytometer. Cells are passed through a laser and the number of cells are plotted as a function of fluorescence intensity. Cells in a population typically have two fluorescent peaks, representing cells in either G1 or G2 phases of the cell cycle. Flow cytometry plots for yeast with the following ploidy levels are shown: haploid (1N), diploid (2N), triploid (3N), tetraploid (4N), and a near-tetraploid aneuploid.

B) Chromosome copy number is determined with whole genome sequencing (WGS) and microarray comparative genome hybridization (aCGH). Y-axis represents Log_2 fold change of sequence reads relative to reference sequence, and chromosome number increases from left to right starting with chromosome I and ending with chromosome XVI (X-axis).

Chromosome copy number plots for *S. cerevisiae* with the following ploidy levels indicate euploid genome for haploid (1N), diploid (2N), triploid (3N), and tetraploid (4N). However the near-tetraploid isolate (bottom panel) is aneuploid for ChrXII (pentasomic), ChrXIV (trisomic) and contains a segmental aneuploidy of ChrIV. Figures generated from data obtained in (20).

C) Allele frequencies obtained from whole genome sequencing data also can be used to determine the ploidy of a strain. Y-axis is the heterozygous allele frequencies ranging from zero to one, plotted as a function of chromosome number starting with chromosome I and ending with chromosome XVI (X-axis). Allele frequency plot of example haploid strain with SNPs at allele frequencies at 1.0; diploid strain with SNPs at allele frequencies of 0.5 and 1.0; triploid strain with SNPs at allele frequencies of 0.33 and 0.66; and tetraploid strain with SNPs at allele frequencies at 0.25, 0.5, 0.75, and 1.0. Images obtained from reference (16).

D) A diploid strain that is trisomic (three copies of a chromosome) for chromosome XII (left panel). Interestingly, the allele frequency plot has SNPs at allele frequencies of 0.5 and 1.0 for all chromosome except ChrXII, which is at allele frequencies of 0.33 and 0.66, supporting that this chromosome is aneuploid (right panel).

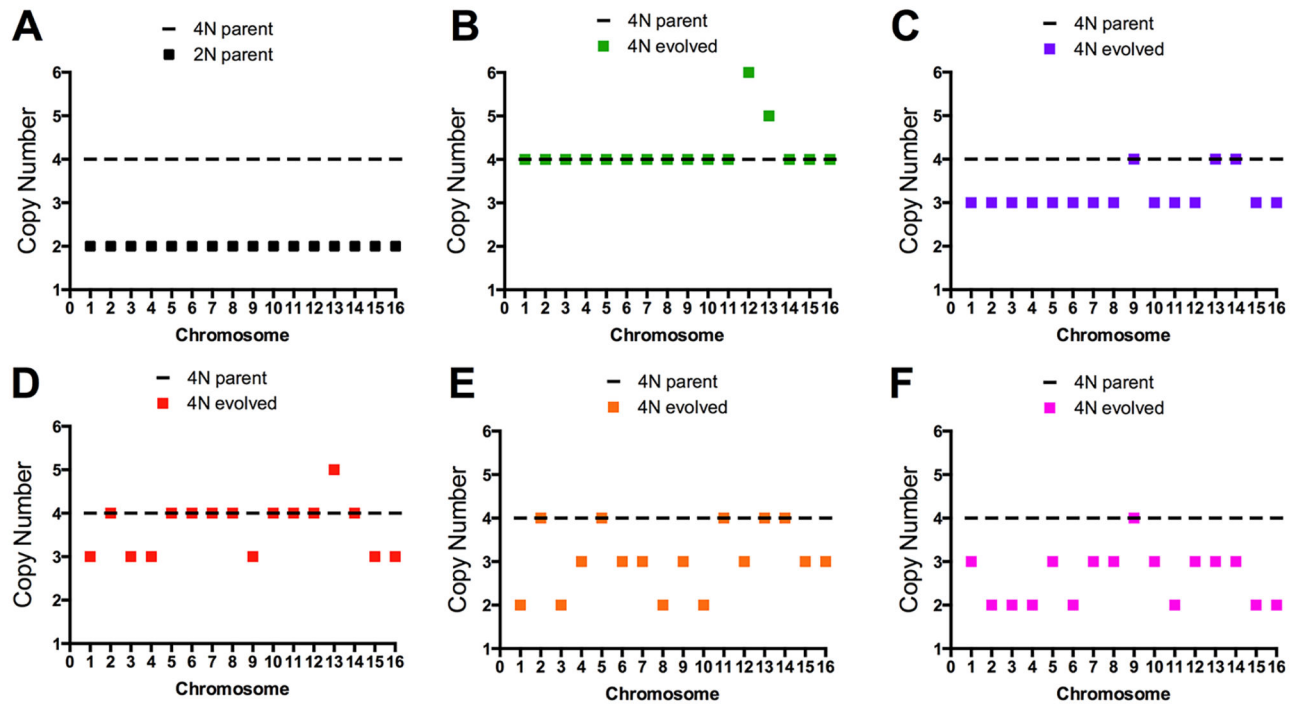


Figure 2. Many polyploid-evolved clones are highly aneuploid

Chromosome copy number was determined by whole genome sequencing and plotted for the A) Parental diploid (2N) and tetraploid (4N) strains and different tetraploid evolved clones after 250 generations in raffinose medium. Adaptation resulted in clones with B) increased chromosome copies, C) approximately trisomic copies of every chromosome (~3N), or highly aneuploid genomes (D–F). Figures generated from data obtained from the Supplementary data Table 1 in reference (20).

Table 1 Summary of experimental evolution studies in fungi and the ploidy and aneuploidy associated with different environmental stresses

Ploidy levels of haploid (1N), diploid (2N), triploid (3N), tetraploid (4N) are euploid states, while aneuploidy is indicated if known.

Summary of experimental evolution studies in fungi and the ploidy and aneuploidy levels associated with different environmental stress

Fungi	Stress	Timeframe	Ploidy Change/Aneuploidy	Reference
<i>S. cerevisiae</i>	YPD, high Salt	1800 Generations	1N → 2N and 4N → 2N	(19)
<i>S. cerevisiae</i>	YPD	186 Generations	4N → 2N and 3N → 2N	(105)
<i>S. cerevisiae</i>	Raffinose	250 Generations	4N → 4N-2N Aneuploidies common 2N → 2N and 1N → 1N (no ploidy change)	(20)
<i>S. cerevisiae</i>	Ethanol	200 Generations	1N → 2N and 4N → 2N	(118)
<i>S. cerevisiae</i>	Low Glucose (LiAc transformation)	88–132 Generations	1N → 2N	(119; 21)
<i>C. albicans</i>	L-sorbose, Pre-sporulation at 37°C	5–7 days	4N → 2N and Aneuploid 2N	(9; 52)
<i>C. albicans</i>	L-sorbose	2 days	2N → 2N monosomic for Chr5	(56)
<i>C. albicans</i>	YPD	28 days	4N → 4N, 3N, or 2N 2N → 2N	(54)
<i>C. albicans</i>	Rich Media and Nitrogen depletion	140 Generations	1N → 2N and 4N → 2N	(121)
<i>C. albicans</i>	Minimal Media and Phosphate depletion	140 Generations	1N → 1N and 4N → 2N	(121)
<i>C. albicans</i>	Fluconazole (2–10 µg/ml)	8–12 hrs	2N → 4N, 8N, 16N Trimeras (20% of cells in the population)	(62)
<i>C. albicans</i>	Fluconazole (100 mg disk)	12–16 hrs	2N → 1N	(123)
<i>C. albicans</i>	Fluconazole (increasing MIC)	330 Generations	2N → 2N-3N Aneuploidies common: whole chromosome, isochromosomes, and telomere-telomere fusions	(59; 61; 46)
<i>C. albicans</i>	Murine host	1–5 days	2N → 1N and Aneuploid 1N	(47)
<i>C. albicans</i>	Mutine host	Single passage	2N → 2N Aneuploid	(60)
<i>C. albicans</i>	Heat Shock (51°C)	90 seconds	4N → 2N	(55)
<i>C. tropicalis</i>	L-sorbose	8–10 days	4N → 2N	(104)
<i>C. tropicalis</i>	Rich Media	>120 Generations	4N → 2N	(104)
<i>C. neoformans</i>	Fluconazole (32 µg/mL)	3–5 days	1N → 1N Aneuploidies common	(42; 124)
<i>C. neoformans</i>	Fluconazole (8 µg/mL)	48–96 hrs	Polyloid Titan cells → 1N and 2N Aneuploidies common	(43)
<i>C. neoformans</i>	YPD, oxidative and nitrosative stress	48 hrs	Polyloid Titan cells → 1N	(43)
<i>C. neoformans</i>	Murine	3 days	1N → Polyloid Titan cells (20% of population)	(38)
<i>C. neoformans</i>	Unisexual Reproduction	~2 weeks	1N → 2N and Aneuploidies common	(35)

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Fungi	Stress	Timeframe	Ploidy Change/Aneuploidy	Reference
<i>A. gossypii</i>	NaCl, ZnSO ₄ , Fluconazole (325 nM), Heat (37°C)	6 hrs	Polyploid and Aneuploid → Euploid 1N (distribution of ploidities shifted towards haploid)	(63)
<i>A. gossypii</i>	Caffeine	6 hrs	Polyploid and Aneuploid → Polyploid and Aneuploid	(63)