

1 **Title:** The fitness cost of mismatch repair mutators in *Saccharomyces*

2 *cerevisiae*: partitioning the mutational load

3 **Authors:** Benjamin Galeota-Sprung<sup>1,a</sup>, Breanna Guindon<sup>1</sup>, Paul Sniegowski<sup>1</sup>

4 <sup>1</sup> University of Pennsylvania

5 <sup>a</sup> Corresponding author: [bsprung@gmail.com](mailto:bsprung@gmail.com);

6 changes for biorxiv: inserted figures and table into text; removed phone

7 number

8 **Running title:** The fitness cost of mismatch repair mutators

9 **Word count:** 6712

10 **Abstract:** Mutational load is the depression in a population's mean fitness  
11 that results from the continual influx of deleterious mutations. Here, we  
12 directly estimate the mutational load in a population of haploid  
13 *Saccharomyces cerevisiae* that are deficient for mismatch repair. We partition  
14 the load in haploids into two components. To estimate the load due to  
15 nonlethal mutations, we measure the competitive fitness of hundreds of  
16 randomly selected clones from both mismatch repair-deficient and -  
17 proficient populations. Computation of the mean clone fitness for the  
18 mismatch repair-deficient strain permits an estimation of the nonlethal load,  
19 and the histogram of fitness provides an interesting visualization of a loaded  
20 population. In a separate experiment, in order to estimate the load due to

21 lethal mutations (i.e. the lethal mutation rate), we manipulate thousands of  
22 individual pairs of mother and daughter cells and track their fates. These two  
23 approaches yield point estimates for the two contributors to load, and the  
24 addition of these estimates is nearly equal to the separately measured short-  
25 term competitive fitness deficit for the mismatch repair-deficient strain. This  
26 correspondence suggests that there is no need to invoke direct fitness effects  
27 to explain the fitness difference between mismatch repair-deficient and -  
28 proficient strains. Assays in diploids are consistent with deleterious  
29 mutations in diploids tending towards recessivity. These results enhance our  
30 understanding of mutational load, a central population genetics concept, and  
31 we discuss their implications for the evolution of mutation rates.

32 **Keywords:** mutational load, experimental evolution, evolution of mutation  
33 rates, yeast

## 34 **Introduction**

35 An evolving population experiences a continual influx of mutations, the vast  
36 majority of which, excluding neutral mutations, are likely to be deleterious  
37 (Fisher 1930). A deleterious allele in a haploid population will attain an  
38 equilibrium frequency that is the quotient of the mutation rate to that allele  
39 and the selection coefficient against it (Danforth 1923). The influx of  
40 deleterious mutations causes a depression in the population's mean fitness  
41 that is termed the mutational load (Muller 1950), and the load at equilibrium

42 is equal to the deleterious mutation rate (Haldane 1937). Because all  
43 populations experience mutation, all populations experience load, and a  
44 substantial proportion of the genetic variance for fitness in natural  
45 populations is due to mutational load (Charlesworth 2015).

46 Mutational load is closely connected to the evolution of mutation rates.  
47 Consider an asexual population in which there is genetic variation for the  
48 mutation rate: within such a population, distinct lineages with differing  
49 mutation rates experience differing loads and therefore possess differing  
50 mean fitnesses. In this way a downward selective pressure on the mutation  
51 rate is realized. This pressure is indirect in the sense that modifiers of the  
52 mutation rate are subject to selection without affecting any physiological  
53 property immediately related to fitness. The existence of ancient and highly  
54 conserved systems for replication fidelity (including proofreading, mismatch  
55 repair, and nucleotide excision repair) attests to the persistence of this  
56 selective pressure (Raynes and Sniegowski 2014).

57 In evolving populations, lineages with higher mutation rates (“mutators”) are  
58 continually produced by mutation to any of numerous mutation-rate-  
59 affecting loci. In the absence of beneficial mutations, the expected frequency  
60 of mutators within a population depends on the increase in the deleterious  
61 mutation rate caused by the mutator allele, the rate of mutation from wild  
62 type to mutator, and the mean selective effect of newly arising deleterious  
63 mutations (Johnson 1999; Desai and Fisher 2011). Investigations of natural

64 and clinical isolates of *Escherichia coli* and other bacteria have shown that  
65 mutators of one to two orders of magnitude in strength, often defective in  
66 mismatch repair, are present at low but notable frequencies in many  
67 populations (Jyssum 1960; Gross and Siegel 1981; LeClerc et al. 1996; Matic  
68 et al. 1997; Oliver et al. 2000; Denamur et al. 2002; Richardson et al. 2002;  
69 Trong et al. 2005; Denamur and Matic 2006; Gould et al. 2007; reviewed in  
70 Raynes and Sniegowski 2014). Evolution experiments conducted with *E. coli*  
71 have demonstrated that mutators can displace wild types by virtue of their  
72 increased access to beneficial mutations (Cox and Gibson 1974; Chao and Cox  
73 1983; Sniegowski et al. 1997; Giraud et al. 2001; Shaver et al. 2002; de Visser  
74 and Rozen 2006). Similar findings have been reported for *Saccharomyces*  
75 *cerevisiae* (Thompson et al. 2006; Raynes et al. 2011, 2018). However, in  
76 contrast to findings in prokaryotes, mismatch repair-deficient (henceforth  
77 *mmr*) or other types of strong mutators have not been found in natural *S.*  
78 *cerevisiae* populations (but see Bui et al. 2017; Raghavan et al. 2018), though  
79 weaker variation for the mutation rate has been detected (Gou et al. 2019).  
80 One explanation for this difference could be that *mmr* mutators experience  
81 higher load, compared to the wild-type, in *S. cerevisiae* than they do in *E. coli*.  
82 Indeed, it has been observed by several investigators that haploid *mmr S.*  
83 *cerevisiae* strains decline in frequency in the short term when co-cultured  
84 with wild-type strains (Thompson et al. 2006; Raynes et al. 2011, 2018; Bui  
85 et al. 2017), even if they eventually out-adapt the wild type. While this short-  
86 term deficit of the fitness of *mmr* mutators relative to the wild type has been

87 attributed to increased mutational load in the *mmr* strain, the evidence that  
88 this is the case has been mostly circumstantial (but see Wloch et al. 2001)  
89 because it is generally difficult to rule out an additional direct fitness effect of  
90 any allele thought to cause an indirect fitness effect (Raynes and Sniegowski  
91 2014).

92 In this work, we establish, by short-term competitive fitness assays and in  
93 agreement with prior studies, that an *mmr* haploid *S. cerevisiae* strain is  
94 substantially less fit than an otherwise isogenic *MMR+* (i.e. wild-type) strain.  
95 This fitness difference could be caused solely by load, or solely due to some  
96 direct fitness effect of the *mmr* phenotype; or it could be some combination  
97 of the two. We develop separate assays to measure the components of load  
98 due to nonlethal and lethal deleterious mutations. To estimate the load  
99 caused by nonlethal deleterious mutations, we randomly sampled hundreds  
100 of clones from *mmr* and wild-type populations and measured the competitive  
101 fitness of each. The resulting histogram of the distribution of fitness of the  
102 *mmr* population provides an illustration of the effect of a high mutation rate  
103 on population mean fitness. We find that the means of these distributions  
104 differ, indicating substantial load for the *mmr* strain, but not fully accounting  
105 for the total observed fitness difference between *mmr* and wild type strains.  
106 To estimate the lethal mutation rate under the two different mutational  
107 regimes, we manipulate single cells to track the fate of mother/daughter  
108 duos. We show that these two separately measured components of load—  
109 due to nonlethal and lethal mutations—approximately sum to the measured

110 fitness difference between the strains; hence we find no reason to suppose a  
111 direct fitness effect for *mmr*. Investigations with diploid versions of our  
112 strains provide support for this conclusion. We discuss some implications of  
113 these findings for continued experimental and theoretical investigations into  
114 the evolution of mutation rates.

## 115 **Materials and Methods**

### 116 **Data analysis and figure production**

117 Data processing and analysis was performed in R (R Core Team 2019) and  
118 RStudio (RStudio Team 2015). Graphical output was produced using the  
119 package *ggplot2* (Wickham 2016).

### 120 **Strains**

121 yJHK112, a haploid, prototrophic, heterothallic, MATa, BUD4-corrected,  
122 ymCherry-labeled W303 strain, was used as the haploid wild type in all work  
123 described in this paper. yJHK111, labelled with ymCitrine (a variant of YFP)  
124 and otherwise isogenic to yJHK112, was used as the “reference strain” in all  
125 haploid fitness competitions. These strains have been previously described  
126 (Koschwanez et al. 2013) and were generously provided by the laboratory of  
127 Andrew Murray, Harvard University, Cambridge, MA. An *msh2Δ* derivative of  
128 yJHK112, in which the wild-type *MSH2* allele was replaced with a kanMX  
129 geneticin (G418) resistance cassette (Wach et al. 1994), was used as the

130 haploid *mmr* mutator strain in all work described in this paper. This strain  
131 was generously provided by Yevgeniy Raynes of the laboratory of Dan  
132 Weinreich, Brown University, Providence, RI and has been previously  
133 described (Raynes et al. 2018). The kanMX cassette has been shown to not  
134 have a negative effect on growth (Baganz et al. 1997; Goldstein and McCusker  
135 1999).

136 We constructed diploid versions of each of the three above strains by  
137 transforming (Gietz and Schiestl 2007) each with plasmid pRY003,  
138 temporarily providing a functional HO locus allowing mating type switching  
139 and subsequent mating. pRY003 was a gift from John McCusker (Addgene  
140 plasmid #81043; <http://n2t.net/addgene:81043>; RRID:Addgene\_81043). The  
141 diploid state of resulting isolates was confirmed by (1) ability to produce  
142 tetrads after plating to sporulation media; (2) by flow cytometry for total  
143 genomic content (following Gerstein et al. 2006); and (3) by the presence of a  
144 PCR product for both the *MATa* and *MAT $\alpha$*  loci. The *mmr* diploids would not  
145 sporulate, but were confirmed to be diploids by the other two methods.

## 146 **Growth conditions**

147 The liquid medium for all fitness competitions was synthetic dextrose (SD)  
148 minimal media containing yeast nitrogen base at a concentration of 6.7 g/L  
149 and glucose at a concentration of 1.5 g/L (0.15%), supplemented with  
150 tetracycline (15 mg/L) and ampicillin (100 mg/L). Fitness competitions were  
151 conducted in volumes of 200  $\mu$ l media in deep polypropylene 96-well plates

152 (Nunc 260251) sealed with flexible caps (Nunc 276002) and shaken at 1000  
153 rpm with an orbit of 3mm (Corning LSE 6780-4) at a temperature of 30 C.  
154 Initial growth in liquid for the lethal event assays was performed in SD as  
155 described above but without antibiotics, in flasks shaken at 200 rpm at 30C.  
156 Growth on agar SD (2% glucose, no antibiotics) plates for the lethal assays  
157 took place at room temperature, approximately 24 C.

### 158 **Competitive fitness assays and isolation of clones**

159 Yeast, when grown by batch transfer with glucose as the carbon source,  
160 follow a relatively complex cycle of lag, fermentation, and respiration, and  
161 fitness benefits “accrued” in one phase (e.g. respiration) may not be  
162 “realized” until the next (e.g. the lag following the next transfer) (Li et al.  
163 2018). Therefore we conducted short-term competitive fitness assays  
164 between wild-type and *mmr* genotypes in which strains were mixed for one  
165 growth cycle prior to measuring frequencies (essentially, following Gallet et  
166 al. (2012)). The fitness assays were conducted as follows, with the interval  
167 between each consecutive day spanning 24 hours. Day 1: wild-type, *mmr*, and  
168 the *YFP+* reference strain were inoculated from frozen stock into single wells.  
169 Day 2: each strain was transferred to a new well with fresh media, diluting  
170 1/100. Day 3: competing strains were mixed 1:1 by volume and transferred  
171 to new wells with fresh media, diluting 1/100, to create between 6 to 8  
172 replicate competitions. Day 4: competitions were transferred to new wells  
173 with fresh media, diluting 1/100, and the frequencies of the competitor and



174 reference strain were assayed by flow cytometry (Guava EasyCyte).  
175 Discrimination between strains was performed on the SSC/GRN scatter plot.  
176 Day 5: the frequencies of the competitor and reference strain were again  
177 assayed by flow cytometry. The population density at the end of a 24-hour  
178 cycle was  $\sim 2 \times 10^7$  cells/mL; the census population size was thus  $\sim 4 \times 10^6$   
179 at transfer and  $\sim 4 \times 10^4$  just after transfer.

180 The change in frequencies between Days 4 and 5 was used to calculate a  
181 selection coefficient  $s$ . Under a continuous model of growth (Crow and  
182 Kimura 1970 p. 193)

$$183 \quad s = \frac{1}{t} \ln \frac{p_t(1-p_0)}{p_0(1-p_t)}$$

184 from which a relative fitness  $w = 1 + s$  follows. The number of generations,  
185  $t$ , was assumed to be  $\log_2 100$ , or approximately 6.64.  $p_0$  and  $p_t$  are the  
186 starting and ending frequencies of the genotype being tested (i.e. the  
187 frequencies at Days 4 and 5 in the above procedure). The resulting selection  
188 coefficients represent differences in Malthusian parameter (that is, the log of  
189 Wrightian fitness) scaled per generation of growth.

190 We conducted fitness competitions using this procedure in 8 separate blocks,  
191 each with multiple replicates as described above. Each block was begun on a  
192 different date. For each block, we computed the fitness of the *mmr* strain  
193 relative to the fitness of the wild-type strain by subtracting their mutual  
194 relative fitnesses to the reference strain. Each block included competitions in

195 both haploid and diploid genotypes. Our final point estimate of the fitness  
196 difference between *mmr* and wild-type strains is the mean difference across  
197 all blocks, and the 95% confidence intervals (as shown in Fig. 2) were  
198 computed from the set of point estimates according to the t-distribution.

199 In 2 of the 8 fitness competition blocks, we randomly sampled individual  
200 clones. To do so, we additionally propagated the haploid wild-type and *mmr*  
201 strains on Day 3 in addition to mixing them 1:1 with the reference strain.  
202 Then, on Day 4, we plated these cultures, diluting appropriately, to YPD agar  
203 plates. After sufficient incubation, the resulting colonies were picked by pipet  
204 tip into wells containing 200 ul YPD, grown for 24 hours, and frozen down by  
205 mixing 1:1 with 30% glycerol and storing at -80 C until needed for fitness  
206 assays. The random selection of colonies was ensured by either (1) picking  
207 all colonies on a given plate or (2) picking colonies concentrically from a  
208 randomly placed point.

209 Fitness assays for sets of isolated clones were conducted on a single 96-well  
210 plate, which allowed us to assay the fitnesses of 88 clones (some wells being  
211 reserved for various purposes) or fewer per run. We followed essentially the  
212 same procedure as the 5-day competition described above, except that  
213 frequencies were estimated at Day 3 and Day 4 instead of Day 4 and Day 5.  
214 This modification was made because some clones had such reduced fitnesses  
215 that an extra day of growth after mixing 1:1 with the reference strain caused  
216 the starting frequency of the clone to depart too greatly from 50%.

217 The expected variance in measured fitness due to random sampling effects  
218 during flow cytometry was computed by means of a simple simulation in  
219 which the true frequency of each genotype at the start and end of the fitness  
220 competition was replaced by a random binomial variable. 10,000 replicates  
221 of this simulation were run.

## 222 Calculation of nonlethal load

223 Mutational load is classically defined (Bürger 1998) as

$$224 \quad L = \frac{w_{max} - \bar{w}}{w_{max}}$$

225 where  $\bar{w}$  is the mean fitness of the population and  $w_{max}$  is the fitness of the  
226 fittest genotype.

227 We measured all fitnesses relative to a common fluorescent strain, as  
228 described above. We define the unloaded fitness of each genotype as equal to  
229 1 and we expect no beneficial mutations to rise to appreciable frequency in  
230 the short course of these experiments. Hence,  $w_{max} = 1$  and thus

$$231 \quad L = 1 - \bar{w}$$

232 We expect measured selection coefficients to be distributed approximately  
233 normally around the true value, because of various sources of error including  
234 binomial sampling error, drift, instrument noise, environmental  
235 perturbations to individual wells (within-batch effects) and among 96-well  
236 plates (across-batch effects). To eliminate across-batch effects, for each run

237 we adjusted all measured fitnesses by a constant  $c$  such that the mode fitness  
238 is 1 (equivalently, such that the mode selection coefficient is zero). Once this  
239 adjustment has been made,

$$240 \quad L = 1 - \text{mean}(W)$$

241 where  $W$  is the vector of all sampled clone fitnesses, or equivalently

$$242 \quad L = \text{mean}(S)$$

243 where  $S$  is the vector of all sampled clone selection coefficients. A 95%  
244 confidence interval for the nonlethal load was computed by bootstrapping  
245 from the measured fitnesses of all sampled clones: for 10,000 replicates,  
246 fitnesses were sampled randomly with replacement and the mean computed;  
247 from this empirical distribution, the 0.025 and 0.975 quantiles formed the  
248 bounds of the confidence interval.

## 249 **Lethal event assay**

250 Strains were inoculated from frozen stock into 6 mL SD in a flask, and  
251 transferred to fresh media after 24 hours, diluting 1/100. After an overnight  
252 of growth, a streak from the culture was made onto an SD agar plate and five  
253 single cells with nascent buds were physically isolated by means of a Zeiss  
254 (West Germany) micromanipulating microscope fitted with a Singer  
255 Instruments (Somerset, UK) dissecting needle. These cells were periodically  
256 checked over the next few hours and the daughters physically separated once  
257 developed. These daughters became the founders of microcolonies that were

258 allowed to grow at room temperature for ~20 hours, reaching an average  
259 size of 23.3 cells (22.8 for the wild type, 23.8 for *mmr*, difference not  
260 significant at  $p > 0.6$ ). These microcolonies were then dissected into a  
261 gridlike arrangement of single cells (step 1 in Fig. 1). These cells were then  
262 checked at intervals of one to two hours and daughters separated as soon as  
263 possible (step 2 in Fig. 1). The colonies resulting from these  
264 mother/daughter duos were checked at ~24, ~48, and in some cases ~72  
265 and ~96 hours after separation. A lethal event was recorded when the  
266 growth of a mother or daughter lineage ceased. In such cases cessation of  
267 growth was sometimes immediate and sometimes occurred after a few  
268 generations. In the latter cases the growth was generally markedly slowed by  
269 the first observation. In a few cases, slow but unceasing growth was noted:  
270 these are presumed to be cases in which a strongly deleterious mutation  
271 occurred, though we stress that this assay was not designed to detect  
272 nonlethal deleterious mutations.

273 As described in the Discussion, the difference in the rate of lethal events  
274 between the wild type and *mmr* strains was used as the estimate for the  
275 lethal mutation rate for the *mmr* strain. A 95% confidence interval for this  
276 difference in rates was computed by the *prop.test* function in R (Newcombe  
277 1998).

## 278 **Fluctuation assays**

279 To measure the mutation rate to 5-fluoroorotic acid (5-FOA) resistance, we  
280 employed the following procedure. Strains of interest were inoculated into  
281 10 mL YPD, grown in flasks with shaking at 30 C for ~24 hours, and then  
282 transferred to 30 mL fresh YPD diluting such that ~200 cells were passaged,  
283 in replicates of 5. After ~48 hours of growth, each replicate was plated  
284 without dilution to SD + 5-FOA (1 g/L) agar plates to estimate density and  
285 absolute number of resistant, and plated with a  $10^{-5}$  dilution to YPD agar  
286 plates to estimate total population density and absolute number. Plates were  
287 counted after ~48 hours of growth and mutation rates were estimated using  
288 the maximum likelihood method of Gerrish (2008). For each round of  
289 fluctuation tests, we estimated mutation rates for both wild-type and *mmr*  
290 strains simultaneously in order to minimize the influence of any uncontrolled  
291 sources of variation.

## 292 **Homopolymers per gene**

293 The per-base rate of homopolymeric runs of various lengths in *S. cerevisiae*  
294 coding regions were computed by a custom Python script. The *S. cerevisiae*  
295 S288C reference genome was downloaded from [www.yeastgenome.org](http://www.yeastgenome.org).

## 296 **Results**

### 297 **Mutation rate elevation in *mmr* strain**

298 To confirm that the *mmr* (*msh2Δ*) strain is a mutator, we conducted  
299 fluctuation tests using resistance to 5-FOA as the selectable phenotype.  
300 Averaged across replicate fluctuation tests, we found a 20.8-fold increase  
301 (95% CI: 13.4- to 28.3-fold) in the mutation rate for the *mmr* strain relative  
302 to the wild-type (Fig. S1). This is likely an underestimate of the effective  
303 genome-wide increase in the mutation rate because *mmr* mutators have a  
304 greatly elevated indel rate for homopolymeric runs (Lang et al. 2013), of  
305 which *URA3*, the main locus involved in this fluctuation test, is relatively  
306 devoid (Fig. S4).

### 307 **Fitness disadvantage of *mmr* compared to wild-type**

308 We competed *mmr* and wild-type strains against a common YFP+ reference  
309 strain. Averaged across 8 separate blocks of fitness competitions, we found  
310 the mutator to be less fit than the wild-type, with an average fitness deficit,  
311 expressed as a selection coefficient per generation, of ~2.25% (95% CI:  
312 1.98% to 2.53%) (Fig. 2).

### 313 **Estimation of nonlethal load**

314 We randomly sampled individual clones from both *mmr* and wild-type  
315 populations and measured the competitive fitness of each clone. The sampled

316 fitness distributions are shown in Fig 3. The *mmr* strain's fitness distribution  
317 has a prominent left tail of less fit individuals. We calculated the load as the  
318 difference between the mode and the mean fitness; this is approximately  
319 zero for the wild-type strain and ~1.65% (95% CI: 2.19% to 1.08%) for the  
320 *mmr* strain. The difference in load between the two strains is significant ( $p <$   
321  $10^{-8}$ ). The fitness distributions for the *mmr* and wild-type are significantly  
322 different in shape (Kolmogorov-Smirnov test;  $p < 10^{-8}$ ), while the fitness  
323 distribution for the wild-type strain is not significantly different from a  
324 normal distribution with the same mean and variance (Kolmogorov-Smirnov  
325 test;  $p > 0.05$ ).

### 326 **Estimation of lethal mutation rate**

327 To assay lethal mutation rates, we manipulated single *S. cerevisiae* cells,  
328 separating mother/daughter duos and tracking events in which one member  
329 of the duo failed to found a colony. The procedure is shown in Fig. 1 and  
330 explicated more fully in the methods. Assaying over 2200 duos for each  
331 strain, we found a rate of lethal events per newly replicated genome of 0.31%  
332 (95% CI: 0.018% to 0.055%) in the wild-type strain and 0.76% (95% CI:  
333 0.53% to 1.07%) in the *mmr* strain (Table 1). The difference between these  
334 rates is 0.0044 (95% CI: 0.0012 to 0.0077). Because the observed rate of  
335 lethal events in the wild type is much higher than the expected lethal  
336 mutation rate, we take this difference as our estimate of the lethal mutation



337 rate in the *mmr* strain (see the Discussion for elaboration on this point).

338 Photographs of representative lethal events are shown in Fig. S3.

339 In our assay we followed separated duos that contained a suspected lethal  
340 until growth ceased. In some cases growth never ceased, but doubling times  
341 were very slow compared to the usual growth rate; such cases were not  
342 counted as lethal events but are tallied separately in Table 1. We also  
343 detected cases in which both members of a duo were lethal, or both showed  
344 strongly reduced growth, and also cases in which the mother cell never  
345 divided. Because such events probably stemmed from a mutation that  
346 occurred prior to the division that created the duo under observation, we  
347 excluded these events from our analysis.

## 348 **Results in diploids**

349 From the haploid strains, we constructed diploid *mmr* and wild-type strains.  
350 We calculate the nonlethal load in the *mmr* diploid strain as  $\sim 0$  for the wild-  
351 type diploid strain and 0.30% (95% CI: -0.02% to 0.55%) (Fig S2) for the  
352 *mmr* strain—substantially less, by about 80%, than the equivalent load in the  
353 in *mmr* haploid strain (difference significant at  $p < 10^{-4}$ ). The diploid wild-  
354 type and *mmr* distributions differ significantly in shape (Kolmogorov-  
355 Smirnov test;  $p < 10^{-4}$ ) but do not differ significantly in mean ( $p > 0.05$ ).  
356 We also measured the difference in population fitness between wild-type and  
357 *mmr* diploid strains via short-term competitive fitness assays. We found that

358 the *mmr* diploid is less fit than the wild-type diploid by a selection coefficient  
359 of ~1.69% (95% CI: 1.40% to 1.94%) (Fig. 4). This difference, though larger  
360 than expected, is smaller than the fitness difference between wild-type and  
361 *mmr* haploid strains by 25% (haploid-to-diploid difference significant at  $p <$   
362 0.004).

## 363 Discussion

364 Prior work has shown that, over the short term, haploid *mmr S. cerevisiae*  
365 strains decline in frequency when competed with a strain that is wild-type  
366 for the mutation rate (Thompson et al. 2006; Raynes et al. 2011, 2018; Bui et  
367 al. 2017). Consistent with these findings, we find a fitness disadvantage,  
368 expressed as a selection coefficient per generation, of ~2.3% for *mmr*  
369 haploids in short-term fitness competitions (Fig. 2). The magnitude of this  
370 selective disadvantage is similar to that in other reports, including Raynes et  
371 al. (2011) (2.4% cost), Raynes et al. (2018) (3.3% cost), and Wloch et al.  
372 (2001) (4.6% cost, though this is a noncompetitive measure of absolute  
373 growth rate).

374 The deleterious mutations that cause load include both lethal and non-lethal  
375 mutations. There is no fundamental theoretical distinction between these  
376 two classes of mutation insofar as their contribution to load is concerned: in  
377 many population genetic models, all members of an asexual population who  
378 are not of the least-loaded class are considered to be doomed (Rice 2002).  
379 However, their different manifestations require different experimental

380 techniques. We therefore developed separate approaches to measure these  
381 two components of load.

### 382 **Load due to nonlethal mutations**

383 We measured the short-term competitive fitnesses of 640 randomly selected  
384 haploid clones. The histogram and QQ plot for the haploid wild-type  
385 populations (Fig. 3A) suggest that, apart from one less-fit clone, the  
386 distribution of fitness for the wild-type strain is essentially normal. The  
387 normality of the distribution is consistent with nearly all wild-type clones  
388 having the same genotype and thus the same expected fitness, along with  
389 many small sources of error in estimation of fitness. One such source of error  
390 is drift over the course of the short-term fitness competition. The formula  
391 derived by Gallet et al. (2012) suggests that the expected variance in fitness  
392 measurement due to drift given our experimental parameters is  $\sim 2 \times 10^{-6}$ . A  
393 larger source of variance is due to sampling error: in the fitness  
394 competitions, we estimate the relative frequencies of the competitors at two  
395 time points, sampling  $\sim 8000$  cells per time point. We carried out simulations  
396 that suggest that the expected variance due to sampling error is  $\sim 2.3 \times 10^{-5}$ .  
397 These two sources of variance, summed, make up about 20% of the observed  
398 variance in selection coefficient. The remainder of the variance probably  
399 stems from small-scale environmental variation and other unknown sources  
400 of error.

401 In contrast to the results in wild types, the fitness histogram and QQ plot for  
402 the haploid *mmr* strain (Fig. 3B) are not reflective of a normal distribution.  
403 Instead, a prominent left tail of less fit clones demonstrates the effect of  
404 mutational load. The mean selection coefficient is  $\sim -1.7\%$ , which is the  
405 quantification of the reduction in population mean fitness due to nonlethal  
406 load. This reduction accounts for a substantial portion ( $\sim 75\%$ ) of the  
407 measured competitive fitness difference (Fig. 2) between the two strains.

408 Our estimate of the nonlethal load ( $\sim 1.7\%$ ) reflects the average per-  
409 generation growth deficit of the mutator subpopulation due to the  
410 accumulation of deleterious mutations up to the point of random sampling of  
411 clones. We note that this is an estimate of the load at a nonequilibrium state,  
412 and is expected to be less than the full load achieved when mutation-  
413 selection balance is reached for all loci. Direct observation of mutation-  
414 selection equilibrium in a laboratory setting would be challenging because  
415 experimental populations rapidly generate adaptive mutations. Our strain-  
416 to-strain fitness assays, which found (Fig. 2) a fitness deficit of  $\sim 2.3\%$  for the  
417 *mmr* strain relative to the wild type, are likewise reflective of a  
418 nonequilibrium state. Since both estimates are derived from the same  
419 nonequilibrium populations, they are directly comparable.

420 Selection coefficients of about the magnitude we observe here cause changes  
421 in relative frequency that are extremely rapid in evolutionary terms. For  
422 example, a selective deficit of 2% would cause a decline from 50% frequency

423 to 20% frequency in 70 generations. Observing a rapid initial decline of  
424 haploid *mmr* *S. cerevisiae* strains in competition with wild-types, some  
425 investigators (e.g. Grimberg and Zeyl 2005) have attributed the observed  
426 fitness difference to an unknown direct cost (i.e. a pleiotropic effect) while  
427 others (e.g. Raynes et al. 2018) have assumed that mutational load fully  
428 explains the dynamics. The question has remained open, in part because it  
429 has been nearly impossible to definitively rule out a direct fitness effect of  
430 being *mmr*—any attempt to measure such an effect will be confounded by  
431 the indirect fitness effects. By quantifying the indirect fitness effects  
432 (i.e. load) we seek to determine if a direct effect need be invoked to explain  
433 the observed experimental dynamics.

434 It is not surprising that the nonlethal load accounts for only a portion of the  
435 observed fitness difference. The nonlethal load assay relies on the growth of  
436 deleterious mutants in order to measure their fitness and thus cannot detect  
437 mutants who do not grow, i.e. lethal mutations. In order to measure this  
438 portion of the load, we designed an assay in which lethal events are directly  
439 observed.

#### 440 **Load due to lethal mutations**

441 The lethal mutation rate has long been a matter of interest (e.g. Dobzhansky  
442 and Wright 1941). By observing 4435 mother-daughter pairs (duos), we  
443 found a rate of lethal events of 0.0076 and 0.0031 for the *mmr* and wild-type  
444 strain, respectively.

445 Our observed wild-type lethal event rate, 0.0031, is on the order of estimates  
446 for the genomic mutation rate itself (Drake 1991; Lynch et al. 2008; Zhu et al.  
447 2014; Sharp et al. 2018) and therefore cannot plausibly reflect the rate of  
448 lethal mutations. Our interpretation is that, for the wild-type, all or most  
449 observed lethal events were not caused by genomic mutations and are  
450 instead best considered to be non-mutational deaths, perhaps caused by fine-  
451 scale environmental fluctuations, experimental manipulation, or other  
452 stochastic sources of insult and stress. Observations of relatively high rates of  
453 cell death, too high to be due to lethal mutation, are not uncommon.  
454 Replicative aging studies of *S. cerevisiae* often observe low but substantial  
455 rates of cell death even in very young mother cells (e.g. Chiocchetti et al.  
456 2007; Shcheprova et al. 2008). Rates of cell death on the order of our  
457 observed rate for the wild-type strain have also been observed in young  
458 bacterial cells (Wang et al. 2010), suggesting that relatively high rates of non-  
459 mutational, non-age-related deaths are common among microbes. Our assay  
460 design ensured that colonies were young (the oldest cell in a microcolony  
461 was on average ~4.3 generations old) and we did not observe a bias in lethal  
462 events towards mothers (Table S1), so we do not attribute the observed  
463 lethal events to senescence. In fact, we observed, across both strains, a bias  
464 towards the lethal event occurring in the daughter cell. This difference was  
465 not statistically significant ( $p = 0.14$ ), although within the *mmr* strain only  
466 we observed 10 lethal events in mothers and 23 in daughters ( $p = 0.04$ ). The  
467 observed bias towards daughters dying, if not a sampling effect, could be

468 attributable to smaller daughter cells being relatively more vulnerable to  
469 stress. Indeed, increased vulnerability of daughters to environmental sources  
470 of stress has been previously reported (Knorre et al. 2010).

471 An *a priori* estimation of the wild-type lethal mutation rate can be made as  
472 follows. Lang and Murray (2008) conducted careful estimations of the rate of  
473 loss-of-function mutations to the *CAN1* locus in a similar background (W303)  
474 as the strains used in this work. Multiplying this rate,  $1.5 \times 10^{-7}$ , by the  
475 number of genes thought to be essential for viability,  $\sim 1100$  (Giaever et al.  
476 2002), and accounting for the fact that *CAN1* is longer than the average  
477 essential gene gives an expected lethal rate in wild-type haploids of  
478  $1.5 \times 10^{-5}$ . This estimate is on the lower end but within the range of  
479 observed rates of accumulation of recessive lethals in several experiments  
480 conducted with diploids (Wloch et al. 2001; Hall and Joseph 2010; Nishant et  
481 al. 2010; Zhu et al. 2014; Jasmin and Lenormand 2016). Such a rate would  
482 suggest that we expected to observe about 0.3 lethal mutations in the wild-  
483 type strain in our experiment; we actually observed 14. Therefore, we  
484 consider the observed rate of lethal events in the wild-type to be an estimate  
485 of the rate of non-mutational deaths. The corresponding rate for the *mmr*  
486 strain is 0.0076 (difference significant at  $p < 0.001$ ). Making the assumption  
487 that non-mutational deaths equally affect both strains, we take the difference  
488 between the wild-type and *mmr* lethal event rates, 0.0044 (95% CI: 0.0012 to  
489 0.0077), as the estimate of the lethal mutation rate in the *mmr* strain. We  
490 note that our empirical result is fairly close to the figure obtained by

491 multiplying the wild-type *a priori* estimate,  $1.5 \times 10^{-5}$ , by the average fold  
492 increase in *CAN1* loss-of-function mutation rate for *mmr* strains in a  
493 collection of published reports (44-fold; see Table S4). A slightly different  
494 methodology, taking the average *CAN1* loss-of-function rate of *mmr* strains  
495 from published reports ( $1.5 \times 10^{-5}$ ; Table S4) and multiplying by 1100  
496 essential genes yields a somewhat higher expected lethal mutation rate of  
497  $\sim 0.015$ .

498 In many of the lethal events that we observed, growth did not immediately  
499 cease but continued for a few generations (Table S2) before halting. Limited  
500 growth for a few generations after an ultimately lethal mutation occurs has  
501 previously been observed (Mortimer 1955). We also observed morphological  
502 defects in several lethal events; one such instance is shown in the bottom  
503 panel of Fig. S3. We note that some lethal mutations that we observed could  
504 be due to chromosomal losses during mitosis (aneuploidies), but that  
505 knocking out *MSH2* has not been observed to greatly increase the rate of such  
506 events in haploids (Serero et al. 2014).

### 507 **Diploid findings**

508 We measured the short-term competitive fitnesses of 573 randomly selected  
509 diploid clones. The distribution of fitness for *mmr* diploids (Fig. S2) suggests  
510 that they are substantially less loaded than *mmr* haploids, as would be  
511 expected if dominance attenuates the deleterious effects of new mutations.  
512 We calculate the nonlethal load in *mmr* diploids as  $\sim 0.3\%$ , as opposed to



513 ~1.7% in *mmr* haploids: that is, ~80% of the load has gone away following  
514 diploidization. One interpretation of this finding is that deleterious mutations  
515 tend to be recessive in diploids. Thus, comparison of the fitness distributions  
516 of *mmr* diploids and *mmr* haploids is consistent with a high mutation rate  
517 and diploidy shielding the effects of deleterious mutations.

518 The sampled wild-type diploid clones included more low-fitness individuals  
519 than the wild-type haploids (compare Fig. S2A to Fig. 3A). We cannot fully  
520 explain this observation; one possible explanation is that diploids are more  
521 prone than haploids to nondisjunctions causing aneuploid chromosomes, a  
522 notion for which there is some experimental support (Sharp et al. 2018).

523 The relative difference in short-term competitive fitness between wild-type  
524 and *mmr* strains is narrowed by 26% in diploids (Fig. 4). It is somewhat  
525 surprising, given that the nonlethal loads are not very different between  
526 wild-type and *mmr* diploids, that this figure is not larger. One possibility is  
527 that the diploid mutator fixed a deleterious mutation during the process of  
528 diploidization, which would account for the discrepancy between the  
529 reduction in nonlethal load (82%) and the reduction in total fitness  
530 difference (26%) in diploids compared to haploids. Another formal  
531 possibility is that diploid mutators have a higher lethal mutation rate than  
532 haploid mutators, but we cannot posit a causative mechanism for such an  
533 effect.

## 534 **Considering the two loads together**

535 The total fitness difference between the haploid wild-type and *mmr* strains  
536 could be a consequence of greater mutational load for the *mmr* strain, a  
537 direct effect of the *msh2Δ* deletion, or a combination of the two. The addition  
538 of the lethal and nonlethal loads ( $0.0166 + 0.0044 = 0.0210$ ) is approximately  
539 7% smaller than the measured fitness difference (0.0225), and the difference  
540 is not significant (Fig. S5). The difference may simply be due to sampling  
541 error, or due to underestimation of one or the other of the loads. The  
542 nonlethal load may be slightly underestimated because clones were isolated  
543 by plating at the beginning of the growth cycle during which competitive  
544 fitness was measured. The load may have continued to increase somewhat  
545 during this growth cycle.

546 The broad equivalence of the sum of the loads, on the one hand, and the  
547 strain-to-strain competitive fitness, on the other, is consistent with the  
548 hypothesis that the total fitness difference is solely due to mutational load.  
549 Hence, although we cannot rule out the existence of a small direct fitness  
550 effect, these findings suggest that there is no need to invoke direct effects in  
551 explaining the fitness difference between the *mmr* and wild-type haploid  
552 strains.

553 The proportion of deleterious mutations that are lethal may also be inflated  
554 by an underestimation of the total deleterious mutation rate. The load is  
555 equal to the deleterious mutation rate only when the population is in

556 mutation-selection balance. This equilibrium is reached instantly for lethal  
557 mutations, quickly for deleterious mutations of large effect, and very slowly  
558 for deleterious mutations of slight effect (Johnson 1999). The *mmr*  
559 populations in our assays experienced, including the initial process of  
560 transformation and growth before frozen storage, about 60 generations of  
561 growth, which is enough time to achieve mutation-selection balance for  
562 deleterious mutations of relatively large effect, but not for deleterious  
563 mutations of slight effect. Hence, our estimate of the total load (2.1-2.3%)  
564 should be considered an estimate of the lower limit for the deleterious  
565 mutation rate for *mmr* haploids.

## 566 **Comparison to results in bacteria**

567 Insofar as *S. cerevisiae* and *E. coli* are two model organisms, from different  
568 domains of life, with which many evolution experiments have been  
569 performed, it is interesting to compare the loads of mismatch repair  
570 mutators in both. It appears that in *E. coli* the relative fitness deficit for *mmr*  
571 strains is smaller than it is in haploid *S. cerevisiae*. For instance, Shaver et al.  
572 (2002) did not detect a fitness difference between *mmr* and wild-type  
573 strains, de Visser and Rozen (2006) did not observe an initial decline in *mutS*  
574 frequency when that genotype was competed with the wild type at different  
575 starting ratios, and Boe et al. (2000) estimated at most a 1% selective  
576 disadvantage for *mmr* mutators. In this context it is relevant to note that  
577 there are several reports of *mmr* genotypes in natural *E. coli* isolates (LeClerc

578 et al. 1996; Matic et al. 1997; Denamur et al. 2002), as well as in other types  
579 of bacteria (Oliver et al. 2000; Richardson et al. 2002; Trong et al. 2005;  
580 Gould et al. 2007). In *S. cerevisiae*, in contrast, no functionally *mmr* natural  
581 isolates have yet been found (though see Raghavan et al. 2018). Such  
582 observations suggest that *E. coli* may be relatively more robust than *S.*  
583 *cerevisiae* to the lack of a functional mismatch repair system. One reason for  
584 this difference could be that the genomic mutation rate in *E. coli* is lower than  
585 that of *S. cerevisiae* by a factor of about 4 (Lee et al. 2012, Lynch et al. (2008);  
586 Zhu et al. 2014; Sharp et al. 2018). If there is a similar absolute difference in  
587 deleterious mutation rate, then even if the relative fold increase in the  
588 deleterious mutation rate caused by the lack of mismatch repair is also equal  
589 in both organisms, the absolute difference in load, which is what controls the  
590 evolutionary dynamics, will be larger in *S. cerevisiae* than in *E. coli*. Another  
591 possible factor is differences in the spectrum and genomic substrate of  
592 mutations. In both *E. coli* and *S. cerevisiae*, the indel rate is greatly increased  
593 in *mmr* lineages, and the rate of indels is strongly elevated in homopolymeric  
594 repeats (HPRs). Both the relative increase from wild-type to *mmr* and the  
595 absolute indel rate in *mmr* are higher, and scale upwards faster with HPR  
596 length, in *S. cerevisiae* than in *E. coli* (Schaaper and Dunn 1991; Tran et al.  
597 1997; Gragg et al. 2002; Lee et al. 2012; Lang et al. 2013). Examining all  
598 coding sequences in the *E. coli* and *S. cerevisiae* genomes, we find that there  
599 are significantly more homopolymeric repeats per coding genome, per gene,  
600 and per coding base in *S. cerevisiae* than in *E. coli* (Table S5). *S. cerevisiae* that

601 are *mmr* are therefore relatively more burdened by indels than are *mmr E.*  
602 *coli* which could account for both the apparent larger fitness difference  
603 between *MMR+* and *mmr* and the corresponding apparent contrast in  
604 occurrence in natural isolates. We caution that this particular analysis is  
605 speculative in nature at this time: one important caveat is that, while this  
606 study and others have found large fitness differences between wild-type and  
607 *mmr* haploids, *S. cerevisiae* spend most of their time in nature as diploids, in  
608 which the fitness deficit of *mmr* lineages might be less severe: classically,  
609 equilibrium mutational load is halved in the recessive case compared to the  
610 additive, or haploid, case (Kimura et al. 1963). However, while estimates of  
611 the rate of outcrossing in *S. cerevisiae* are very low (Ruderfer et al. 2006), the  
612 rate of sporulation, which entails a haploid stage, is not known, and evidence  
613 of extensive inbreeding and loss of heterozygosity (Peter et al. 2018) suggest  
614 that it is relatively frequent. Recessive deleterious mutations may thus be  
615 frequently exposed to selection in natural *S. cerevisiae* populations by both  
616 the haploid life cycle stage and loss of heterozygosity from inbreeding,  
617 suggesting that diploidy may not be as much of a shield for *mmr* lineages as it  
618 otherwise would be. A second caveat is that, even if there is no direct fitness  
619 effect of *mmr* in haploids, there could be such an effect in diploids, perhaps  
620 due to misregulation of the frequency of recombination events (reviewed in  
621 Surtees et al. 2004; George and Alani 2012).

## 622 **Conclusions and future directions**

623 We have found that the indirect fitness effects of strong modifiers for  
624 mutation rates are substantial in haploid *S. cerevisiae*, and that it is not  
625 necessary to postulate direct fitness effects in order to explain the selective  
626 disadvantage of the lack of a functional mismatch repair pathway. This  
627 finding is probably most relevant to experimental inquiries of the dynamics  
628 of mutation rate evolution in which *S. cerevisiae* is the model organism.

629 We have also reported findings relevant to fundamental questions about  
630 mutational dynamics, including the lethal mutation rate and the relative ratio  
631 of lethal and nonlethal deleterious mutations. By sampling the fitnesses of  
632 many individuals we have clearly demonstrated mutational load in an *mmr*  
633 population, and from the load we are able to estimate a lower limit for the  
634 deleterious mutation rate. We sampled hundreds of clones and were able to  
635 obtain a clear picture of the left tail of the fitness distribution for the *mmr*  
636 strain, but not for the wild-type strain. If the fitnesses of tens of thousands of  
637 clones could be measured, much could be learned about load and other  
638 evolutionary dynamics at wild-type mutation rates; such experiments may  
639 become possible as methods for high-throughput measurements continue to  
640 advance.

641 A limitation of this study is that we captured a snapshot of mutational load at  
642 a particular point in time in an evolving population. It would be interesting to  
643 observe, at a fine scale, how the distribution of fitnesses changes over time as

644 a population approaches mutation-selection balance, adapts, and experiences  
645 other population genetic processes.

## 646 **Acknowledgments**

647 This research was facilitated by a National Aeronautics and Space  
648 Administration grant (NNA15BB04A) to P.D.S. and a National Science  
649 Foundation Graduate Research Fellowship to B.G.S.

650 We thank the laboratories of Andrew Murray (Harvard University,  
651 Cambridge, MA) and Dan Weinreich (Brown University, Providence, RI) for  
652 their generous sharing of yeast strains.

## 653 **Conflict of interest**

654 The authors declare that there are no competing financial interests in  
655 relation to the work described.

## 656 **Data archiving**

657 The authors plan to upload the raw data upon which this study is based to  
658 Dryad.

## 659 **References**

- 660 Baganz F, Hayes A, Marren D, Gardner DCJ, Oliver SG (1997) Suitability of  
661 replacement markers for functional analysis studies in *Saccharomyces*  
662 *cerevisiae*. *Yeast* 13: 1563–1573.
- 663 Boe L, Danielsen M, Knudsen S, Petersen JB, Maymann J, Jensen PR (2000)  
664 The frequency of mutators in populations of *Escherichia coli*. *Mutation*  
665 *Research/Fundamental and Molecular Mechanisms of Mutagenesis* 448: 47–  
666 55.
- 667 Bui DT, Friedrich A, Al-Sweel N, Liti G, Schacherer J, Aquadro CF et al. (2017)  
668 Mismatch repair incompatibilities in diverse yeast populations. *Genetics* 205:  
669 1459–1471.
- 670 Bürger R (1998) Mathematical properties of mutation-selection models.  
671 *Genetica* 102: 279.
- 672 Chao L, Cox EC (1983) Competition between high and low mutating strains of  
673 *Escherichia coli*. *Evolution* 37: 125.
- 674 Charlesworth B (2015) Causes of natural variation in fitness: Evidence from  
675 studies of *Drosophila* populations. *PNAS* 112: 1662–1669.
- 676 Chiocchetti A, Zhou J, Zhu H, Karl T, Haubenreisser O, Rinnerthaler M et al.  
677 (2007) Ribosomal proteins Rpl10 and Rps6 are potent regulators of yeast  
678 replicative life span. *Experimental Gerontology* 42: 275–286.



- 679 Cox EC, Gibson TC (1974) Selection for high mutation rates in chemostats.  
680 *Genetics* 77: 169–184.
- 681 Crow JF, Kimura M (1970) *An Introduction to Population Genetics Theory*.  
682 Harper & Row: New York.
- 683 Danforth CH (1923) *Eugenics, Genetics and the Family: Scientific Papers of*  
684 *the Second International Congress of Eugenics ; Held at American Museum of*  
685 *Natural History, New York. September 22-28, 1921. In: Williams & Wilkins*  
686 *Company.*
- 687 de Visser JAGM, Rozen DE (2006) Clonal interference and the periodic  
688 selection of new beneficial mutations in *Escherichia coli*. *Genetics* 172: 2093–  
689 2100.
- 690 Denamur E, Matic I (2006) Evolution of mutation rates in bacteria. *Molecular*  
691 *Microbiology* 60: 820–827.
- 692 Denamur E, Bonacorsi S, Giraud A, Duriez P, Hilali F, Amorin C et al. (2002)  
693 High frequency of mutator strains among human uropathogenic *Escherichia*  
694 *coli* isolates. *J Bacteriol* 184: 605–609.
- 695 Desai MM, Fisher DS (2011) The balance between mutators and nonmutators  
696 in asexual populations. *Genetics* 188: 997–1014.
- 697 Dobzhansky T, Wright S (1941) *Genetics of natural populations. V. Relations*  
698 *between mutation rate and accumulation of lethals in populations of*  
699 *Drosophila pseudoobscura*. *Genetics* 26: 23.

- 700 Drake JW (1991) A constant rate of spontaneous mutation in DNA-based  
701 microbes. *Proceedings of the National Academy of Sciences* 88: 7160–7164.
- 702 Fisher R (1930) *The genetical theory of natural selection*. Clarendon Press:  
703 Oxford.
- 704 Gallet R, Cooper TF, Elena SF, Lenormand T (2012) Measuring selection  
705 coefficients below 10<sup>-3</sup>: Method, questions, and prospects. *Genetics* 190:  
706 175–186.
- 707 George CM, Alani E (2012) Multiple cellular mechanisms prevent  
708 chromosomal rearrangements involving repetitive DNA. *Critical Reviews in*  
709 *Biochemistry and Molecular Biology* 47: 297–313.
- 710 Gerrish P (2008) A simple formula for obtaining markedly improved  
711 mutation rate estimates. *Genetics* 180: 1773–1778.
- 712 Gerstein AC, Chun H-JE, Grant A, Otto SP (2006) Genomic convergence  
713 toward diploidy in *Saccharomyces cerevisiae*. *PLOS Genetics* 2: e145.
- 714 Giaever G, Chu AM, Ni L, Connelly C, Riles L, Véronneau S et al. (2002)  
715 Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418:  
716 387–391.
- 717 Gietz R, Schiestl R (2007) Quick and easy yeast transformation using the  
718 LiAc/SS carrier DNA/PEG method. *Nature Protocols* 2: 35–7.

719 Giraud A, Matic I, Tenaillon O, Clara A, Radman M, Fons M et al. (2001) Costs  
720 and Benefits of High Mutation Rates: Adaptive Evolution of Bacteria in the  
721 Mouse Gut. *Science* 291: 2606–2608.

722 Goldstein AL, McCusker JH (1999) Three new dominant drug resistance  
723 cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* 15: 1541–  
724 1553.

725 Gou L, Bloom JS, Kruglyak L (2019) The genetic basis of mutation rate  
726 variation in yeast. *Genetics* 211: 731–740.

727 Gould CV, Sniegowski PD, Shchepetov M, Metlay JP, Weiser JN (2007)  
728 Identifying mutator phenotypes among fluoroquinolone-resistant strains of  
729 *Streptococcus pneumoniae* using fluctuation analysis. *Antimicrobial Agents*  
730 *and Chemotherapy* 51: 3225–3229.

731 Gragg H, Harfe BD, Jinks-Robertson S (2002) Base composition of  
732 mononucleotide runs affects DNA polymerase slippage and removal of  
733 frameshift intermediates by mismatch repair in *Saccharomyces cerevisiae*.  
734 *Molecular and Cellular Biology* 22: 8756–8762.

735 Grimberg B, Zeyl C (2005) The effects of sex and mutation rate on adaptation  
736 in test tubes and to mouse hosts by *Saccharomyces cerevisiae*. *Evolution* 59:  
737 431–438.

738 Gross MD, Siegel EC (1981) Incidence of mutator strains in *Escherichia coli*  
739 and coliforms in nature. *Mutation Research Letters* 91: 107–110.

- 740 Haldane JBS (1937) The effect of variation of fitness. *The American Naturalist*  
741 71: 337–349.
- 742 Hall DW, Joseph SB (2010) A high frequency of beneficial mutations across  
743 multiple fitness components in *Saccharomyces cerevisiae*. *Genetics* 185:  
744 1397–1409.
- 745 Jasmin J-N, Lenormand T (2016) Accelerating mutational load is not due to  
746 synergistic epistasis or mutator alleles in mutation accumulation lines of  
747 yeast. *Genetics* 202: 751–763.
- 748 Johnson T (1999) The approach to mutation–Selection balance in an infinite  
749 asexual population, and the evolution of mutation rates. *Proceedings of the*  
750 *Royal Society of London B: Biological Sciences* 266: 2389–2397.
- 751 Jyssum K (1960) Observations on two types of genetic instability in  
752 *Escherichia coli*. *Acta Pathologica Microbiologica Scandinavica* 48: 113–120.
- 753 Kimura M, Maruyama T, Crow JF (1963) The Mutation Load in Small  
754 Populations. *Genetics* 48: 1303–1312.
- 755 Knorre DA, Kulemzina IA, Sorokin MI, Kochmak SA, Bocharova NA, Sokolov  
756 SS et al. (2010) Sir2-dependent daughter-to-mother transport of the  
757 damaged proteins in yeast is required to prevent high stress sensitivity of the  
758 daughters. *Cell Cycle* 9: 4501–4505.
- 759 Koschwanez JH, Foster KR, Murray AW (2013) Improved use of a public good  
760 selects for the evolution of undifferentiated multicellularity. *eLife* 2.

- 761 Lang GI, Murray AW (2008) Estimating the per-base-pair mutation rate in  
762 the yeast *Saccharomyces cerevisiae*. *Genetics* 178: 67–82.
- 763 Lang GI, Parsons L, Gammie AE (2013) Mutation rates, spectra, and genome-  
764 wide distribution of spontaneous mutations in mismatch repair deficient  
765 yeast. *G3: Genes, Genomes, Genetics* 3: 1453–1465.
- 766 LeClerc JE, Li B, Payne WL, Cebula TA (1996) High mutation frequencies  
767 among *Escherichia coli* and *Salmonella* pathogens. *Science* 274: 1208–1211.
- 768 Lee H, Popodi E, Tang H, Foster PL (2012) Rate and molecular spectrum of  
769 spontaneous mutations in the bacterium *Escherichia coli* as determined by  
770 whole-genome sequencing. *Proceedings of the National Academy of Sciences*  
771 109: E2774–E2783.
- 772 Li Y, Venkataram S, Agarwala A, Dunn B, Petrov DA, Sherlock G et al. (2018)  
773 Hidden Complexity of Yeast Adaptation under Simple Evolutionary  
774 Conditions. *Current Biology* 28: 515–525.e6.
- 775 Lynch M, Sung W, Morris K, Coffey N, Landry CR, Dopman EB et al. (2008) A  
776 genome-wide view of the spectrum of spontaneous mutations in yeast. *PNAS*  
777 105: 9272–9277.
- 778 Matic I, Radman M, Taddei F, Picard B, Doit C, Bingen E et al. (1997) Highly  
779 variable mutation rates in commensal and pathogenic *Escherichia coli*.  
780 *Science* 277: 1833–1834.

- 781 Mortimer RK (1955) Evidence for two types of x-ray-induced lethal damage  
782 in *Saccharomyces cerevisiae*. *Radiation Research* 2: 361–368.
- 783 Muller HJ (1950) Our load of mutations. *Am J Hum Genet* 2: 111–176.
- 784 Newcombe RG (1998) Interval estimation for the difference between  
785 independent proportions: Comparison of eleven methods. *Statistics in*  
786 *Medicine* 17: 873–890.
- 787 Nishant KT, Wei W, Mancera E, Argueso JL, Schlattl A, Delhomme N et al.  
788 (2010) The baker's yeast diploid genome is remarkably stable in vegetative  
789 growth and meiosis. *PLOS Genetics* 6: e1001109.
- 790 Oliver A, Cantón R, Campo P, Baquero F, Blázquez J (2000) High frequency of  
791 hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection.  
792 *Science* 288: 1251–1253.
- 793 Peter J, Chiara MD, Friedrich A, Yue J-X, Pflieger D, Bergström A et al. (2018)  
794 Genome evolution across 1,011 *Saccharomyces cerevisiae* isolates. *Nature*  
795 556: 339.
- 796 R Core Team (2019) R: A Language and Environment for Statistical  
797 Computing. R Foundation for Statistical Computing: Vienna, Austria.
- 798 Raghavan V, Bui DT, Al-Sweel N, Friedrich A, Schacherer J, Aquadro CF et al.  
799 (2018) Incompatibilities in mismatch repair genes MLH1-PMS1 contribute to  
800 a wide range of mutation rates in human isolates of baker's yeast. *Genetics*  
801 210: 1253–1266.

- 802 Raynes Y, Sniegowski PD (2014) Experimental evolution and the dynamics of  
803 genomic mutation rate modifiers. *Heredity* 113: 375–380.
- 804 Raynes Y, Gazzara MR, Sniegowski PD (2011) Mutator dynamics in sexual  
805 and asexual experimental populations of yeast. *BMC evolutionary biology* 11:  
806 158.
- 807 Raynes Y, Wylie CS, Sniegowski PD, Weinreich DM (2018) Sign of selection on  
808 mutation rate modifiers depends on population size. *PNAS* 115: 3422–3427.
- 809 Rice WR (2002) Experimental tests of the adaptive significance of sexual  
810 recombination. *Nature reviews Genetics* 3: 241.
- 811 Richardson AR, Yu Z, Popovic T, Stojiljkovic I (2002) Mutator clones of  
812 *Neisseria meningitidis* in epidemic serogroup A disease. *PNAS* 99: 6103–  
813 6107.
- 814 RStudio Team (2015) RStudio: Integrated development environment for R.  
815 RStudio, Inc.: Boston, MA.
- 816 Ruderfer DM, Pratt SC, Seidel HS, Kruglyak L (2006) Population genomic  
817 analysis of outcrossing and recombination in yeast. *Nature Genetics* 38:  
818 1077–1081.
- 819 Schaaper RM, Dunn RL (1991) Spontaneous mutation in the *Escherichia coli*  
820 *lacI* gene. *Genetics* 129: 317–326.

- 821 Serero A, Jubin C, Loeillet S, Legoix-Né P, Nicolas AG (2014) Mutational  
822 landscape of yeast mutator strains. *PNAS* 111: 1897–1902.
- 823 Sharp NP, Sandell L, James CG, Otto SP (2018) The genome-wide rate and  
824 spectrum of spontaneous mutations differ between haploid and diploid  
825 yeast. *PNAS* 115: E5046–E5055.
- 826 Shaver AC, Dombrowski PG, Sweeney JY, Treis T, Zappala RM, Sniegowski PD  
827 (2002) Fitness evolution and the rise of mutator alleles in experimental  
828 *Escherichia coli* populations. *Genetics* 162: 557–566.
- 829 Shcheprova Z, Baldi S, Frei SB, Gonnet G, Barral Y (2008) A mechanism for  
830 asymmetric segregation of age during yeast budding. *Nature* 454: 728–734.
- 831 Sniegowski PD, Gerrish PJ, Lenski RE (1997) Evolution of high mutation rates  
832 in experimental populations of *E. coli*. *Nature* 387: 703–705.
- 833 Surtees JA, Argueso JL, Alani E (2004) Mismatch repair proteins: Key  
834 regulators of genetic recombination. *CGR* 107: 146–159.
- 835 Thompson DA, Desai MM, Murray AW (2006) Ploidy controls the success of  
836 mutators and nature of mutations during budding yeast evolution. *Current*  
837 *Biology* 16: 1581–1590.
- 838 Tran HT, Keen JD, Kricker M, Resnick MA, Gordenin DA (1997)  
839 Hypermutability of homonucleotide runs in mismatch repair and DNA  
840 polymerase proofreading yeast mutants. *Molecular and Cellular Biology* 17:  
841 2859–2865.



- 842 Trong HN, Prunier A-L, Leclercq R (2005) Hypermutable and  
843 fluoroquinolone-resistant clinical isolates of *Staphylococcus aureus*.  
844 *Antimicrobial Agents and Chemotherapy* 49: 2098–2101.
- 845 Wach A, Brachat A, Pöhlmann R, Philippsen P (1994) New heterologous  
846 modules for classical or PCR-based gene disruptions in *Saccharomyces*  
847 *cerevisiae*. *Yeast* 10: 1793–1808.
- 848 Wang P, Robert L, Pelletier J, Dang WL, Taddei F, Wright A et al. (2010)  
849 Robust growth of *Escherichia coli*. *Current Biology* 20: 1099–1103.
- 850 Wickham H (2016) *Ggplot2: Elegant Graphics for Data Analysis*. Springer.
- 851 Wloch DM, Szafraniec K, Borts RH, Korona R (2001) Direct estimate of the  
852 mutation rate and the distribution of fitness effects in the yeast  
853 *Saccharomyces cerevisiae*. *Genetics* 159: 441–452.
- 854 Zhu YO, Siegal ML, Hall DW, Petrov DA (2014) Precise estimates of mutation  
855 rate and spectrum in yeast. *Proceedings of the National Academy of Sciences*  
856 111: E2310–E2318.
- 857

## 858 **Figures and Tables**

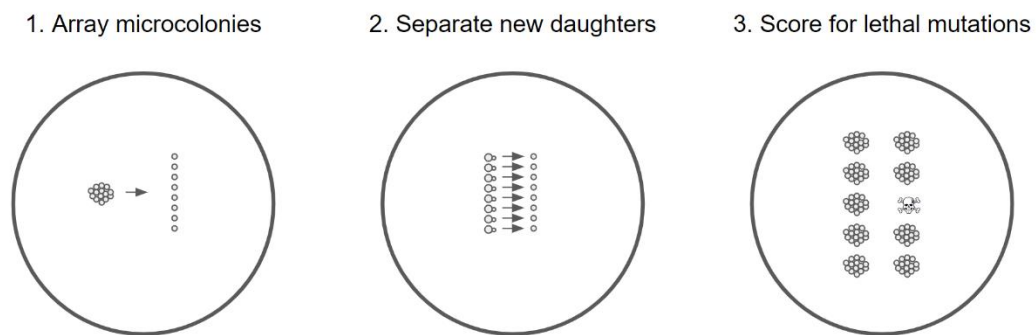
859 **Table 1**

	Wild type		<i>mmr</i>		
<b>Event</b>	<b>Count</b>	<b>Rate</b>	<b>Count</b>	<b>Rate</b>	<b>p-value</b>
Both OK	2235	0.9967	2145	0.9908	0.0006
One lethal	14	0.0031	33	0.0076	0.0049
One strongly reduced growth	1	0.0002	7	0.0016	0.0363

860 Counts and frequencies of events of interest in the lethal assay. "Both OK" means that both  
861 mother and daughter cell grew into normal colonies. "One lethal" means that the lineage  
862 founded by either the mother or daughter cell ceased to grow within the observation period.  
863 "One strongly reduced growth" means that either the mother or daughter lineage was  
864 observed to grow noticeably slowly. Other events--both members of the duo lethal or  
865 strongly reduced growth, or the mother never budding--were not included in the analysis  
866 and are not displayed here. The p-values reflect the statistical significance of the difference  
867 in rates between wild type and *mmr* strains and were obtained by Fisher's exact test. Note  
868 that events are displayed per duo while rates are calculated per individual.

869

870 **Figure 1**



871

872 Schematic of lethal assay. The arraying in step 1 and separation in step 2

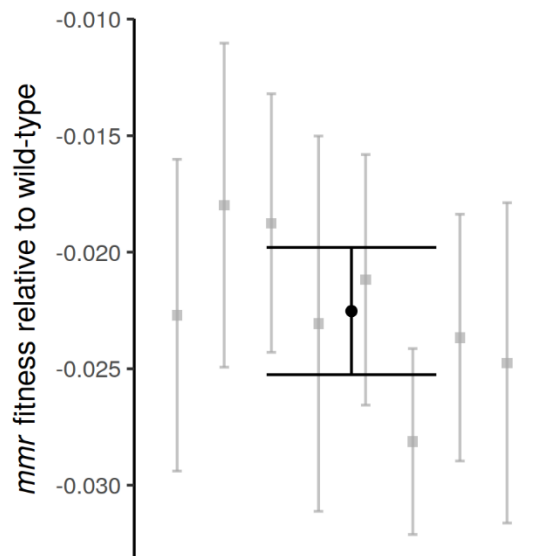
873 were performed by micromanipulation. Microcolonies in step 1 comprised

874 on average 23 cells, and were founded by new daughter cells that had

875 themselves been isolated by micromanipulation.

876

877 **Figure 2**

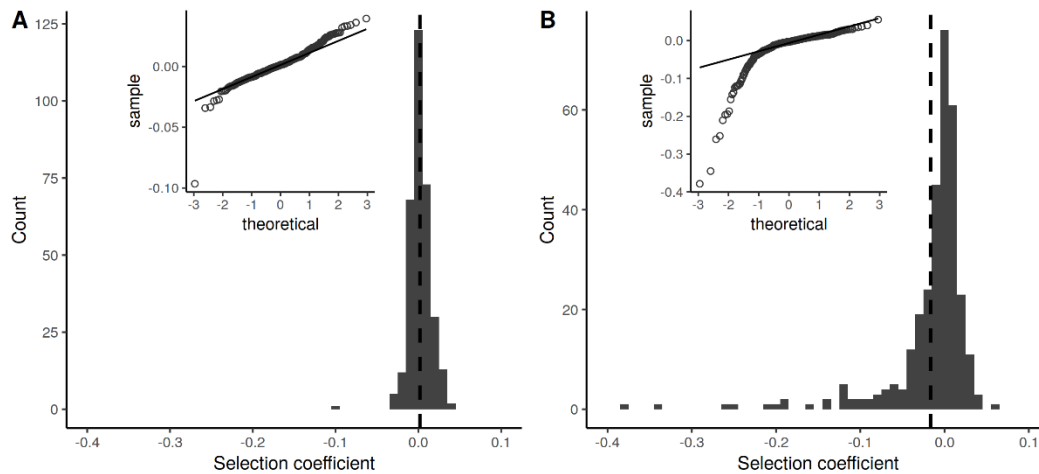


878

879 The average competitive fitness deficit (in black, error bars are 95%  
880 confidence intervals) of the mutator strain relative to the wild-type,  
881 expressed as selection coefficient, is 2.3%. Fitness competitions were  
882 conducted in a series of 8 blocks, shown in gray. The two strains were not  
883 competed directly against each other; within each block, each was competed  
884 separately against an otherwise isogenic *MMR+* *YFP+* strain. Each block  
885 contained between 5 and 8 replicate competitions.

886

887 **Figure 3**

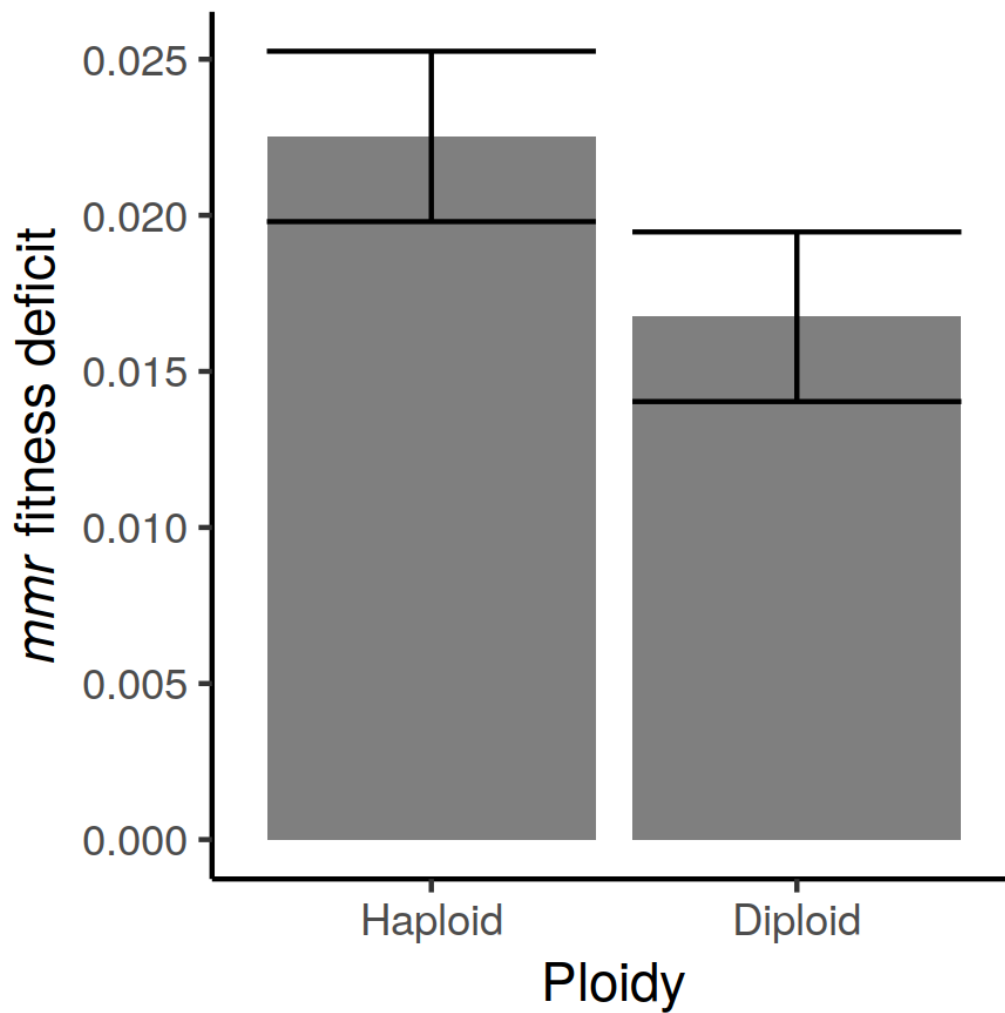


888

889 Distributions of fitness in haploid wild-types (**A**) and *mmr* mutators (**B**). We  
890 measured the fitnesses of 327 wild-type and 313 *mmr* clones. Fitnesses were  
891 measured in competition with an *MMR+* *YFP+* reference strain otherwise  
892 isogenic to the wild-type, as described in Methods. Dashed vertical lines  
893 indicate the mean. The load is  $\sim 0$  for wild-types; for *mmr* mutators it is  
894  $\sim 1.7\%$ . QQ plots of the fitness distributions are shown as insets. The two  
895 distributions differ significantly (Kolmogorov-Smirnov test,  $p < 10^{-8}$ ).

896

897 **Figure 4**



898

899 Change in *mmr* fitness disadvantage with ploidy. The fitness deficit, relative

900 to the wild type, decreased by 26% from haploid to diploid strains ( $p <$

901 0.004).