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THE WILHELMINE E. KEY INVITED REVIEW

Ploidy and the Causes of Genomic Evolution

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Biography of Sarah Otto (2007 Wilhelmine Key Lecturer)

The core of my work focuses on the evolutionary forces that act on the structure of the genome. In particular, I study the evolution of three fundamental aspects of any genome: (1) the number of copies of each gene present, (2) the number of chromosomes within the genome, and (3) recombination rates among genes. My research uses mathematical models to determine how selection acts on the genomic variation produced by mutations to shape the changes that occur over time in these fundamentally important biological characteristics.

Abstract

Genomes vary dramatically in size and in content. This variation is driven in part by numerous polyploidization events that have happened over the course of eukaryotic evolution. Experimental evolution studies, primarily using the yeast *Saccharomyces cerevisiae*, provide insights into the immediate fitness effects of ploidy mutations, the ability of organisms of different ploidy levels to mask deleterious mutations, the impact of ploidy on rates of adaptation, and the relative roles of selection versus drift in shaping ploidy evolution. We review these experimental evolution studies and present new data on differences in maximal growth rate for cells of different ploidy levels.

Key words: *Saccharomyces cerevisiae, experimental evolution, ploidy, mutation, rate of adaptation, selection, drift, growth rate*

Humans have long marveled at the incredible diversity present in the natural world for a number of phenotypic traits (e.g., body structures and size, method of locomotion, thermal tolerance, etc.). Genetic analyses have revealed that such diversity also extends to the level of the genome, with variation in genome size, chromosome number, gene order, and content observed among even closely related species. These observations have raised questions about the causes of and constraints on genomic diversity. Experimental evolution with single-celled microbes offers the opportunity to witness evolutionary shifts in genomic diversity over short periods of time as thousands of cell generations can be tracked over the course of a single year. In addition, by manipulating the genome and determining the consequences, studies with microbes promise to shed light on the selective forces acting on genomic variation.

In this paper, we describe results of experimental evolution studies exploring genome size evolution and report some new results on the evolution of maximal growth rates as a function of ploidy. Our focus is on studies that have examined transitions among ploidy levels as well as the evolutionary consequences of ploidy, defined as the number of copies of homologous chromosomes carried by an organism. Lest we view "*The Causes of Evolution*" as different in kind between genomic evolution and genic evolution, we frame this paper within the context of the

book of Haldane (1932) by this title. This book provided an accessible and popular account of the mathematical underpinnings of evolutionary change, developed primarily by Haldane, Fisher, and Wright in the 1920s and 1930s, following the rediscovery of Mendelian genetics. Haldane's book describes how evolution is shaped by the interplay between mutation, which supplies the "material" basis of change, and selection, seen as the strongest force effecting changes across an entire population. Haldane pointed out the importance of a wide array of factors to the outcome of evolution, including deleterious and beneficial mutation rates, recombination rate, population size, rate of drift, and strength of selection. These theoretical insights continue to inform our understanding of evolution, as much today in the era of genomics as they did when first popularized.

In the next section, we briefly describe genomic diversity in ploidy levels. The interested reader is referred to the much more extensive reviews by Lewis (1980), Ramsey and Schemske (1998, 2002), Otto and Whitton (2000), and Gregory (2004). We then proceed to 4 topics that have been the subject of laboratory experiments investigating ploidy evolution in microbes:

- *The material basis of ploidy change: mutation.*
- *The impact of ploidy on the accumulation of deleterious mutations.*
- *The impact of ploidy on the rate of adaptation.*
- *Random genetic drift and the efficacy of selection on ploidy levels.*

As detailed below, these sections pay homage to Haldane and describe how his insights into the causes of evolution apply to ploidy evolution.

Background: Variation in Ploidy Level

The number of chromosome sets (ploidy) of an organism is a fundamental trait that differs among species and even within a species (Lewis 1980). Although the majority of species are functionally haploid (1 chromosome set, e.g., bacteria, several fungi, several protists, moss) or diploid (2 sets, e.g., most vertebrates, ferns, seed plants, several other fungi, and protists), there are also a substantial number of species that have become polyploid (more than 2 sets, e.g., coffee, sugar, wheat, the frog *Hyla versicolor*, and the red viscacha rat *Tympanoctomys barrerae*). Plant species in particular seem prone to polyploidization, as evidenced by the large excess of species whose gametic chromosome number is even rather than odd (Otto and Whitton 2000). Recent estimates from several genera of flowering plants suggest that lineages polyploidize at a rate that is approximately 10% of the rate of speciation (Meyers and Levin 2006). Although early studies estimated that 57% (Grant 1963) to 70% (Goldblatt 1980; Masterson 1994) of angiosperm species had a polyploidization event at some point in their evolutionary history, recent genomic analyses have revealed more and more cases of ancient polyploidization events ("paleopolyploidy"). The earliest of the detected events traces back to the base of the angiosperms and suggests that all flowering plants are ancient polyploids (Bowers et al. 2003; Blanc and Wolfe 2004).

Although polyploidization is less common in animals, hundreds of polyploidization events have been documented (Otto and Whitton 2000; Gregory and Mable 2005), and this number is likely to rise, especially among invertebrates, as genomic analyses extend beyond model systems. As with plants, comparative genomic analyses have revealed cases of paleopolyploidy in animals, including near the base of the vertebrate tree of life (Dehal and Boore 2005; Freeling and Thomas 2006).

In fungi, there is a long tradition of studying ploidy variation, especially as it relates to variation in the relative timing of meiosis and syngamy during the alternation of generations (e.g., Fowell 1969; Raper and Flexer 1970; see also Rogers 1973 on polyploid fungi). On the one hand are groups, such as the budding yeast *Saccharomyces*, that are prone to mating whenever compatible cells (MAT*a* and MATα) encounter one another and thus are primarily diploid. On the other hand are groups, such as the fission yeast *Schizosaccharomyces*, that sporulate soon after mating and hence are primarily haploid. This is, however, a coarse classification; a survey of 68 natural isolates of *Saccharomyces cerevisiae* from "Evolution Canyon" at Mount Carmel, Israel, revealed extensive variation in ploidy level, with diploid (31%), triploid (10%), and tetraploid lineages (59%; Ezov et al. 2006). And, again, evidence exists for ancient polyploidization events, most famously in the hemiascomycete lineage giving rise to *S. cerevisiae* and its close relatives (Wong et al. 2002).

Genomic analyses of yeast and other species that have undergone ancient polyploidization events have yielded insight into the long-term fate of duplicated genes. Naively, one would predict that duplicated genes are free to accumulate mutations and should eventually decay and be lost; yet, many anciently duplicated genes persist. For example, ~8% of genes duplicated by the ancient polyploidization in yeast ~100 million years ago remain in the genome of *S. cerevisiae* (Seoighe and Wolfe 1999). Explanations for the long-term survival of gene duplicates include 1) surviving duplicates are found when gene copies are immediately and actively preserved (e.g., when selection favors increased copy number), 2) surviving duplicates are those that take on novel functions before decay ("neofunctionalization"), and 3) surviving duplicates are those that lose nonoverlapping functions so that both copies must be retained for full function ("subfunctionalization"). Which of these outcomes is most likely for a particular class of genes depends on the interplay between selection, mutation, and genetic drift (see Walsh 2003; Lynch 2007). Data from yeast indicate that highly expressed genes are more often retained in duplicate (Seoighe and Wolfe 1999), and there is some evidence that purifying selection acts against amino acid mutations in newly duplicated gene pairs (Kondrashov et al. 2002), consistent with the first explanation that selection acts to preserve some gene duplicates right from their initial formation. By examining the number of protein–protein partners, He and Zhang (2005) showed that pairs of duplicated genes in yeast are more likely to have more interaction partners than singleton genes, a result consistent with neofunctionalization, but they also showed that duplicated genes had partitioned the

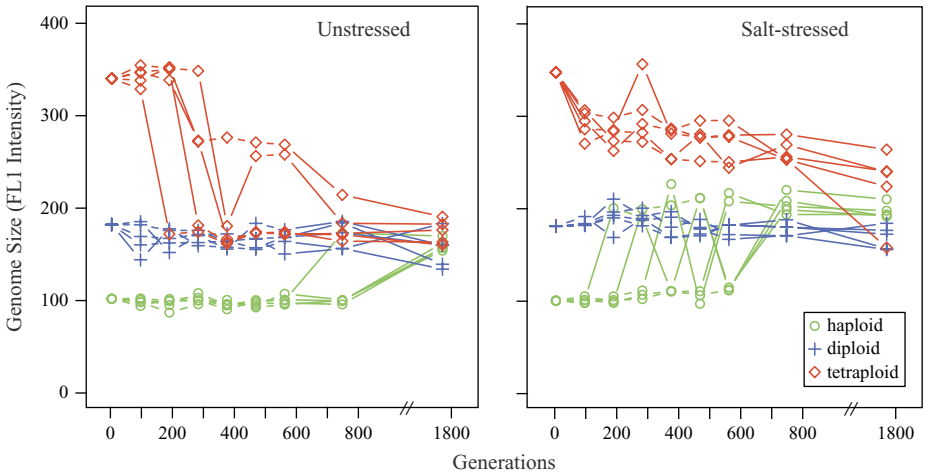

Figure 1. Genomic convergence to diploidy over 1800 generations of asexual evolution in *Saccharomyces cerevisiae* (adapted from Figure 1 of Gerstein et al. 2006). Five replicate lines were established at each of 3 ploidy levels: haploid (circle), diploid (plus sign), and tetraploid (diamond; all lines initially isogenic with one another), in each of 2 environments: unstressed and salt-stressed. Each data point represents approximate genome size measured by flow cytometry of a single colony from a line, with connections among dots representing colonies sampled from the same lineage at different time points (the observed fluctuations over time likely represent sampling from a polymorphic line). Note that flow cytometry was performed in batches involving all lines with the same initial ploidy (i.e., the same symbol), and so numerical comparisons are most meaningful within an initial ploidy level.

original subfunctions carried out by the unduplicated ancestral gene, consistent with subfunctionalization. Thus, empirical studies in yeast support a mixed model of evolutionary forces acting to preserve duplicated genes over the long term.

In the remainder of this article, we focus on experimental evolution studies that use microbes to gain insight into shorter term processes shaping ploidy evolution.

The Material Basis of Ploidy Change: Mutation

> "The material on which selection acts must be supplied by mutation."
>
> —Haldane (1932, p. 60)

For polyploidization to occur, 2 rare events must occur: a mutation must form a polyploid individual, and this genomic variant must become established within the population. Polyploidization results primarily from 3 mutational mechanisms: gametic nonreduction (the production of unreduced gametes caused by an error in meiosis), somatic doubling (the production of a cell with twice the normal chromosome number caused by an error in mitosis), and polyspermy (syngamy of multiple gametes). Gametic nonreduction is thought to be particularly important in the origin of polyploid plants. Polyploid gametes arise spontaneously at a rate of ~0.5%, (Web table 1 in Ramsey and Schemske 1998), although this rate rises substantially under certain environmental circumstances (e.g., cold shock, nutritional stress, herbivory) and following hybridization (rising to 26.3%, Web table 1 in Ramsey and Schemske 1998). Polyploid zygotes are observed at roughly similar frequencies in vertebrates (0.9% of chicken embryos are triploid or tetraploid, Bloom 1972; 5.3% of spontaneous human abortions are triploid or tetraploid, Creasy et al. 1976) and arise primarily from gametic nonreduction or polyspermy. Once formed, low viability and/or low fertility often hamper the establishment of polyploids. In particular, triploids have low, but not negligible, fertility (31.9%, Ramsey and Schemske 1998), and newly formed tetraploids also have low fitness in many cases (Web table 7 in Ramsey and Schemske 2002). Nevertheless, the recurrent establishment of polyploids implies that polyploidization occasionally alters phenotype in beneficial ways, ameliorating any fertility disadvantage and allowing rare polyploid individuals to persist and replicate.

Given the drastic change to genomic structure, we might expect mutations involving polyploidy to cause a more devastating reduction in fitness. Yet as Haldane (1932, p. 29) pointed out, "the number of genes of all sorts is increased equally" in polyploids, whereas "the balance is upset" in aneuploids (individuals that have a chromosome complement not an exact multiple of the haploid number). Furthermore, all organisms have evolved to be tolerant of changes in ploidy level because of the doubling and halving of genome size each cell division. As a consequence, shifts in ploidy level are often surprisingly well tolerated.

Witnessing spontaneous polyploidization events and tracking their establishment is difficult in nature but is possible in laboratory experiments with single-celled organisms. In a batch culture experiment with *S. cerevisiae*, we found that replicate haploid and tetraploid lines, evolved independently for 1800 generations, converged toward [diploidy, (Figure 1 and Gerstein et al. 2006)]. This convergence occurred in 2 different evolutionary environments, an unstressful environment (using a standard rich

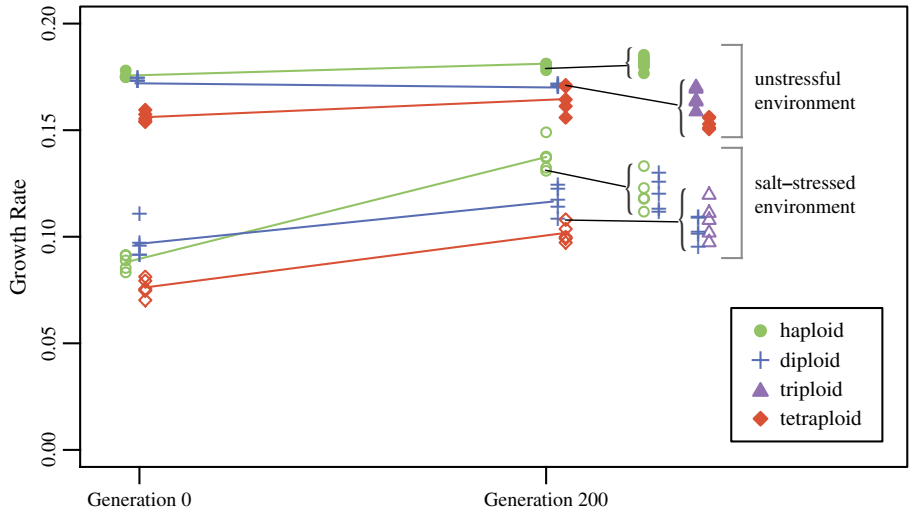

Figure 2. Maximal growth rates of haploid (circle), diploid (plus sign), and tetraploid (diamond) *Saccharomyces cerevisiae* exhibited by ancestral populations (generation 0) and evolved populations (generation 200). Replicate lines were propagated independently in YPD (5 lines for each initial ploidy level; solid symbols) and in YPD + salt (5 lines for each initial ploidy level; hollow symbols). Each point at generations 0 and 200 represents the average value of the maximal growth rate in the Bioscreen C measured in 2 replicate population samples from a line. Because ploidy may have changed over time, 25 colonies were assessed for ploidy level by FACScan at 200 generations. Ten colonies, uniformly representing the observed ploidy levels, were then assessed for growth rate (growth rates shown after braces; see Appendix for methods). The symbols used for these colonies are as before, with the addition of triploid colonies (triangles).

medium, YPD) and a salt-stressed environment (YPD + 0.6 M NaCl). We can use this experiment to ask 1) what were the mutations altering ploidy and 2) what evolutionary forces allowed the persistence and spread of ploidy variants. We will examine these 2 questions separately, considering first the initially tetraploid populations and then the initially haploid populations.

Although it is often thought that polyploidization is an irreversible mutation (Meyers and Levin 2006), a decline in ploidy level was observed by Gerstein et al. (2006), with initially autotetraploid lines evolving toward diploidy (with some aneuploidy). The mutation(s) responsible is, however, unknown at present. It was not a regular meiotic event because the tetraploids were MAT*a* (not MAT*a*/MATα) and because the 4 homologues of each chromosome would have been physically separated (rather than 2 pairs of sister chromatids). Furthermore, as seen in Figure 1, triploid-sized individuals were often sampled at intermediate time points, which is unexpected from a regular mitotic or meiotic reductive division. Starting a second batch culture experiment with these triploid-sized individuals, Gerstein et al. (2008) showed that triploid-sized yeast are also able to lose a complement of chromosomes, becoming diploid (or near diploid). The timescale of genome size reduction (within 200 generations) argues against the independent loss of 16 chromosomes (the haploid number of chromosomes in *S. cerevisiae*). Use of comparative genomic microarrays also revealed that patterns of chromosome loss were consistent with a concerted, rather than random, mechanism. Once mutants with a lower ploidy level had arisen, their spread is not very surprising, given that tetraploid yeast typically grow more slowly than both haploids and diploids (generation 0 in Figure 2; Mable 2001) and exhibit mitotic defects related to scaling mismatches in the components of the spindle apparatus (Storchova et al. 2006). Similarly rapid declines in ploidy have been observed in the closely related yeast, *Candida albicans* (tetraploid to diploid transition; Bennett and Johnson 2003), and in *Aspergillus nidulans* (diploid to haploid transition; Schoustra et al. 2007).

Because the strains used by Gerstein et al. (2006) do not produce a membrane-bound transporter critical for releasing the pheromone (encoded by STE6) and showed no evidence of mating (i.e., no MAT*a*/MATα diploids were found), the evolutionary transition from haploidy to diploidy likely occurred via a somatic doubling event (chromosome replication not followed by division). The rate at which diploids took over initially haploid populations depended on the environment. Diploid cells arose and spread more rapidly in the salt-stressed lines (Figure 1b) than in the unstressed lines (Figure 1a). Other studies have also observed diploid (or near diploid) *S. cerevisiae* mutants arising within haploid lines under a variety of environmental conditions and at varying frequencies (Mable 2001; Zeyl et al. 2003; Lynch et al. 2008). These findings suggest that different environments either increase the rate of ploidy mutations and/or alter the strength of selection favoring an increase in ploidy.

To determine if the transition from haploidy to diploidy caused an immediate increase in fitness, we measured growth rates using the microbiology workstation Bioscreen

C (ThermoLabsystems), which samples the optical density of cultures over time (for methods, see Appendix). The 48 h growth trajectories were obtained, and the maximum growth rate was estimated for ancestral haploid and diploid lines. Because the ancestral diploids were created by inserting MAT*a* and MATα plasmids that contained the functional ste6 gene into haploid cells, crossing, and selecting for plasmid loss, the diploid and haploid ancestral lines should be virtually isogenic, save for the difference in ploidy and the rare mutation that might have arisen during the handful of generations required to produce the diploid line. Interestingly, there was no clear growth advantage to diploids. In YPD, ancestral haploids grew significantly faster than diploids ($t_8 = 4.193$, $P = 0.0030$; generation 0 in Figure 2), whereas in YPD + salt, diploids grew slightly but not significantly faster than haploids ($t_8 = -2.185$, $P = 0.0604$). The growth rate data are thus consistent with the observation that diploidy took over more rapidly in the salt-stressed environment (Figure 1), but they fail to explain why diploid cells took over at all in the unstressed environment. Competition experiments performed on the same strains by Mable (2001) also failed to find a significant advantage of diploids over haploids in YPD, although small competitive differences cannot be ruled out. Similarly, a recent study by Dickinson (2008) failed to identify significant differences in competitive fitness between haploid and diploid lines, using an assay that should detect fitness differences $>\sim 0.5\%$. Ongoing experiments are underway to determine whether fitness components other than maximal growth rate play a role (such as the ability to survive during stationary phase or the ability to resume growth once placed in new medium).

In the above growth rate assay, there was a significant interaction between ploidy and environment ($F_1 = 8.307$, $P = 0.0108$) with diploids performing relatively better than haploids in the salt-stressed environment. One possibility for the relatively higher growth rate of diploids in salt-stressed environments is that their surface area to volume ratio is lower than that of haploids (Mable 2001). Thus, diploids cells might require less energy to maintain favorable intracellular ion concentrations. More generally, cells of higher ploidy might be fitter in environments carrying toxic substances because of their relatively smaller surface area in contact with the external environment. The reverse has also been suggested: When nutrients are limiting, haploids may gain a fitness advantage because of their higher surface area to volume ratio (Adams and Hansche 1974; Weiss et al. 1975; Lewis 1985). This "nutrient limitation hypothesis" has been tested with mixed results (reviewed by Mable 2001). One difficulty is that experimental tests of this hypothesis are often conducted on genetically different haploid and diploid strains (either across the genome or at the mating-type locus itself). Using isogenic strains at all loci (including the mating locus), Mable (2001) found that haploids had a growth rate advantage in rich medium (YPD) but not in minimal medium, counter to the nutrient limitation hypothesis. That said, not all forms of nutrient limitation are equivalent. Adams and Hansche (1974) argued that haploids and diploids should differ little when growth is limited by sugars, which are actively transported and whose metabolism depends on enzyme concentrations internal to the cell ("utilization limited"). Rather, they argued that haploids would outcompete diploids under conditions when growth is limited by nutrients transported across the membrane. In particular, they observed that when the limiting nutrient was organic phosphorous (which is hydrolyzed by an extracellular enzyme, acid phosphatase), haploids outcompeted diploids, consistent with the nutrient limitation hypothesis. More research is necessary to define the environmental conditions favoring different ploidy levels and the relationship of these conditions to cell geometry and transport.

The Impact of Ploidy on the Accumulation of Deleterious Mutations

> "polyploidy may be preserved because it protects against mutations in some thousands of loci at once. . ."
>
> —Haldane (1933)

Haldane (1937) demonstrated that the long-term impact of deleterious mutations on the mean fitness of a population depends almost entirely on the genome-wide deleterious mutation rate and not on the selective disadvantage of the mutations. Specifically, in the absence of epistasis and given that mutations reduce fitness to some extent in heterozygotes, the equilibrium mean fitness of a population is reduced by approximately $c\,U$ (the "mutation load"), where c is the ploidy level and U is the mutation rate per haploid genome (assumed to be $<<1$ in this approximation). Thus, all else being equal (e.g., equivalent mutation rates per base pair, which appears to be true at least in *S. cerevisiae*; Ohnishi et al. 2004), haploids will have the lowest mutation load, despite the fact that deleterious mutations are "masked" to some extent in diploids and polyploids. Masked mutations persist for longer and reach higher frequencies in diploids and polyploids before being eliminated by selection. Ultimately, for every mutation that enters into a population, selection must eliminate a mutation, and this selective process will have the least negative impact on haploids because of their lower mutation rate per individual per generation.

Although masking mutations provides no long-term benefit to higher ploidy levels, masking can temporarily raise the mean fitness of a newly formed polyploid population (Otto and Whitton 2000). This occurs because tetraploids initially carry deleterious mutations at a frequency expected within the diploid populations from which they arose. Neotetraploids may thus benefit from the additional masking capacity afforded by having 4 copies of every gene but not yet suffer from the burden of carrying a higher equilibrium frequency of mutations. As shown in Figure 3 of Otto and Whitton (2000), masking can raise the mean fitness of tetraploids relative to diploid populations for hundreds to thousands of generations when deleterious mutations are partially recessive.

Empirical results have supported the theory that masking of mutations can, at least temporarily, increase fitness of

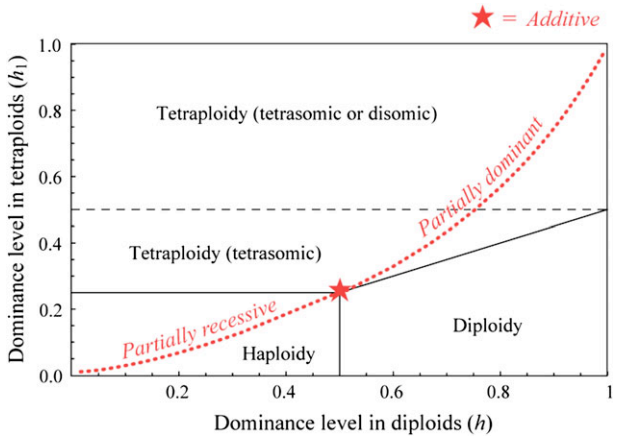

Figure 3. The ploidy with the highest rate of adaptation in a sexual population depends on the dominance of beneficial mutations (adapted from Otto and Whitton 2000). When beneficial mutations are partially to fully dominant, populations with higher ploidy levels are expected to adapt faster because such populations are more likely to carry new beneficial mutations, all else being equal. When beneficial mutations are partially to fully recessive, lower ploidy levels are expected to adapt faster because such populations better reveal the fitness benefits of new mutations.

diploid heterozygotes. Using mutator strains of *S. cerevisiae*, Korona (1999) compared the fitness of mutation-loaded haploid strains with diploid heterozygotes created by mating 2 such haploids. He found that the fitness of loaded diploids was significantly higher than that of loaded haploids (loaded haploids had a fitness of ≈0.75, whereas loaded diploids had a fitness of ≈0.95, measured by their maximum growth rate relative to nonmutant lines). Mable and Otto (2001) also assessed the masking ability of higher ploidy levels by subjecting isogenic haploid, diploid, and tetraploid yeast to ethane methyl sulphonate (EMS), a DNA-damaging agent. In 3 experiments involving 2 different strains of *S. cerevisiae*, Mable and Otto (2001) found that the growth rate of haploid populations was much more severely reduced by EMS than that of diploid or tetraploid populations, as expected by the inability of haploids to mask deleterious mutations. Tetraploid lines, however, exhibited a reduction in growth rate that was comparable to diploid lines following EMS, suggesting that higher ploidy might not substantially improve the ability of a cell to mask deleterious mutations. In addition to the immediate effects of EMS, one would expect that fitness should recover most rapidly in haploids because mutations are more directly exposed to selection. This expectation was not borne out. Instead, growth rates rose over time at similar rates in haploids and diploids. That said, severely deleterious mutations would have been immediately eliminated in haploids, leaving only mildly deleterious mutations to contribute to the observed patterns (see Figure 3 in Mable and Otto 2001). As mentioned by the authors, there are a number of caveats to this study. In particular, the rate of EMS uptake and the ability to repair EMS damage might depend on ploidy, and the fitness effects of deleterious mutations might be exacerbated in tetraploids because of the stress induced by high ploidy. In addition, EMS is a toxin that could have directly reduced fitness for a number of cell generations, even in the absence of mutation. Further complicating matters, rapid changes in cell size and some ploidy changes were observed, consistent with diploidization of some haploid lines and ploidy reduction in all tetraploid lines, as found by Gerstein et al. (2006) in the absence of EMS.

The Impact of Ploidy on the Rate of Adaptation

> "Mutation pressure ... will favour ... polyploids, which possess several pairs of sets of genes, so that one gene may be altered without disadvantage, provided its functions can be performed by a gene in one of the other sets of chromosomes"
>
> —Haldane (1932, p. 110)

Early work on adaptation as a function of ploidy level focused on the advantage of having multiple copies of genes at which beneficial mutations could occur. This motivated an early empirical study by Paquin and Adams (1983) using fluctuation assays to assess the rate of fixation of beneficial mutations in haploid and diploid populations. Although they concluded that diploids adapted faster, the inferred rate of fixation was inconsistent with the fitness differences observed among the cells (Dykhuizen 1990). Because the population sizes were so large, the likely explanation was that multiple mutations were segregating simultaneously, selectively interfering with one another, and invalidating the assumption that mutations that initially rise in frequency will ultimately fix.

Although Paquin and Adams (1983) focused on the number of beneficial mutations that arise in haploids and diploids, the rate of adaptation also depends on the fate of those mutations. Indeed, the rate at which fitness increases in a population should depend on 1) the rate of appearance of new beneficial mutations, 2) the fixation probability of these mutations, and 3) the fitness effect of new mutations. In sexual populations, Haldane (1927) calculated the probability of fixation of a beneficial new mutation, A, as approximately twice its selective advantage ($\sim 2s$), assuming that the variance in reproductive success among individuals is approximately Poisson. Extending this theory to polyploids, the rate of fitness increase due to the fixation of new mutations is:

$$\text{Haploids} : \Delta W_{1n} = (N\nu) \times (2s) \times (s),$$

$$\text{Diploids} : \Delta W_{2n} = (2N\nu) \times (2hs) \times (s),$$

$$\text{Tetraploids} : \Delta W_{4n} = \underbrace{(4N\nu)}_{\text{(1) mutation appearance}} \times \underbrace{(2h_1 s)}_{\text{(2) fixation probability}} \times \underbrace{(s)}_{\text{(3) fitness effect}},$$

where N is the population size, ν is the beneficial mutation rate, h and h_1 are the dominance of the new A allele in

diploids (*Aa*) and tetraploids (*Aaaa*), tetraploids are assumed to be tetrasomic, and the population is assumed to be sexual (for other cases, see Otto and Whitton 2000). The ploidy level expected to have the highest rate of adaptation thus depends on the dominance level of mutations in diploids (h) and tetraploids (h_1). As shown in Figure 3, we expect haploids to adapt the fastest when beneficial mutations are partially recessive and polyploids to adapt the fastest when beneficial mutations are partially dominant.

In asexual populations (such as clonally propagated yeast), the situation is complicated by linkage ("clonal interference"; Crow and Kimura 1965). The rate of adaptation still depends on the same 3 factors (rate of appearance, fixation probability, and fitness effect), but the fixation probability of a particular mutation now depends strongly on the genetic background in which it appears and the fitness effects of loci throughout the genome. According to theory, in large asexual populations, the rate-limiting step is the spread to high frequency of beneficial mutations, predicting that haploids should adapt fastest because they best expose mutations to selection (Orr and Otto 1994; Otto and Whitton 2000). In small populations, in contrast, the rate-limiting step becomes the appearance of beneficial mutations, predicting that tetraploids should adapt faster than lower ploidy levels if dominance is high ($h_1/h > 0.7$) because tetraploids are then more likely to bear and express mutations.

A few empirical studies have tested and confirmed these expectations. Zeyl et al. (2003) showed that large asexual haploid populations of *S. cerevisiae* were able to adapt faster than diploids during 2000 generations of batch culture evolution in minimal liquid medium (average fitness increase of diploids relative to haploids was only 0.69, as measured by a competitive fitness assay against the diploid ancestor). In small populations, however, the same strains of haploids and diploids adapted at nearly the same speed, and the advantage of haploidy disappeared (average fitness increase of diploids relative to haploids was 0.97). Anderson et al. (2004) demonstrated that when yeast cells are subjected to low concentrations of the antifungal drug fluconazole, diploids evolved drug resistance faster than haploids. In contrast, when the yeast cells were grown in high concentrations of fluconazole, haploid populations evolved faster. This difference was expected based on the nature of mutations required to grow at different drug concentrations: low fluconazole levels require dominant mutations (favoring diploids, which have double the number of mutational "targets"), whereas high fluconazole levels require recessive mutations (favoring haploids, which are able to fix beneficial recessive mutations more rapidly).

Although haploid strains are predicted to evolve more rapidly when the requisite mutations are partially to fully recessive, mitotic recombination and the occasional production of haploids can facilitate rapid adaptation in initially diploid strains. This phenomenon was recently reported by Schoustra et al. (2007) in *A. nidulans* using strains that carried a costly resistance allele to fludioxonil and that were propagated in fungicide-free medium for 3000 generations. The mycelial growth rate increased slowly in both haploids and diploids, perhaps due to the accumulation of mutations ameliorating the costs of resistance. The fastest rate of response was observed, however, in diploids that reverted to haploidy (4 lines) and in a diploid strain that exhibited loss of heterozygosity (potentially due to mitotic recombination). Dominance tests suggested that these lines had accumulated multiple recessive beneficial mutations. The authors argued that the haploidized diploids (and the diploids that had become homozygous) had the best of both ploidy worlds: They could accumulate mutations in the diploid state, even if some of those mutations were deleterious on their own, but then those mutations could be revealed and positively selected following haploidization.

To assess the generality of these findings across a broader range of ploidy levels, we measured growth rates (for methods, see Appendix) of the haploid, diploid, and tetraploid lines evolved in unstressful and salt-stressed medium from Gerstein et al. (2006). Because of the shifts in ploidy level observed over the 1800-generation experiment, we focused only on changes in growth rate during the first 200 generations of evolution. As this evolution experiment was carried out with a large and asexually reproducing population, we expected that haploids would adapt faster than higher ploidy levels (as seen by Zeyl et al. 2003). As shown in Figure 2, the difference in growth rates between generations 0 and 200 varied significantly among ploidy levels (unstressful environment: $F_{2,12} = 12.80$, $P = 0.0011$; salt stressed: $F_{2,12} = 16.28$, $P < 0.0001$) and among environments ($F_{1,28} = 43.50$, $P < 0.0001$). There was also a significant ploidy-by-environment interaction ($F_{2,1} = 12.46$, $P = 0.0002$). This interaction term was largely driven by a faster increase in growth rate of the haploid lines exposed to high salt (haploid $>$ tetraploid $\approx$ diploid; Tukey test following analysis of variance [ANOVA]). There was also a smaller but significant increase in growth rate among the haploid and tetraploid lines propagated in the unstressful environment (haploid $\approx$ tetraploid $>$ diploid; Tukey test following ANOVA).

The observed increases in growth rate might have been caused, in part, by switches in ploidy level. We thus assessed variation in ploidy and its impact on growth rate within one haploid and one tetraploid line from each environment (Appendix). From these 4 lines (haploid YPD, haploid YPD + salt, tetraploid YPD, and tetraploid YPD + salt), 25 colonies were sampled from the 200 generation time point. Widespread polymorphisms for ploidy were observed in 3 of the 4 lines (Figure 2; ploidy designations are based on flow cytometry and are approximate, see Appendix). The exception was the haploid line evolved in YPD, which remained haploid. Specifically, the following ploidy transitions were observed:

- From the initially haploid line propagated in YPD + salt, both haploid and diploid colonies were isolated at generation 200. Growth rates were assayed for 10 of these colonies (5 haploid and 5 diploid), revealing that the evolved colonies had significantly higher growth rates

than the ancestral haploid populations ($F_{2,12} = 37.01$, $P < 0.0001$). The diploid evolved colonies exhibited lower growth rates than the haploid evolved colonies, but this difference was not significant ($t_8 = 0.283$, $P = 0.784$). Because the diploids exhibited slower growth, if anything, the growth rate improvement observed over time in the initially haploid lines exposed to salt (Figure 2) cannot be explained by a switch to diploidy.

- From the initially tetraploid line propagated in YPD + salt, triploid and diploid colonies were isolated at generation 200. Growth rates were assayed for 10 of these colonies (5 triploid and 5 diploid), and both groups grew significantly faster than the ancestral tetraploid populations (Tukey test following significant ANOVA: $F_{2,12} = 33.71$, $P < 0.0001$). Again, the ploidy difference was not associated with a significant change in growth rate ($t_8 = -1.053$, $P = 0.320$).
- From the initially tetraploid line propagated in YPD, tetraploid and triploid colonies were isolated at generation 200, and growth rates were assayed for 10 of these colonies (5 tetraploid and 5 triploid). In this case, the evolved tetraploid colonies exhibited no improvement in growth over the ancestral tetraploids. In contrast, the evolved triploid colonies grew faster than both ancestral tetraploids and evolved tetraploid colonies isolated from the same population (Tukey test results following significant ANOVA: $F_{2,12} = 16.47$, $P = 0.0004$). Thus, the increase in growth rate in YPD observed in this tetraploid population was strongly associated with a genome size reduction to triploidy (Figure 2).

In summary, we find no evidence that the increases in growth rate observed over time in lines adapting to salt were driven by ploidy shifts. In contrast, the increased growth rate of initially tetraploid lines grown in YPD appeared to be due, in large part, to a reduction in ploidy.

Random Genetic Drift and the Efficacy of Selection on Ploidy Levels

> "Fisher has shown that it is only when [the coefficient of selection] is less than the reciprocal of the number of the whole population that natural selection ceases to be effective."
>
> —Haldane (1932, p. 54)

Although Haldane (1932) envisioned selection as the primary effector of evolutionary change, he also appreciated the limits of selection. As was pointed out by Haldane (see quote above) and more fully discussed in the work of Kimura (1983), selection is a potent force relative to drift only when the selection coefficient is greater than the inverse of the population size. This insight provides us with a method to render selection ineffective, simply by propagating populations at low size, allowing us to observe the evolutionary changes driven by mutation and drift. This idea underlies "mutation accumulation" experiments, where repeated bottlenecks reduce the size of a population and allow deleterious alleles to accumulate with little opposition from selection. Similarly, we can determine the extent to which observed ploidy transitions are driven by selection or mutation by repeating the above evolution experiments at smaller population sizes.

During the 1800-generation batch culture experiment of Gerstein et al. (2006), replicate haploid and tetraploid lines evolved toward diploidy. The initial experiment was conducted at very large population sizes, suggesting that there was something selectively beneficial about diploid cells that arose by mutation within these populations. It is, nevertheless, possible that there was a strong mutational bias driving organisms toward diploidy. To assess this possibility for tetraploid lines, we repeated the experiment in unstressed medium at very small population sizes, by reducing the population to a single cell every 48 h. In the original experiment conducted at large population size, 5 of 5 initially tetraploid lines were reduced in genome size (toward diploidy) by ~570 generations. In contrast, in the experiment conducted at small population size, a reduction in ploidy was present in only 2 of the 10 initially tetraploid lines by this time (Gerstein et al. 2006; Figure 4). This result does not rule out a mutational bias in favor of diploidy, but it does demonstrate that selection is required to explain the repeated and rapid convergence toward diploidy from tetraploidy observed in our experiments at large population sizes. Similar experiments comparing evolution in large and small populations are needed to determine the relative importance of selection versus mutation in driving the transition from haploidy to diploidy. A recent mutation accumulation study by Lynch et al. (2008) found that all 4 haploid lines evolved to near diploid levels within ~4800 cell generations, despite the fact that selection was largely rendered ineffective by repeated bottlenecks. Whether mutation pressure alone could account for the rapid transitions to diploidy within 200 generations that we report here remains to be determined (to do so, the mutation rate from haploidy to diploidy would have to be on the order of 1/200 per cell division).

Further work is needed to identify the exact mechanisms responsible for repeated transitions toward diploidy from both haploid and tetraploid *S. cerevisiae* and to determine the relative roles that selection, mutation, and drift play in driving these transitions.

Conclusion

> "The world is full of mysteries. Life is one. The curious limitations of finite minds are another. It is not the business of an evolutionary theory to explain these mysteries. Such a theory attempts to explain events of the remote past in terms of general laws known to be true in the present, assuming that the past was no more, but no less, mysterious than the present."
>
> —Haldane (1932, p. 3)

Large-scale shifts in genome size and content have accompanied the thousands of polyploidization events during the evolutionary history of eukaryotes. Nevertheless,

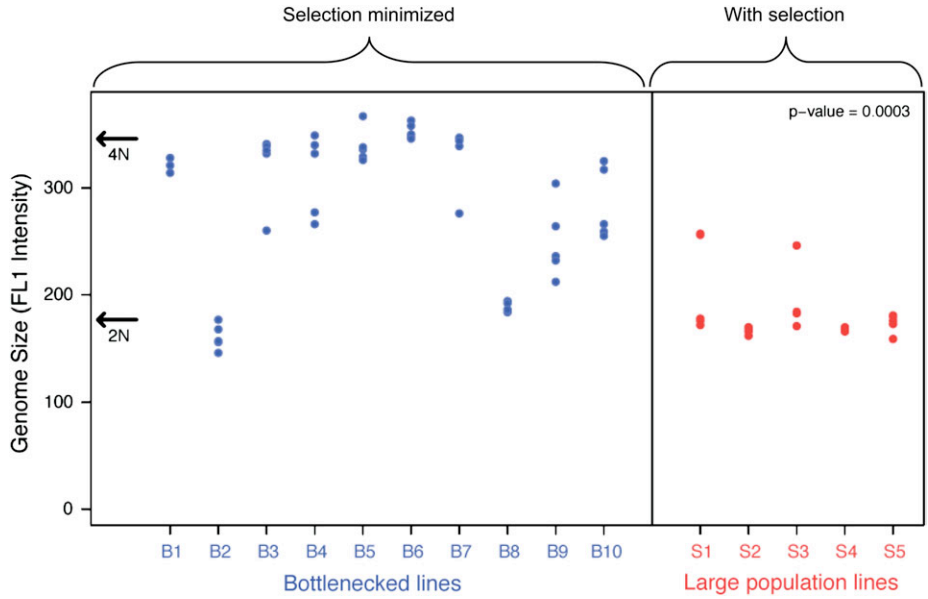

Figure 4. Genome size of 5 colonies isolated from each of 10 tetraploid lines that were evolved asexually at small population sizes for ~572 generations (bottleneck lines) and from each of 5 tetraploid lines evolved at large population sizes for ~558 generations (adapted from Gerstein et al. 2006). Compared with large populations, population bottlenecks cause the fate of mutations to be determined more by chance (inclusion in the colony used to initiate the next plate) and less by selection. Diploid colonies were present in all 5 lines evolved at large population size but only in 2 (B2 and B8) of the 10 bottlenecked lines (Fisher's Exact test, $P = 0.007$). The fact that reductions in genome size were observed significantly more often in populations of large size is consistent with selection favoring transitions from tetraploidy to diploidy.

we still have only a rudimentary understanding of how and why shifts in ploidy occur when they do. Experimental evolution studies with microbes promise to shed light on the rates of ploidy shifts, the immediate impact on fitness of a ploidy shift, the variation in this fitness impact among environments, and the long-term evolutionary consequences to organisms of different ploidy levels. Such studies have demonstrated the remarkably fluid nature of ploidy levels, with switches commonly observed following exposure to mutagens (Mable and Otto 2001) and even after a period of growth in standard medium (Gerstein et al. 2006; Schoustra et al. 2007) or in presporulation medium (Bennett and Johnson 2003). In *S. cerevisiae* and *C. albicans*, these shifts tend to restore the diploid genomic content typical of these species. Similarly, in *A. nidulans*, these shifts tend to restore the haploid genomic content typical of nuclei in this genus (whose mycelia consist of haploid or dikaryotic hyphae). One hypothesis consistent with these results is that selection acts to return ploidy to the level typical of the evolutionary history of an organism because past evolution has adapted the organism to the geometrical, metabolic, and/or physiological properties of that ploidy level, creating inertia that selects against shifts in ploidy.

Ploidy evolution provides an interesting example where shifts in ploidy level need not correspond to what would be optimal in the long term. From a theoretical perspective, although haploids suffer from the lowest mutation load, alleles that modify the life cycle and increase the diploid phase are able to spread within highly recombining populations because diploid individuals are better able to mask the deleterious mutations that currently segregate in the population (Perrot et al. 1991; Otto and Goldstein 1992). Ultimately, however, this masking leads to a higher load of mutations. From an empirical perspective, we found that haploids adapt faster to a stressful environment (YPD + salt) than diploids or tetraploids (Figure 2); yet, diploids spread within haploid populations under these conditions (Figure 1). In fact, diploids spread faster under high-salt conditions than under standard YPD conditions, despite the more dramatic ability of haploids to adapt to salt. This seeming contradiction highlights the fact that evolution is myopic: What ploidy level would adapt fastest in the long term is irrelevant if a different ploidy level can take over in the short term.

The emerging picture is one where ploidy shifts are largely governed by the immediate fitness effects to an organism composed of haploid, diploid, or polyploid cells, with the environment affecting the magnitude and direction of these fitness effects. More experimental work is needed to identify the range of environments most favorable to each ploidy level so that we can better understand the important functional differences between cells with different genome sizes. More experimental work is also needed to determine the fitness effects of allopolyploidization in yeast as almost all the experimental studies have focused on autopolyploids. Because of the potential to combine

favorable alleles that have arisen in different strains into permanent heterozygotes (e.g., see Spofford 1969), allopolyploids may gain a fitness advantage across environments, a prediction that deserves empirical verification in yeast.

More work is also needed to determine the factors driving transitions between haploidy and diploidy in *S. cerevisiae*. Diploids do not exhibit a general advantage over haploids in terms of growth rate, as pointed out early on by Weiss et al. (1975). This begs the question of why diploids spread rapidly and repeatedly when they arise within haploid populations. Are diploids better able to survive as resources dwindle or better able to resume growth when resources are renewed, such that they enjoy a slight competitive advantage? If so, why is this the case? Or do haploids simply undergo frequent mutations to diploidy? Despite the many open questions, or perhaps because of them, future evolutionary experiments with microbes hold great promise as a means to understand when, why, and how ploidy transitions occur.

Appendix

Growth Rate Measurements

Cultures of interest were removed from a −80 °C freezer and either streaked onto YPD plates (to obtain colony isolates) or inoculated directly into 10 ml of the appropriate medium (for population estimates). Colonies were grown on plates for 48 h prior to inoculation into liquid medium. After 48 h of growth in liquid medium at 30 °C with constant shaking at 200 rpm, 100 ul of stationary phase culture was transferred into 10 ml of fresh medium. For all, 150 ul aliquots of this culture were then pipeted into 2 randomly assigned wells of a Bioscreen C plate (Thermo-Labsystems). The plates were cultured in the Bioscreen at 30 °C with continuous shaking (except during measurements). Optical density measurements were taken automatically every half an hour for 48 h.

An analysis program was written by R. FitzJohn in the R programing language (R Development Core Team 2006). Splines were fit through the log-transformed optical density data to obtain the spline with the highest slope using the loess function. We interpret and record this as the maximal growth rate for each well. As 2 replicate measures were taken for each sample of interest, growth rates were averaged prior to statistical analyses.

Growth rate data were analyzed by *t*-test and by 1-way and 2-way ANOVAs as indicated. All statistical analyses were performed using JMP 4.0 (SAS Institute).

Ploidy Measurements

For Figure 2, ploidy was measured as described in Gerstein et al. (2006, 2008). Flow cytometry was performed on 25 colonies isolated from one haploid and one tetraploid line at generation 200 from both the unstressful and salt-stressed environments. In brief, the FL1 intensities from 30 000 cells dyed with Sytox green dye were measured on culture initiated from a single colony grown up for 48 h in YPD using a FACSCalibur (Becton Dickinson Immunocytometry Systems). Ancestral haploid, diploid, and tetraploid colonies were used as standards to assess ploidy levels among evolved colonies. Comaprative genomic hybridization to microarrays (Gerstein et al. 2006, 2008) has demonstrated that cells are typically euploidy, or nearly so. Culture samples from each isolated colony were frozen at −80 °C prior to flow cytometry analysis for use in further experiments (including the growth rate assays described above).

From the 25 colonies isolated at 200 generations, we selected 10 colonies that equally represented the different ploidy levels present at that time. Populations were found to be either monomorphic for ploidy (haploids grown in YPD) or dimorphic (haploids grown in YPD + salt, both tetraploid lines). For the 3 dimorphic populations, colonies from different ploidy levels were grouped, and 5 colonies were randomly selected from each ploidy group for growth rate analyses.

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