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Article

Ploidy Controls the Success of Mutators and Nature of Mutations during Budding Yeast Evolution

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Summary

Background: We used the budding yeast *Saccharomyces cerevisiae* to ask how elevated mutation rates affect the evolution of asexual eukaryotic populations. Mismatch repair defective and nonmutator strains were competed during adaptation to four laboratory environments (rich medium, low glucose, high salt, and a nonfermentable carbon source).
Results: In diploids, mutators have an advantage over nonmutators in all conditions, and mutators that win competitions are on average fitter than nonmutator winners. In contrast, haploid mutators have no advantage when competed against haploid nonmutators, and haploid mutator winners are less fit than nonmutator winners. The diploid mutator winners were all superior to their ancestors both in the condition they had adapted to, and in two of the other conditions. This phenotype was due to a mutation or class of mutations that confers a large growth advantage during the respiratory phase of yeast cultures that precedes stationary phase. This generalist mutation(s) was not selected in diploid nonmutator strains or in haploid strains, which adapt primarily by fixing specialist (condition-specific) mutations. In diploid mutators, such mutations also occur, and the majority accumulates after the fixation of the generalist mutation.
Conclusions: We conclude that the advantage of mutators depends on ploidy and that diploid mutators can give rise to beneficial mutations that are inaccessible to nonmutators and haploid mutators.

Introduction

Mutations that elevate the spontaneous mutation rate can accelerate evolutionary adaptation [1]. We refer to cells and populations that contain such mutations as mutators and to their counterparts that lack these mutations and have lower mutation rates as nonmutators. In bacterial populations, mutators outcompete nonmutators when mixtures of the strains are exposed to novel environments [2–5]. Mutators also appear in nonmutator cultures that are subjected to long-term selection, most likely because mutators that are originally present at low frequencies acquire beneficial mutations that carry the mutator to victory, a process referred to as hitchhiking [6–10].

These results suggest that mutators may play a large role in evolution, especially in populations that must frequently adapt to new or rapidly changing environments. However, understanding the circumstances under which mutators are likely to succeed and their general effects on evolution is a difficult problem. Mutators increase deleterious as well as beneficial mutations, and because they alter DNA metabolism, they change the spectrum of mutations [11, 12]. Therefore, their effects on adaptation are complex and depend on mutator strength, population size, ploidy, the kind and intensity of selection, the frequency of sex, and the availability of beneficial mutations [1, 13–17]. Several of these effects have been studied theoretically, and experimental studies of mutator bacteria have explored others [1, 10].

Little is known about the mutator effect in eukaryotic populations, apart from studies of cancer [18, 19]. In eukaryotes, the benefits of mutators are likely to depend on ploidy. Analyzing the systematic gene deletions in budding yeast suggests that most deleterious mutations are recessive, implying that mutators produce more deleterious mutations in haploids than they do in diploids [20]. Therefore, if many beneficial mutations are dominant or semidominant, increasing the mutation rate should confer a larger advantage in diploid populations than it does in haploid ones [21].

We used the budding yeast, *Saccharomyces cerevisiae*, to test this prediction, compare the advantage of mutators in different selective conditions, and study the effect of mutators on adaptation. We found that mutators have an advantage in diploid populations, but not in haploid ones. We also found that diploid mutators acquire a distinct class of mutations that provides a selective advantage in several different conditions.

Results

We used laboratory evolution of budding yeast to study competitions between mutators and nonmutators. Yeast proliferates rapidly, is genetically tractable, and can be maintained as a haploid or diploid, and its mutation rate can be genetically manipulated. Our mutator strains lacked the mismatch repair gene *MSH2*. They thus lack an essential component of the mismatch repair machinery, increasing their frequency of point mutations, especially in repeats of mono-, di-, and trinucleotides [11, 22]. In addition, *msh2Δ* strains have a lower barrier to recombination between similar sequences located in different parts of the genome, leading to a higher frequency of chromosomal rearrangements [12, 23].

In each experiment, mutator and nonmutator cells were mixed and grown together for about 350 generations. We refer to these experiments as competition experiments since mutator and nonmutator populations evolved together in a common environment. Multiple,

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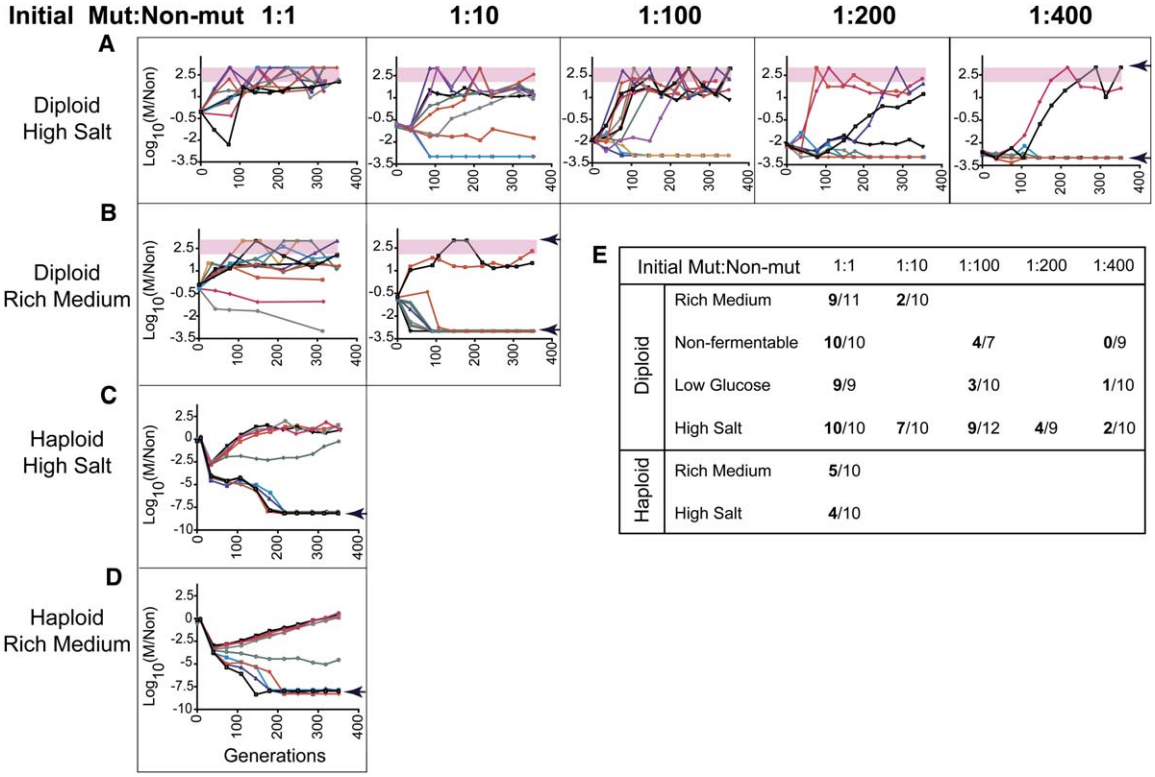

E

	Initial Mut:Non-mut	1:1	1:10	1:100	1:200	1:400
Diploid	Rich Medium	**9**/11	**2**/10			
	Non-fermentable	**10**/10		**4**/7		**0**/9
	Low Glucose	**9**/9		**3**/10		**1**/10
	High Salt	**10**/10	**7**/10	**9**/12	**4**/9	**2**/10
Haploid	Rich Medium	**5**/10				
	High Salt	**4**/10				

Figure 1. Mutator versus Nonmutator in High Salt and Rich Medium

The mutator and nonmutator strains differed by genetic markers that did not lead to fitness differences on the selective medium but allowed the two populations to be distinguished on analytical media. The results of several independent mutator (M) versus nonmutator (Non) replicates competed at the indicated initial M:Non ratios in high salt (A).

(B) Competitions in rich medium at the initial M:Non ratios 1:1 and 1:10. Haploid 1:1 M versus Non in high salt (C) and rich medium (D). The error bars (standard deviation [SD]) were removed from all panels for clarity. These data sets are shown with error bars in Figures S2–S12. Arrows indicate values of $\log_{10}$(M/Non) where either no mutator (−2.99) or nonmutator (3.0) cells were detected, and frequencies were estimated as 1/(2n) where n, the number of colonies tested, was 500 (A and B). In (C) and (D), the arrows indicate $\log_{10}$(M/Non) values of −7.87–−8.28, where the frequency of mutators was estimated using $n = 3.65 \times 10^7 - 9.6 \times 10^7$. Note the different scale of the y axis for (C) and (D) that reflects these higher resolution data sets. The pink bar shows the 99% lower confidence interval for the maximal mutator:nonmutator ratio. It represents the range of ratios at which the probability of seeing no nonmutator colonies is greater than 0.01. We use a 99% confidence interval since many time courses contain multiple time points for which no nonmutator colonies were observed.

(E) Mutator winners table: the first number in bold is the number of independent competitions in which the mutator genotype is in the majority at the end of the experiment. The second number is the total number of competitions in the data set.

replicate populations of mutator (*msh2Δ*) and nonmutator (*MSH2*) populations were mixed at a specified ratio, inoculated into selective medium, and allowed to proliferate for 16 hr. Populations were then diluted 10- to 30-fold, giving effective population sizes ranging from 9×10^6 to 2×10^7, depending on the selective condition. We chose large population sizes to minimize the contribution of genetic drift. We classified the winner as the majority genotype at the end of the experiment. In 105 of the 146 competitions the final winner:loser ratio exceeded 100, in 33 it was between 100 and 50, and in only 8 was it less than 50.

Diploid Mutators Have a Large Advantage over Nonmutators

In diploid populations, we expect the deleterious effect of mutators to be smaller since many deleterious mutations are recessive. However, the effect of diploidy on beneficial mutations is hard to predict, since we do not know how these are distributed between fully recessive, semidominant, and fully dominant mutations.

We examined initial diploid mutator:nonmutator ratios that ranged from 1:1 to 1:400 in four different selective conditions: the rich medium (yeast extract, peptone, 2% glucose [YPD]) in which laboratory populations of budding yeast are most commonly grown, a mixture of nonfermentable carbon sources (yeast extract, peptone, 2% glycerol, and 2% ethanol [YPEG]), high salt (YPD + 0.75 M NaCl), and low glucose (yeast extract, peptone, 0.05% glucose). The strains we used are isogenic with the laboratory strain W303 [24], which has been cultivated on rich medium for an unknown but large number of cell divisions. In contrast, laboratory strains are not usually propagated in the other media, and we thus refer to nonfermentable carbon sources, high salt, and low glucose as novel selections.

For each selective condition, we set up and followed several independent, replicate experiments. A subset of this data is shown in Figure 1 with the remainder appearing in the Supplemental Experimental Procedures in the Supplemental Data available with this article online. Figure 1A shows the outcome of competitions

between mutator and nonmutator strains selected in high salt. When mutators and nonmutators start at a 1:1 ratio, the mutators won all 10 competitions; they won 9 out of 12 at a 100-fold numerical disadvantage and still won a minority of competitions at 1:400. The changing ratio of mutators to nonmutators also influenced the dynamics of the competition with the mutators winning more slowly when they started at a smaller fraction of the population.

We measured the rate of mutation to 5-fluoroorotic acid drug resistance in the nonmutator and mutator populations as 1.4×10^{-7} and 1.4×10^{-6} per cell per generation, respectively (G. Lang and A.W.M., unpublished data). The mutation rate of *msh2Δ* cells matches values measured by others [25], but our nonmutator strain has a higher rate than those of other laboratory strain backgrounds. Thus, in our strains, the removal of Msh2 only raises the mutation rate 10-fold. The ability of mutators to beat nonmutators at ratios as low as 1:400 suggests that mutators can have an advantage over nonmutators that is substantially greater than the extent to which they elevate the genome-wide mutation rate, a point we return to in the Discussion.

Figure 1B shows the outcome of competitions in rich medium. In contrast to the results with high salt, the mutators failed to win all the competitions at a 1:1 mutator:nonmutator ratio and lost the majority at 1:10. At this ratio, the difference between mutators winning most of the competitions in high salt and losing most of them in rich medium is statistically significant ($p = 0.025$, chi-square test). Although mutators won fewer competitions in rich medium, when they did win, they won as fast they did in high salt. Figure 1E tabulates the results from the nonfermentable carbon sources and low-glucose competitions and reveals that in both these conditions mutators won more often than they did in rich medium (Figure S1).

Haploid Mutators Behave Differently from Diploid Mutators

We competed haploid mutators with haploid nonmutators to ask whether ploidy affected the advantage of mutators. In both high salt and rich medium, haploid mutators won roughly half of the competitions that started at a 1:1 mutator:nonmutator ratio. For high salt, the diploid mutators won significantly more competitions against their nonmutator counterparts than the haploid mutators did against theirs ($p = 0.003$, chi-square test). The combined results for rich medium and high salt, where diploid mutators win 19 out of 21 competitions and haploids win 9 out of 20, are also significant ($p = 0.002$, chi-square test).

The dynamics of the haploid and diploid competitions differed. In 1:1 mutator:nonmutator, diploid competitions, the abundance of the mutators had increased by the first time point ($\approx$30 generations) in most cultures. In contrast, in 1:1 haploid competitions, the abundance of the mutators had fallen at least 100-fold by the same time point, even in those trials that the mutators were destined to eventually win (Figures 1C and 1D). This result supports the widely held belief that most deleterious mutations are recessive, making the harmful effects of mutators greater in haploids than in diploids and explaining why mutators are less likely to outcompete nonmutators in haploid populations.

Diploid Mutators Accumulate Generalist Mutations

Two features of the 1:1 mutator:nonmutator, diploid competitions surprised us: mutators won competitions on rich medium, where the naïve expectation is that beneficial mutations should be hard to find, and the speed with which mutators won competitions was similar in all four conditions. Both observations suggested that the mutators might be acquiring a generalist class of mutation that gave them an advantage in several different environments, rather than specialist mutations that improved fitness only in the environment where the selection had occurred.

If diploid mutators acquire a generalist mutation, their fitness in the selective condition and other environments will increase at the same time. To test this prediction, we measured the fitness of our populations at several time points, testing them both in the environment they had been evolved in and in the other three environments we examined. We measured fitness by mixing an ancestral diploid strain marked with yellow fluorescent protein (YFP) with the unlabeled evolved population and measuring the initial ratio of the two strains using a fluorescence-activated cell sorter, competing the ancestral and evolved populations for 8 to 15 generations, and then measuring the final ratio of the strains. We used the initial and final ratios to compute the fitness of the evolved strain relative to the ancestor (see Experimental Procedures).

When we used rich medium to measure the fitnesses of evolved populations, the results were strikingly similar both amongst replicate populations from a single environment and between populations evolved in different environments. In all 16 diploid populations which began at 1:1 mutator:nonmutator, there was a rapid initial jump in fitness from 1.02 (the mean fitness of the unlabeled ancestral clones is >1 which reflects the fitness cost of expressing YFP in the reference strain) to a mean of 1.36, which occurred within $\approx$30 generations from the beginning of the experiment, and there was little subsequent change in fitness. The simplest interpretation of these results is that a single beneficial mutation with a large selective advantage occurred early in each mutator population and fixed rapidly and that this mutation is beneficial in several conditions. Subsequent beneficial mutations were of much smaller or negligible effect. Figure 2E shows the mean changes in fitness on rich medium at the end of the experiment. All of the evolved populations are significantly fitter than the ancestor, but the fitness of the populations evolved under different conditions are not significantly different from each other, including those populations that were evolved for more than 300 generations on rich medium.

Figure 2B shows the fitnesses of the same populations, measured on low-glucose medium. The results are subtly different from measuring the fitnesses of the same populations in rich medium. In low glucose, all the populations had a similar rapid initial increase in fitness, but the populations evolved in nonfermentable carbon sources and low glucose showed a continuing, slower increase in fitness, whereas the populations evolved in rich medium and high salt did not increase

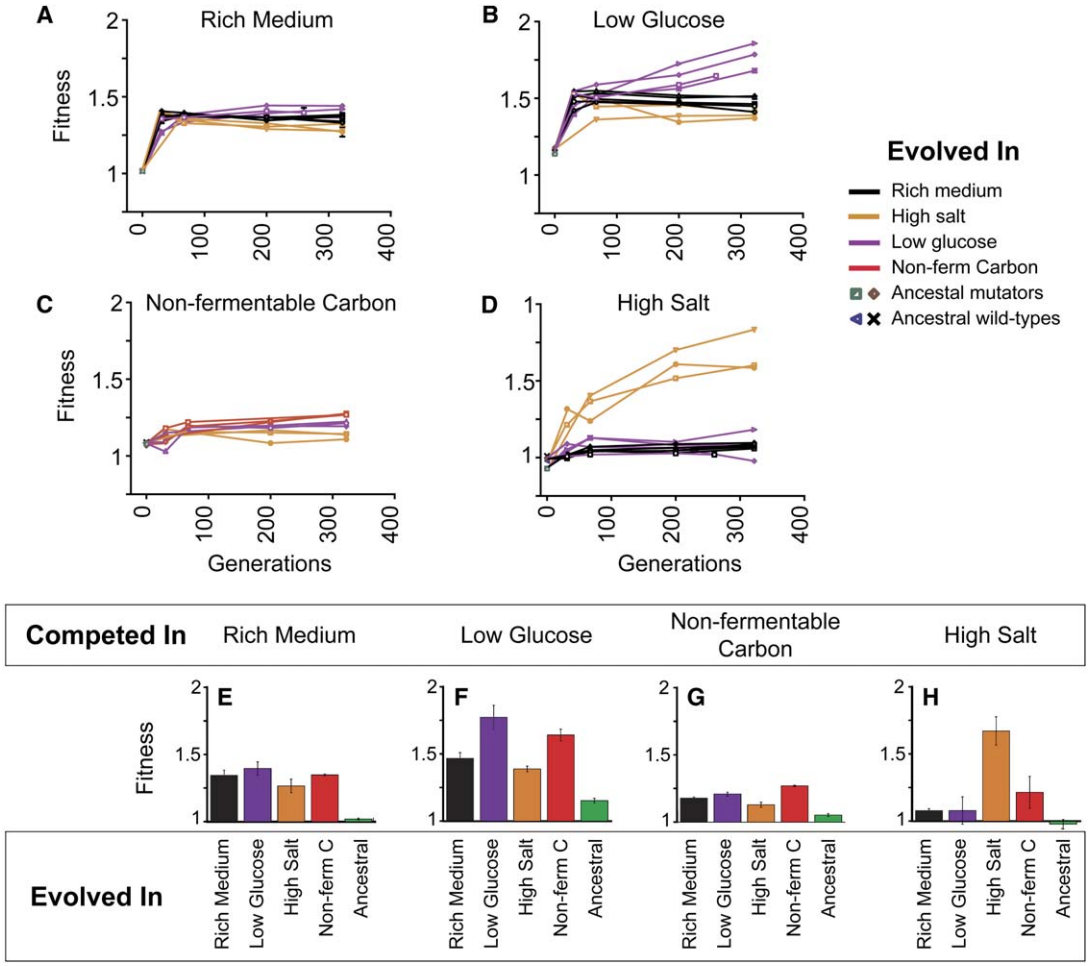

Figure 2. Fitness of Evolved Diploid Mutator Populations over Time and across Conditions

The mean population selective advantage (*s*) was assayed from competitions in which mutators won. The fitness of the ancestral reference is 1.0 and that of evolved populations is 1+ *s*. Time points from several populations originally evolved in rich medium, low glucose, and high salt were each competed with the ancestral reference in rich medium (A), low glucose (B), and high salt (D). Data for the populations evolved on the nonfermentable carbon source are in Figure S13.

(C) Time points from several populations originally evolved in the nonfermentable carbon source, low glucose, and high salt were each competed with the ancestral reference in the nonfermentable carbon source. Data for the rich medium populations are in Figure S13. Ancestral mutator clones (A and B) and ancestral nonmutator clones (A and B) were assayed in all conditions as zero generation controls. Error bars are ± standard error (SEM).

(E, F, G, and H) Summary plots of the data for each assay condition where each column is the average of the mean population fitness for the ≈350 generation (final) time points for all populations evolved in each condition. The ancestral column is the average of the mean population fitness for all four ancestral clones. The error bars are ± SD.

in fitness after the initial jump. Figure 2F shows the final mean fitness of the populations, measured on low glucose. All of the evolved populations were significantly fitter than the ancestor, but the mean fitness of the populations evolved in low glucose was significantly greater than those of populations evolved in the other three environments. We conclude that an early mutation (or mutations) occurs under all selections that begin at a 1:1 mutator:nonmutator ratio, giving rise to a fitness advantage on low glucose, and that only populations evolved on nonfermentable carbon and low glucose accumulate subsequent smaller effect mutations that lead to a continuing fitness increase in this environment.

Figure 2C analyzes populations fitnesses measured on nonfermentable carbon sources. On nonfermentable carbon sources, strains evolved in all four conditions showed a smaller initial fitness increase than those seen when the same strains were assayed on rich medium or low glucose, but there was a subsequent slow increase in fitness in the populations selected on nonfermentable carbon sources. Figure 2G shows the mean changes in fitness on nonfermentable carbon sources at the end of the experiment. All of the evolved populations are significantly fitter than the ancestor, but the mean fitness of the populations evolved in nonfermentable carbon sources was significantly higher than those evolved in the other three conditions.

Figure 2D shows that the population fitnesses measured on high salt were qualitatively different from those measured in the other three environments. The populations evolved in high salt had an initial, large fitness increase (mean = 1.31), followed by a continuing gradual increase in fitness. In high salt, the populations evolved in the other three conditions did not show the initial

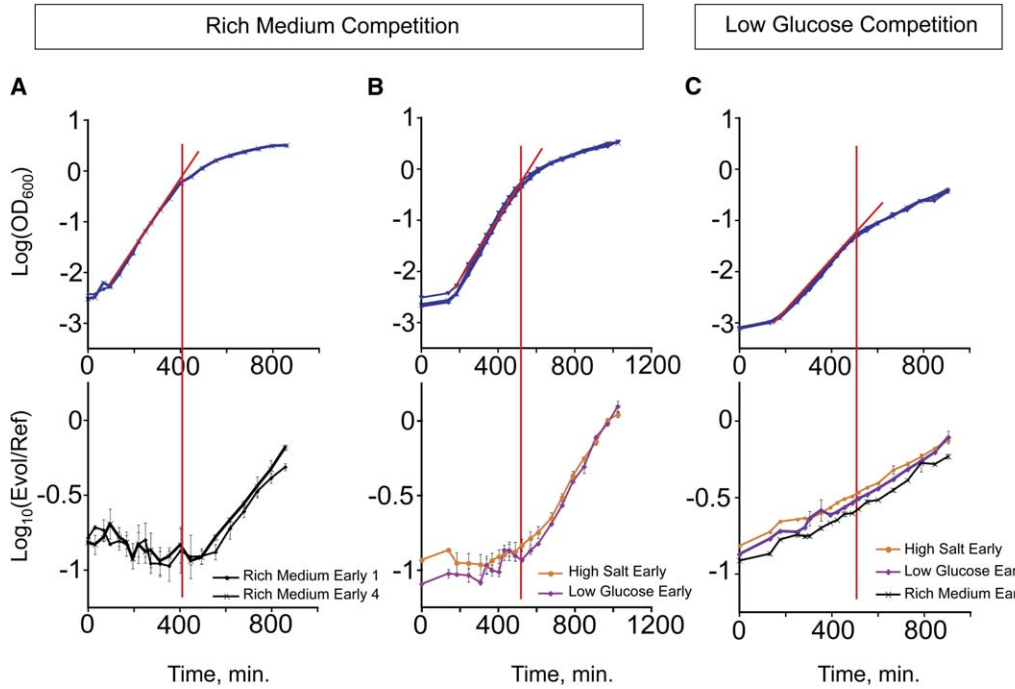

Figure 3. Evolved Populations with the Generalist Phenotype Have Their Greatest Advantage after Exponential Growth Ceases

Top panels: growth curves, log (O.D. 600), were generated for mixtures composed of a minority of an evolved population with the generalist phenotype and a majority of the reference strain. Bottom panels: measurements by flow cytometry of the evolved/reference ratio ($\log_{10}$(Evol/Ref)) for the indicated times. The vertical lines demarcate the end of exponential growth to the left and the beginning of a slower growth rate to the right, a transition called the diauxic shift.

(A) Populations from two mutator lines that had won competitions in rich medium at an early (≈30 generation) time point competed with the reference in rich medium.

(B) Mutators that had won after ≈30 generations in low glucose and high salt competed with reference in rich medium.

(C) The early populations from cultures evolved on rich medium, low glucose, and high salt were competed with the reference in low glucose. Error bars are ± SEM.

fitness jump and never exceeded the fitness of their ancestors in high salt (Figure 2H).

We conclude that diploid mutators populations fix an unusual class of mutations. These mutations appear early in all competitions won by diploid mutators in all four different environments. They confer a large selective advantage in three environments (rich medium, low glucose, and nonfermentable carbon sources). Their magnitude depends on the environment they are assayed in, and their effects are very similar in 16 independent populations despite the fact they were selected in four different environments.

Although the first mutations in 1:1 competitions have rather general advantages, later mutations do not. For every test environment except rich medium, the fittest strains at the end of our experiments were always those evolved in the test environment. This effect is most prominent for strains tested in high salt.

The third conclusion is that fitness evolves reproducibly in diploid, mutator populations evolved in the same environment. Even after the initial fitness jump caused by the generalist class of mutations, fitness increased at similar rates in independent mutator populations.

The Generalist Class of Mutations Confers Its Advantage during Respiration

The generalist class of mutations found in diploid mutator winners confers a large and consistent fitness advantage. This advantage varies depending on the condition in which the winners are tested, ranging from a mean of 1.14 when cells are grown on nonfermentable carbon sources to 1.45 on low-glucose medium. We did not find such large-effect mutations in populations that had been evolved in low glucose, but were never allowed to cease exponential proliferation (M.M.D., unpublished data), suggesting that the generalist mutations might confer their advantage after the diauxic shift, the period when cell proliferation slows as cells stop fermenting and begin oxidizing the ethanol that they have produced during fermentation (as well as any remaining glucose) [26].

To test this prediction, we mixed a minority of unlabeled diploid, mutator winners with a majority of fluorescently labeled ancestral cells and followed their proliferation. The mutator populations all came from early in the evolution experiments, at a point when we believe that the generalist class of mutation accounts for almost all of the selective advantage of the mutators. During the time course, the overall size of the combined populations was monitored by optical density and the ratio of the two ancestral and evolved cells was quantified by flow cytometry.

Figure 3A shows the ancestral reference strains competed with two different diploid mutators that had evolved on rich medium. After a short lag, the culture grows exponentially for 6 hr. During this period, the ratio

of evolved to reference cells remains constant. At 400 min, cell proliferation slows, marking the diauxic shift. Shortly afterwards, the fraction of the evolved cells begins to increase, and over the next 400 min the ratio of evolved to reference cells increases 3- to 4-fold, confirming that the evolved populations have a large advantage over their ancestors after the diauxic shift.

Figure 3B shows a similar experiment, but in this case the mutator populations had evolved on high salt or low glucose and were competed with the reference in rich medium. The outcome of this experiment is strikingly similar to the one where the evolved cells had been evolved on rich medium: the evolved cells have no detectable advantage during exponential growth but show a strong and continuing advantage after the diauxic shift.

Figure 3C measures the performance of those same populations evolved in rich medium, high salt, and low glucose, respectively, competed with the reference on low-glucose medium. In this environment, the reference cells proliferate more slowly than they do in rich medium, and the evolved cells continually increase in number relative to the reference cells both before and after the diauxic shift.

In Diploids, Mutators Evolve Differently from Nonmutators

We examined the behavior of diploid, nonmutator populations that had won competitions. Nonmutator populations that had won in low glucose and high salt were competed against the ancestral reference strain. Figure 4 shows how the fitness of diploid, nonmutator winners changed when they were evolved in low glucose and high salt. The fitness of the strains was measured in rich medium, low glucose, and high salt, and in each panel the data for diploid, mutator winners (Figure 2) is reproduced for comparison.

The nonmutator winners differed from their mutator counterparts in four ways. First, the behavior of different nonmutator winners evolved under the same conditions differed from each other, whereas one mutator winner behaved much like another. Second, the early jump in fitness seen in every mutator winner was not seen in any of the nonmutator winners. Third, in most assays, the mutator winner displayed higher average final fitnesses than the nonmutator winners. Finally, the generalist class of mutations found in all the mutator populations was not present in the nonmutator populations. This is most clearly shown by the populations evolved in high salt. By 60 generations, all of the mutator winners show fitnesses at least 30% greater than that of their ancestors, when measured in all four environments. If the generalist class of mutation occurred in nonmutators, it might appear later in these populations, and we therefore examined nonmutator winners, evolved in and analyzed on high salt, when their fitness had also increased by 30% (about 200 generations). But when assayed on low glucose, none of the nonmutator winners evolved on high salt showed a fitness increase of more than 5% on low glucose (Figure 4).

Comparing Haploid and Diploid Populations

In diploids, mutators win more contests than nonmutators, and mutator winners get fitter faster than nonmutator winners. We asked if these trends held in haploids.

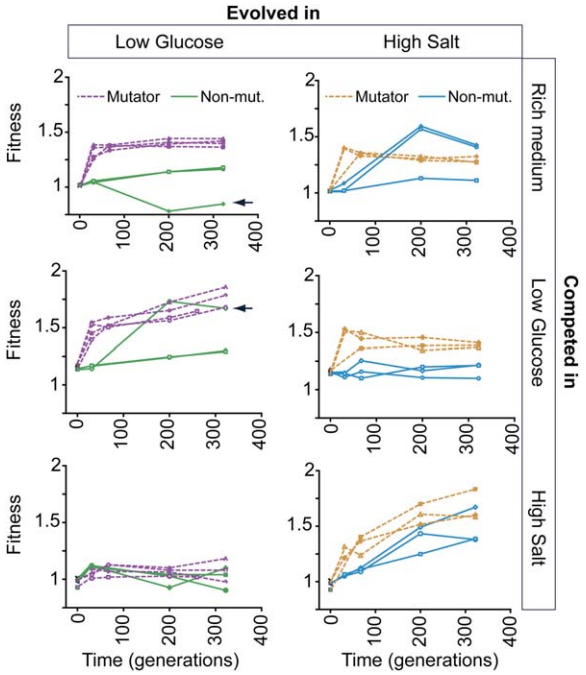

Figure 4. Fitness of Mutator Winners versus Nonmutator Winners

The mean population fitness was assayed from competitions in which nonmutators won. Left panels: time points from several nonmutator winner populations originally evolved in low glucose were each competed with the reference strain in rich medium, low glucose, and high salt. Right panels: time points from several nonmutator populations originally evolved in high salt were competed with the reference strain in rich medium, low glucose, and high salt. The relevant data from the mutator winners presented in Figure 2 is shown with dashed lines for direct comparison. Ancestral mutator clones (A and B) and ancestral nonmutator clones (A and B) were assayed in all conditions as zero generation controls. The arrows indicate a nonmutator population that was evolved on low glucose and has a large selective advantage in low glucose combined with a selective disadvantage in rich medium.

We found that when assayed on high salt, the haploid nonmutator winners show a higher average fitness than the mutator winners throughout the course of the evolution (Figures 5A, 5C, and S14). We conclude that in haploids, mutator winners increase in fitness more slowly than nonmutator winners, whereas the reverse is true in diploids.

We asked if generalist mutations appeared in haploids by taking populations evolved in high salt and measuring their fitness on low glucose (Figures 5B and 5D). Over the course of 350 generations, there was no statistically significant increase in the fitness of either the mutator or nonmutator winners in low glucose, showing that the generalist class of mutation does not appear in haploids. We conclude that the types of mutations that are selected in evolution are affected by both ploidy and mutation rate.

Discussion

We competed nonmutator and mutator strains under different conditions. In diploids, mutators can win competitions even when starting at a large numerical disadvantage to nonmutators, whereas in haploid strains, mutators and nonmutators are equally likely to win

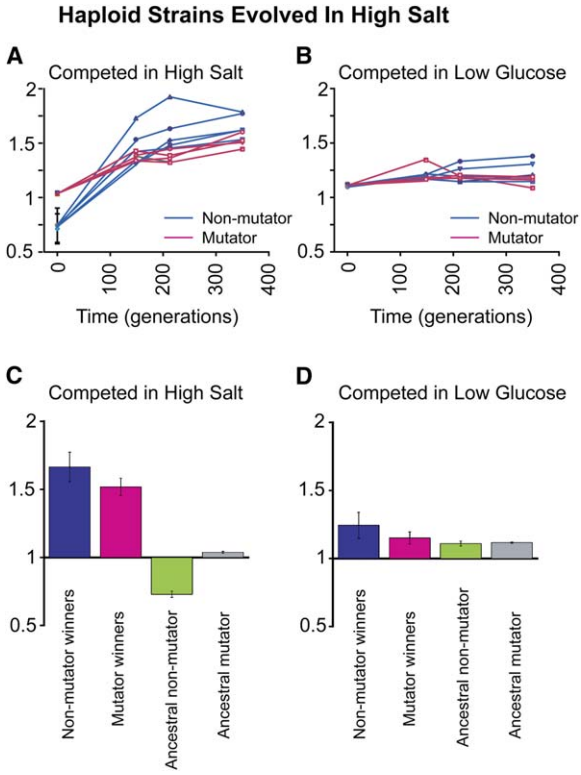

Figure 5. Fitness of the Haploid High-Salt Mutator and Nonmutator Winners

Samples from five nonmutator winners and four mutator winner lines originally evolved in high salt were each competed with the diploid reference strain in high salt (A) and low glucose (B). Samples were taken from the evolving populations at the indicated times, and the mean population fitness of nonmutator winners is compared with that of mutator winners. The ancestral haploid nonmutator (5a, 23c) and mutator clones (24c, 16c) were assayed in both conditions as zero generation controls.

(C and D) Summary plots of the data for each assay condition where each column is the average of the mean population fitness for the ≈350 generation (final) time points for all nonmutator winner and mutator winner lines, respectively. The ancestral nonmutator column is the average of the mean population fitness for the two nonmutator clones. The ancestral mutator column is the average of the mean population fitness for the two mutator clones. The error bars are ± SD. The difference in average mean fitness between nonmutator and mutators winners becomes more marked when the fitness of the evolved populations is compared to that of the ancestral strains, because the nonmutator haploid ancestor is surprisingly but reproducibly less fit than the haploid mutator ancestor relative to the diploid ancestral reference strain.

competitions at a 1:1 ratio. The diploid mutator strains uniquely acquire a generalist class of mutations.

The Advantage of Diploid Mutators

We begin by considering the outcome of diploid competitions. The simplest model is of two populations racing to produce the first beneficial mutation that survives genetic drift and then spreads through the population [27]. In this scenario, later mutations can change the rate at which winners prevail but cannot change which population wins. Thus mutators win when they generate a beneficial mutation before the nonmutators and lose when they do not. This model predicts that the fraction of cultures where mutators win is $r\lambda/(1 + r\lambda)$, where r is the mutator:nonmutator ratio and λ is the elevation in mutation rate in the mutators (which is about 10-fold for our strains). For a 1:10 mutator:nonmutator mixture, r = 0.1, λ = 10, and $r\lambda$ = 1, meaning that a beneficial mutation is equally likely to occur first in the mutator or the nonmutator population and mutators should win half the time. At a mutator:nonmutator ratio of 1:100, the mutators should win 9% of the time, and so on.

This simple hypothesis is contradicted by our data. In the three novel conditions, mutators are more successful than it predicts. At a 1:100 mutator:nonmutator ratio, the mutators win 16 of the 29 competitions in novel conditions. If the race model were correct, the chance of the mutators winning as many as 16 out of 29 competitions at a mutator:nonmutator ratio of 1:100 would be 5×10^{-10} (binomial distribution). Even if the absence of Msh2 elevated the mutation fold 60-fold, this result would still be unlikely ($p = 0.04$). A second prediction is that mutators should fare just as well in rich media as in the other novel conditions, because the number of beneficial mutations available is unimportant in a race: what matters is that mutators get the first mutation faster than the nonmutators. Yet the mutators are much less successful in rich than in novel media ($p < 0.025$ chi-square test; Figure 1E). In the race model, mutators would only win more often in novel conditions if λ for this class of mutations were higher for novel conditions than it was for rich medium. This is unlikely.

We thus need other explanations for the large advantage of the diploid mutator populations adapting to novel conditions. Three factors can alter the race model. The first is that different beneficial mutations have different effects, so the first mutation does not always decide the competition. For example, if the nonmutator population gets the first beneficial mutation but a mutator later gets a larger-effect mutation, the mutators will win. Second, multiple beneficial mutations may be involved [28]. This effect can allow mutators to win even when the nonmutator population gets the first beneficial mutation. If the mutator population gets a later mutation that gives the same benefit, the outcome of the competition is now determined by who gets the second mutation. The two single mutant populations grow at the same rate, but the mutator population has a higher mutation rate and thus a higher probability of producing successive beneficial mutations. The third factor is the existence of deleterious mutations. Since these occur more often in mutators, the mutator fraction of the population will fall continuously, meaning that the later the critical beneficial mutation occurs, the more likely it will appear in a nonmutator [15]. A greater number of beneficial mutations that increase fitness in novel media can account for the greater success of diploid mutators in novel conditions. A beneficial mutation in the mutator population whose effect is too small for it to be likely to win a competition on its own can still be large enough to slow down the decline of the mutators caused by deleterious mutations and thus allow the mutator population to survive long enough to acquire the larger mutations that lead it to triumph. Computer simulations show that the ability of mutators to beat nonmutators depends strongly on the balance between the rate of beneficial and deleterious mutations. A 10-fold decrease in the beneficial mutation rate can take mutators from winning

64% of the competitions starting at 1:100 mutator:nonmutator ratio to winning only 30% of those that start at 1:1 (A.W.M., unpublished data).

The dynamics of our diploid competitions support this model. In rich medium, mutators either won or lost rapidly, as we would expect if small effect beneficial mutations were rare and there is a race between the accumulation of deleterious mutations, which can drive the mutator extinct, and a large effect mutation, which lifts it to victory. In contrast, at very low mutator:nonmutator ratios in novel conditions, the initial mutator:nonmutator ratio was often roughly maintained for long periods until the mutator rapidly won the competition. The simplest interpretation of this long standoff is that the mutator population was accumulating a mixture of deleterious and beneficial mutations that allowed its fitness to increase in step with that of the nonmutator population, until the stand off ended when the generalist class of mutations occurred in the mutator population, leading to a dramatic increase in its fitness. This explanation is supported by our evidence that specialist mutations occur in novel conditions but not in the familiar rich medium (Figure 2). This model also predicts that diploid mutator winners from rich medium competitions should be less fit than nonmutator winners in rich medium, which is what we observe (Figure S14).

Early, small effect mutations may also explain the apparently paradoxical relationship between fitness in high salt and the generalist class of mutations. Diploid, mutator populations that won high salt competitions all contain generalist mutations, but mutator winners from other conditions have no detectable fitness advantage on high salt even though they contain the same class of mutation. This puzzle can be resolved by assuming that the diploid mutator winners on high salt acquired a small beneficial mutation that allowed the generalist mutation to confer a large advantage in high salt. Populations evolved on other media would have no fitness advantage in high salt because they would lack this salt-specific, small effect mutation.

The Influence of Ploidy

In haploid populations, mutators were much less successful than in diploid ones. Even at a 1:1 ratio, they won only half of the competitions in both rich medium and high salt. A simple explanation of this result is that most deleterious mutations are recessive so that haploids bear their cost and diploids do not, giving haploids less time for beneficial mutations to occur before deleterious mutations eliminate the mutators.

This is precisely what we see. In every haploid competition, the mutators fell at least 100-fold in abundance by $\approx$30 generations, and in all their victories, their subsequent rise implies that a beneficial mutation or mutations had already occurred and begun to fix. If it had not, the mutators were doomed to extinction. The speed at which the mutators fall is surprising, it implies a deleterious mutation rate of $\approx$0.15, given the fact that fitness of the mutator and nonmutator starting populations is similar, when measured against the fluorescently labeled reference strain. In diploid competitions, this initial fall in the abundance of mutators was less frequent and less pronounced even in competitions where the mutators won late or not at all. Our computer simulations show that increasing the deleterious mutation rate has a stronger effect than reducing the beneficial rate (A.W.M., unpublished data), so that even if diploidy reduced beneficial and deleterious mutation rates equally, the advantage of mutators would be much less in haploid than diploid populations.

We examined how ploidy affected the overall fitness increase of mutator and nonmutator populations evolved in high salt. In high salt, the relative fitness of the four populations was haploid nonmutator winners > diploid mutator winners $\geq$ diploid nonmutator winners > haploid mutator winners (Figure S14). These relative fitness rankings are all significant ($p < 0.05$), except that diploid nonmutator winners are not significantly less-fit than diploid mutator winners ($p = 0.07$). The low fitness of the haploid mutator winners is consistent with the idea that recessive deleterious mutations impose a large burden on haploid mutators, whereas the high fitness of haploid nonmutator winners suggests that at least some of the beneficial mutations are recessive, and is consistent with the previously observed advantage to haploidy in large yeast populations [29].

Novel Mutations

We observed a particular class of generalist mutation in all the diploid mutator winners. Cells carrying this class of mutation had very uniform properties, including a large selective advantage in rich medium, low glucose, and glycerol, and the ability to proliferate more rapidly than ancestral cells after the diauxic shift. This uniformity suggests that all the members of this class of mutations occurred within a single physiological pathway and possibly within a single gene. We used DNA microarrays to analyze the pattern of gene expression in several clones with the generalist phenotype. Although these strains had been evolved in different conditions, all of them showed similar increases in the expression of genes involved in gluconeogenesis (D.A.T., unpublished data).

None of the diploid nonmutator, haploid mutator, or haploid nonmutator winners showed this class of mutation. This observation leads to two conclusions. The absence of the generalist mutations from diploid nonmutators shows that the absence of Msh2 increases the frequency of this class of mutation much more than that of other beneficial mutations and (that is, λ is substantially more than 10 for the generalist mutation). The failure to find these mutations in haploid mutator winners suggests that this mutation is neutral or deleterious in haploids. The *msh2*Δ mutation preferentially elevates two types of events: adding or deleting nucleotides from a run of As, Gs, Cs, or Ts and recombination between related but nonidentical sequences [11, 23, 30]. Several recombination events between transposon-like sequences dispersed throughout the yeast genome were identified in diploid yeast strains adapting to glucose-limited media [31]. Such recombination events could produce chromosomal rearrangements that were strongly advantageous to heterozygous diploids but would be deleterious in haploids. A greater number of repeated elements and thus more opportunities for deleterious chromosome rearrangements in yeast compared to bacterial genomes might explain why our haploid mutators had no advantage compared with the

significant advantage of bacterial mutators adapting to novel environments [2–4].

The unique occurrence of the generalist class of mutation in diploid mutators indicates that changes in DNA metabolism can have large effects on the types of mutations that are selected. This observation has implications for understanding the role of genetic instability in cancer progression. Malignant tumors must acquire several successive genetic changes, producing a selection for a mutator and other forms of genetic instability [19, 32, 33]. In humans heterozygous for mutations at the *hMSH2* locus, rare mutations that inactivate the second *hMSH2* allele produces mutator cells [19] that go on to cause hereditary nonpolyposis colorectal cancer, by accumulating mutations that allow cancer cells to proliferate when their normal counterparts do not. Our results suggest that the different forms of genetic instability in different cancers are likely to preferentially elevate the frequency of mutations in different genes, explaining why cancers that arise from different forms of genetic instability are phenotypically distinct.

Conclusions

Our results reveal that many factors determine who wins in asexual contests between nonmutator and mutator strains. These include the ploidy of the strains, the nature of the selection, and the initial ratio between mutator and nonmutator. Under the right combination of factors, mutators can take over populations very quickly. If such combinations have occurred often, mutators are likely to have played an important role in evolution, especially in populations where sex is rare.

Experimental Procedures

Our methods are outlined here and presented in more detail in the Supplemental Experimental Procedures.

Yeast Media and Strains

Our yeast strains were adapted in the following media: rich medium (YPD—1% yeast extract, 2% peptone, 2% glucose), high salt (YPD plus 0.75 M NaCl), low glucose (1% yeast extract, 2% peptone, 0.05% glucose), and a combination of nonfermentable carbon sources (YPEG—1% yeast extract, 2% peptone, 2% glycerol, 2% ethanol).

All strains were derivatives of the W303 background [24] (Table S1). Two ancestral mutator (*msh2Δ*) and two isogenic nonmutator (*MSH2*) diploid strains were constructed by mating the colonies derived from *MSH2* and *msh2Δ* spores that were the products of an *msh2Δ/MSH2* diploid. The proliferation of both haploids and diploids before the start of our evolutions was minimized to reduce the accumulation of mutations before the beginning of our experiments. The nonmutator reference strain, used for measuring fitnesses, expressed YFP fused to the C terminus of Cwp2. The expression of this protein confers a modest selective disadvantage in low glucose ($\approx$0.12 per ancestral reference generation), but since all fitnesses were measured relative to the same ancestor, this does not affect estimates of the difference between the fitnesses of different evolved populations.

Evolution and Fitness Measurements

Frozen aliquots of the ancestral strains were thawed, patched onto YPD plates, and allowed to grow overnight at 30°C. These cells were incubated at 30°C overnight in a nonfermentable carbon source to eliminate petite mutants, which lack mitochondrial function. Mutator and nonmutator cells were combined into the selection medium at the appropriate ratio to yield a final total cell concentration of 1×10^7 cells/ml. Cultures were incubated at 30°C and transferred once a day by inoculating 3×10^6 cells into 3 ml of fresh media. The number of generations/day at the beginning of the experiment was 5.6, 3, 4.9, and 5.1 in rich medium, low glucose, nonfermentable carbon source, and high-salt media, respectively, and by 100 generations of adaptation, the final cell density had increased in the majority of cultures to the point that it permitted 6.9, 3.6, 6.9, and 6.3 generations/day. The effective population size was calculated as n = population bottleneck (3×10^6 cells) × number of generations between transfers [34]. The fraction of mutator cells in each culture was evaluated at 30–50 generation intervals by serial dilution and plating to rich media. These plates were then replica plated to YPD plates containing 100 μg/ml of Clonat, which selects for *msh2Δ* cells. For high-resolution data sets, the frequency of mutator cells was determined by comparing the plating efficiency of the population on YPD and YPD plus Clonat plates. Each frequency was derived from two independent serial dilutions. Each time the fraction of mutators was measured, 1.5 ml of each culture was mixed with 0.5 ml of 50% glycerol and frozen at −80°C.

We measured the fitness of our evolved lines in all four selection conditions by competing them against the ancestral reference strain, expressing Cwp2::YFP, under the exact conditions in which the original adaptation experiments were conducted. The reference strain and evolved population competitors were mixed, diluted into fresh medium at a final cell concentration of 1×10^7 cells/ml, and allowed to compete for two transfer cycles, which represents 8–15 generations of growth, depending on the strain genotype and the assay conditions. The ratio of the two competitors was quantified at the initial and final time points by flow cytometry. Two to four independent replicates for each fitness measurement were performed. The selective advantage, *s*, or disadvantage of the evolved population was calculated as

$$s = \frac{\ln(E_f/R_f) - \ln(E_i/R_i)}{T}$$

where *E* and *R* are the numbers of evolved and reference cells, the subscripts refer to final and initial populations, and *T* is the number of generations that reference cells have proliferated during the competition.

Supplemental Data

Supplemental Data include 14 figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/16/1581/DC1/.

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