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1 **Mutations in yeast are deleterious on average regardless of the degree of adaptation to**
2 **the testing environment**
3
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11 **Abstract**
12 The role of spontaneous mutations in evolution depends on the distribution of their effects on
13 fitness. Despite a general consensus that new mutations are deleterious on average, a handful
14 of mutation accumulation experiments in diverse organisms instead suggest that of beneficial
15 and deleterious mutations can have comparable fitness impacts, i.e., the product of their
16 respective rates and effects can be roughly equal. We currently lack a general framework for
17 predicting when such a pattern will occur. One idea is that beneficial mutations will be more
18 evident in genotypes that are not well adapted to the testing environment. We tested this
19 prediction experimentally in the laboratory yeast *Saccharomyces cerevisiae* by allowing nine
20 replicate populations to adapt to novel environments with complex sets of stressors. After >1000
21 asexual generations interspersed with 41 rounds of sexual reproduction, we assessed the mean
22 effect of induced mutations on yeast growth in both the environment to which they had been
23 adapting and the alternative novel environment. The mutations were deleterious on average,
24 with the severity depending on the testing environment. However, we find no evidence that the
25 adaptive match between genotype and environment is predictive of mutational fitness effects.

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26 **Introduction**

27 Many mutations are deleterious or neutral, but some serve as the basis for novelty and
28 adaptation. Predicting the distribution of fitness effects of new mutations (DFE) is an important
29 challenge in evolutionary genetics (1,2). Methods are being explored to experimentally
30 characterize the fitness effects of many individual alleles (3–5), and to predict mutational fitness
31 effects based on molecular data (6–8). While these are exciting prospects, it is not clear that we
32 can currently predict basic elements of the DFE, even in model organisms. A key method for
33 studying mutational effects is mutation accumulation (MA), where selection is rendered largely
34 ineffective in replicate lineages by repeated bottlenecking to minimize the effective population
35 size (9–11). Most such studies show a decline in mean fitness under MA, indicating that
36 beneficial mutations must have a much smaller net impact than deleterious mutations, i.e., they
37 occur less frequently, have weaker effects, or both. However, fitness decline is not always
38 observed in MA experiments, even when other evidence confirms that un-selected mutations
39 are accumulating (reviewed in (12)). The simplest interpretation of this pattern is that beneficial
40 mutations were relatively common, with a net impact on fitness similar to that of deleterious
41 mutations (5,13,14). We should ideally be able to predict whether beneficial mutations will be
42 common or scarce, in order to understand the role of mutation in evolution.

43

44 An attractive explanation for the presence of inconsistent consequences of MA has to do with
45 the extent of prior adaptation. Intuitively, a genotype that is well adapted to a given environment
46 would have little beneficial mutational variation available for further adaptation, relative to a
47 poorly-adapted genotype in the same environment—minimal adaptation to the testing
48 environment could perhaps account for the lack of mean fitness decline observed in some MA
49 experiments. Such an outcome might be more likely for organisms that are preserved in
50 freezers or seed banks prior to experimentation (e.g., yeast, plant seeds), as opposed to those
51 maintained continually in lab environments (e.g. *Drosophila*). Using a "fitness landscape"

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52 analogy, a population on a fitness peak would have nowhere to go but down, but one further
53 from the peak would have opportunities for fitness improvement. Such models have been
54 explored extensively, evolving from ideas by Fisher as a part of the eponymous Geometric
55 Model (15) to more detailed mathematical treatments (16–20). Extensions of Fisher's model
56 assuming a Gaussian fitness landscape predict that the current "adaptedness" of a genotype
57 influences the variance of mutational fitness effects but not the average effect; this is because
58 while relatively more beneficial mutations are available to a maladapted genotype, this is offset
59 by an increase in the severity of deleterious mutations (17). Experimental tests of these
60 predictions have yielded somewhat mixed results (21,22), which we review briefly below (see
61 also (12,23)).
62
63 To study the role of adaptation in shaping the DFE, Wang et al. (21) studied the fitness effects
64 of 36 gene disruption alleles back-crossed into *Drosophila melanogaster* genotypes that had
65 previously adapted to one of two alternative environments (cadmium- and salt-enriched larval
66 media diets), when measured in each environment. They found that these alleles were generally
67 deleterious regardless of whether the genetic background was well-adapted to the assay
68 environment or not, and that the environment per se had the largest effect on the mean
69 selection coefficient, rather than evolutionary history: selection against gene disruptions was
70 stronger on average when assessed in the salt environment regardless of genetic background.
71 Genetic background and adaptedness were also found to have no strong effect on the variance
72 in selection coefficients, whereas environment did have an effect on the variance. In another
73 study (22), *Arabidopsis thaliana* lines from the extremes of its natural range (southern France
74 and central Sweden) were used to test the predictions of fitness landscape theory. Founder
75 plants collected from these two range extremes were cultivated for 7-10 generations under MA
76 conditions. They then assessed the change in fitness in these mutant lines in both the founder's
77 original environment ("home") and the other environment ("away"). MA lines with a fitness

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78 difference from the ancestor showed a nearly universal trend of fitness decline. MA lines also
79 performed worse in the novel sites than in those sites where their founding genotype originated,
80 contradicting the prediction that there would be no difference in the mean fitness effects of new
81 mutations. The authors concluded that the mean fitness effect of mutations depends more on
82 the environment than on adaptedness, similar to the Wang et al. (21) study. While variance
83 among lines was positively correlated with how stressful the environment was (and away sites
84 were universally more stressful than home sites for the mutants), among-line variance
85 accounted for very little of the overall variation (22).
86
87 While compelling, these studies naturally have limitations. Wang et al. (21) studied specific gene
88 disruption mutations on a single chromosome. While this allowed for the effects of specific,
89 individual mutations to be assessed, spontaneous mutations have a more diverse molecular
90 spectrum, affecting both genic and non-coding regions, which can both have effects on fitness
91 (1,24). In another study, Weng et al. (22) relied on natural populations of a species with well-
92 documented patterns of local adaptation. The use of natural variation brings with it all the
93 challenges presented by any wild population like standing genetic variation, demographic
94 structure, and an uncertain evolutionary history. Further complicating the picture, several
95 different fitness metrics were used, which produced somewhat inconsistent results. Finally,
96 although they did find increased variance in the more stressful environment as predicted by the
97 Martin and Lenormand model (17), this could have been due to standing genetic variation that
98 only influenced fitness in more stressful environments (25). These studies in very different
99 organisms reflect the challenges of testing the predictions of the fitness landscape model,
100 perhaps explaining why experiments of this kind remain scarce.
101
102 Our goal was to investigate how new mutations affect fitness given alternative histories of
103 adaptation in the budding yeast *Saccharomyces cerevisiae*. In this species there are examples

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104 of MA apparently resulting in little mean fitness decline (14,26), suggesting that the average
105 effect of mutations may be influenced by the environment or genetic background. We followed
106 previous studies by using a reciprocal transplant-style design, which allows effects of adaptation
107 to be distinguished from effects of the environment. We allowed diploid *S. cerevisiae*
108 populations to adapt to novel environments that each contained multiple stressors, with regular
109 opportunities for sexual reproduction within populations to facilitate adaptation (27). We chose
110 to create relatively complex environments to minimize the possibility that adaptation would
111 involve only a single genetic pathway (28,29), which might have idiosyncratic interactions with
112 new mutations. After generating these "locally adapted" populations, with highest fitness in their
113 "home" environments, we measured the fitness consequences of induced mutations in both
114 "home" and "away" environments, for each population. We found that mutations had deleterious
115 effects on average, regardless of whether the genetic background was well- or poorly-adapted
116 to the testing environment.
117

118 **Methods**

119 *Strains, growth measures and media*

120 We obtained the haploid strain FM1282 from C. Hittinger, which was derived from BY4724 and
121 modified to express green fluorescent protein (30). By inducing mating-type switching and
122 mating we generated a homozygous diploid version of this strain with genotype *ho ura3Δ lys2Δ*
123 PTDH3-yEGFP-TCYC1 to use in our experiments; we preserved this strain at –80 C, and
124 hereafter we refer to it as the "ancestral" strain. We cultured the yeast at 30 C with continuous
125 shaking applied to the liquid cultures. We used growth rate assays in several stages of our
126 study, which we conducted in a BioTek Epoch II plate reader, taking regular measurements of
127 optical density (OD) at 600 nm with incubation and shaking. We calculated maximum growth
128 rate in each assay culture by fitting a spline to the log of OD over time using the *loess* function

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129 in *R* with degree = 1 and span = 0.2 and finding the maximum slope of this spline across 99
130 equally-spaced time points.
131
132 Our first goal was to generate three liquid-media environments that resulted in moderately
133 reduced yeast growth rates. Based on literature searches we identified several substances
134 known to affect growth rate and tested their effects when added individually to liquid yeast-
135 peptone-dextrose (YPD) media to find appropriate concentration ranges. In order to generate
136 "complex" environments we combined groups of four substances and performed additional
137 growth rate tests. We chose combinations of substances haphazardly, but we sought to make
138 each environment distinct, and excluded any combinations found to precipitate out of solution.
139 The final media environments used in our experiments contained the following substances
140 dissolved in YPD. We called these environments EnvA, EnvB and EnvC; *EnvA*: boric acid,
141 caffeine, nickel chloride and sodium fluoride; *EnvB*: acetic acid, acetaminophen, lithium chloride
142 and sodium chloride; *EnvC*: chromium potassium sulfate, cupric sulfate, sodium benzoate and
143 zinc chloride. Recipes and concentrations for each environment are given in Table S1. These
144 three environments all produced similar reductions in growth rate of the ancestral strain, relative
145 to its growth rate in YPD (Fig. 1).
146
147 *Adaptation*
148 For each of the three environments we initiated three replicate yeast populations, each growing
149 in 3 mL of liquid media. We passaged each population three times a week (every 2-3 days) by
150 inoculating 3 mL of new media with 30 μL of the previous culture, i.e., a 1:100 dilution. We refer
151 to populations that were serially passaged through these environments as A-evolved, B-
152 evolved, and C-evolved, respectively. At the end of each week, we sampled yeast from each
153 population and assessed their growth rate in their assigned media, alongside the ancestral
154 strain (see below). Relative fitness is calculated as the difference in growth rate between the

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155 ancestor and the evolving populations. This allowed us to monitor the relative fitness of the
156 adapting populations on a regular basis. We froze samples from each population in 15%
157 glycerol at regular intervals throughout this phase as well as at the end of the adaptation. These
158 procedures are depicted in Fig. 2A.
159
160 Every third week we allowed the adapting populations to undergo sexual reproduction within
161 their own population (independent replicate populations were not mixed). We inoculated 3 mL of
162 pre-sporulation medium (1% potassium acetate, 1% yeast extract, 2% peptone), with 30 μL of
163 saturated culture for each replicate population and allowed the cultures to grow for 18-24 h with
164 shaking at 30 C. We then centrifuged these cultures, resuspended the cells in 3 mL sporulation
165 medium (1% potassium acetate) supplemented with amino acids (0.01% lysine and uracil), and
166 incubated them at room temperature on a rotor for 72-120 h. These conditions encourage
167 sporulation but limit the opportunity for asexual growth. We then examined these cultures for the
168 presence of tetrads, but used all cells to inoculate 3 mL of new media, allowing asexual growth
169 to resume in the appropriate media environment for each replicate population. Note that this
170 procedure allowed for sexual reproduction to take place but did not enforce it, as non-sporulated
171 cells were not eliminated. We assume that haploid spores germinated upon returning to nutritive
172 media, and mated to re-form diploid cells, but we also confirmed ploidy at the end of the
173 adaptation phase (see below). During the weeks when sporulation was permitted, we did not
174 freeze any of the adapting populations or conduct fitness assays. The sporulation media did not
175 include our stressful additives, and so we do not include the sporulation periods when
176 calculating the total time spent adapting to the novel media environments. In total, the
177 experimental populations spent 357 days growing asexually in their respective media. Given the
178 number of 100-fold dilutions, 160, there must have been at least $160 log_2(100) = 1063$ rounds of
179 asexual cell division over the course of the experiment. At ten timepoints throughout the
180 adaptation phase we performed cell counts to estimate the size of the experimental populations

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181 just prior to passaging. For the cultures we ultimately used in tests of mutation effects, the
182 average population size at passaging was 7.7×10^8 cells per mL (SE 4.2×10^7, $n = 106$),
183 indicating effective population sizes of at least 7.7×10^6, i.e., the estimated population size
184 immediately following each dilution.
185
186 *Growth rate assays during adaptation*
187 Throughout the adaptation phase of the experiment, we conducted regular assays to track the
188 growth rate of the evolving populations in their respective media environments relative to the
189 ancestor. Prior to each assay, we revived the ancestor strain, which had not undergone any
190 adaptation, from frozen stock and grew it in YPD for two days. We set up a 96 well plate with a
191 stratified treatment arrangement; each plate held 21 wells each of A-evolved, B-evolved, and C-
192 evolved in their respective media types, 9 wells each of the ancestor growing in EnvA, EnvB,
193 and EnvC for comparison, and two "blank" wells per media type, not inoculated with yeast, to
194 detect contamination. We diluted each yeast culture 500-fold to initiate the growth assay, and
195 obtained OD readings every 15 min for 30 h.
196
197 *Growth rate assays following adaptation*
198 Following the adaptation phase of the experiment we assessed whether each population grew
199 more rapidly in its "home" environment than in the alternative, "away" environments. We also
200 included the ancestral strain in these assays for comparison (Fig. 2A). We designed these
201 assays to be similar to the transplantation assays used in other studies to verify that local
202 adaptation had occurred (22), i.e., that the best performing populations in a given environment
203 were those with a history of adaptation to that environment. We conducted these assays using
204 the same methodology and setup as the weekly fitness assays conducted during the adaptation
205 phase of the experiment except that we collected data for 48 h rather than 30 h to account for
206 the possibly slowed growth rates in novel environments. We tested each adapting population in

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207 each of the three environments, effectively "transplanting" lines from familiar "home"
208 environments they had adapted to (e.g., B-adapted populations in Env B) to novel "away"
209 environments(e.g., B-adapted populations in EnvA). We additionally tested the ancestral strain
210 in each environment. As described below (see Results), we found that the expected signature of
211 local adaptation was absent for the C-evolved populations, and so we excluded them from the
212 remainder of the experiment. We repeated this assay using only A-evolved and B-evolved
213 populations in EnvA and EnvB, testing six replicates of each evolved population in each
214 environment, and four replicates per environment for the ancestral strain. Relative growth rate
215 was calculated as the difference in growth rate between an evolved population in a given
216 environment and that of the ancestor in the same environment.
217
218 *Mutagenesis and mutant fitness assay*
219 We counted the number of cells in saturated samples from each population grown in their home
220 environments, and used this number to plate 100–200 single cells on each of three YPD agar
221 plates. We then placed two plates in a biosafety cabinet, approximately 84 cm from a germicidal
222 UVC bulb, and irradiated them for 10 seconds with their lids removed, with the remaining plate
223 serving as a control. Following 2 d of incubation we counted colonies on irradiated and non-
224 irradiated plates, and observed an expected 40-70% reduction in colony counts for all irradiated
225 plates (31), representing a significant effect of UV on cell survival. This is taken to indicate that
226 mutagenesis was effective in generating non-lethal mutations in the surviving cells. For each
227 strain we chose one irradiated plate at random and picked several colonies from this plate and
228 from the the non-irradiated plate using sterile toothpicks. To prevent bias, we picked colonies
229 from the center of the plate moving outwards. We suspended each colony in sorbitol before
230 dividing the volume into two 1.5 mL centrifuge tubes, followed by a 10-fold dilution in sorbitol.
231 These colonies each represent a mutant line and will be referred to as "mutants" and mutant or
232 mutagenized "genotypes." We centrifuged these tubes, removed the sorbitol, and resuspended

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233 the cells from one tube in EnvA and the other in EnvB. In this way we were able to measure the
234 growth rate of each mutant genotype, as well as the non-mutagenized control, in both EnvA and
235 EnvB. We measured growth rates using the same procedures as the fitness and transplantation
236 assays described above, and collected data for 48 h. In total, we measured the growth rate of
237 162 mutagenized genotypes and 114 non-mutagenized genotypes in both EnvA and EnvB, for a
238 total of 552 measurements. Here, relative growth rate is the difference in growth rate between a
239 mutant in a given environment and a non-mutagenized control with the same evolutionary
240 history in the same environment.
241
242 A class of mutation that may occur in irradiated yeast is respiratory deficiency, resulting in the
243 "petite" phenotype (32,33). This could be problematic for our fitness assays because if this type
244 of mutation is common it could have an outsized impact on fitness relative to other mutations.
245 To determine how frequently our mutagenesis protocol produced mutants with the petite
246 phenotype we repeated the UV mutagenesis procedure and selected mutant colonies following
247 the same procedure used for the mutant fitness assays. We then patched these mutants onto
248 YPD agar plates alongside non-mutagenized controls. After 3 d of growth at 30 C, we replica
249 plated onto YPG (yeast peptone glycerol) agar plates, a non-fermentable medium on which
250 petite strains cannot grow, allowing us to determine the frequency of petites.
251
252 *Flow cytometry*
253 While we allowed for sexual reproduction (sporulation) periodically throughout the adaptation
254 phase of our experiment, we expected the resulting haploid spores to rapidly germinate and
255 mate with one another upon encountering nutritive media, thereby restoring the diploid state.
256 However, unicellular fungi have been known to change ploidy during adaptation (34,35), and so
257 we confirmed ploidy using flow cytometry for each experimental population following adaptation,
258 using known haploid and known diploid versions of the ancestral strain for comparison.

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259 Following overnight growth, we combined 200 µl of each culture with 800 µl pure water, pelleted
260 cells, and gently resuspended in 1 mL cold 70% ethanol. After 1 h incubation at room
261 temperature we pelleted and washed cells twice with 1 mL sodium citrate (50 mM, pH 7), then
262 added 25 µL of RNAse A (10 mg/mL) and incubated overnight at 37 C. We then pelleted and
263 resuspended cells in sodium citrate and added 30 µL of 50 µM SYTOX green nucleic acid stain
264 (Invitrogen S7020). Finally, we incubated samples in the dark overnight and performed analysis
265 of particle fluorescence and size on an Attune NxT V6 flow cytometer (ThermoFisher). We
266 removed particles with extreme size or shape from the dataset, and visually compared the
267 fluorescence profiles of known haploids and diploids with those of our experimental populations.
268 These analyses confirmed that all nine of our experimental populations consisted of cells in the
269 diploid state by the end of the adaptation phase (Fig. S1).
270

271 **Results**

272 *Adaptation*

273 Throughout the adaptation phase of the experiment, we visually confirmed the presence of
274 tetrads in all adapting populations during each week of sporulation, indicating that some
275 propensity to undergo sexual reproduction was maintained. After 372 days of asexual growth
276 (>1000 generations) in the novel media environments (not including time spent in sporulation
277 media), all populations of A-evolved, B-evolved, and C-evolved showed a significant
278 improvement in fitness when compared to the ancestral strain grown in the same environment
279 (Fig. 3): in a mixed-effect model of growth rate accounting for random effects of population,
280 environment, and assay block, we find a highly significant interaction between evolution status
281 (adapting or ancestral), and time (days of evolution) (likelihood ratio test (LRT) χ^2 = 125.13, $P <$
282 10^{-15}). A random effect of assay plate location did not have a significant impact on our results
283 when factored into the linear models (LRT: χ^2 = 5.20, P = 0.074), and was omitted. To

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284 summarize, the experimental populations all performed better than the unadapted ancestors in
285 their respective media types.
286
287 *Transplantation assay*
288 We performed a transplantation assay to identify signatures of "local adaptation". Our initial
289 assay tested each permutation of evolved populations and ancestral, unadapted yeast with
290 each of the environments EnvA, EnvB, and EnvC (Fig. 4A). Using a mixed-effect model of
291 growth rate with random effects of population and assay block we found a highly significant
292 interaction effect between evolutionary history (i.e., the environment in which a population
293 evolved) and the test environment (excluding the ancestor; LRT, $\chi^2 = 67.892$, $P < 1 \times 10^{-15}$). We
294 then analyzed the effect of evolutionary history in each test environment separately, and
295 detected significant effects of evolutionary history in EnvA (LRT, $\chi^2 = 3.8743$, $P = 0.049$) and
296 EnvB (LRT, $\chi^2 = 6.97$, $P = 0.0083$). In EnvC we did not detect such an effect (LRT, $\chi^2 = 0.7511$,
297 $P = 0.39$), meaning that populations that had evolved in EnvC did not outperform other evolving
298 populations in that environment (Fig. 4A). Notably, populations evolving in EnvC also showed
299 the weakest improvement in relative growth rate in our weekly fitness assays (Fig. 3).
300
301 Because the C-evolved populations failed to show a signature of local adaptation, we repeated
302 the transplantation assay with only A-evolved and B-evolved populations and EnvA and EnvB
303 (Fig. 4B). Repeating our analyses with mixed effect linear models with replicate population and
304 plate as random effects, we again found a highly significant genotype by environment
305 interaction effect on growth rate (excluding the ancestor; LRT, $\chi^2 = 298.21$, $P < 10^{-15}$). The
306 testing environment had a significant effect on growth rate for both A-evolved (LRT, $\chi^2 = 34.893$,
307 $P < 10^{-8}$) and B-evolved (LRT, $\chi^2 = 225.02$, $P < 10^{-15}$) populations, where growth was highest in
308 the environment to which each population had adapted (Fig. 4B). We reanalyzed the data using
309 the *emmeans* package in *R* to obtain post-hoc contrasts accounting for multiple testing, pooling

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310 the replicates of the adapting populations within treatment groups after determining the replicate
311 populations did not differ statistically (all $\chi^2 < 3.12$, all $P > 0.077$). Contrasts showed that each
312 adapted population performed better than unadapted ancestors in EnvA ($t = 5.170$, $P < 0.0001$)
313 and EnvB ($t = 10.438$, $P = 0.0067$). We then asked if the evolved populations performed
314 differently from the ancestor in non-familiar environments (e.g. A-evolved grown in EnvB). The
315 adapted populations did not perform significantly differently than the ancestors in these
316 comparisons (A-evolved vs. ancestor in EnvB: $t = 1.445$, $P = 0.47$; B-evolved vs. ancestor in
317 EnvA: $t = -1.713$ $P = 0.45$). This shows that adaptation to a given environment did not result in
318 significantly improved fitness in another environment with a different set of stressors. This can
319 also be taken as an indication that general lab adaptation played little to no role in the fitness of
320 the adapted populations.
321
322 *Mutant fitness*
323 Our assessment of respiratory deficiency in mutagenized cells indicated that this type of
324 mutation was not widespread: about 2% of mutagenized cells formed petite colonies (8/407
325 colonies tested), and this was not significantly higher than the petite frequency in the non-
326 mutagenized treatment (0/141 colonies tested; Fisher's exact test, $P = 0.212$). Among the
327 mutagenized colonies we used for our fitness assay, we would therefore expect only two or
328 three petites, and so this type of mutation should not have undue influence on our results.
329
330 We measured the fitness of mutagenized genotypes and their non-mutagenized counterparts.
331 Each genotype was measured twice: once in EnvA and once in EnvB. To compare groups we
332 used bootstrapping with 10 000 replicates, retaining the paired nature of the data, i.e., the fact
333 that each genotype was measured both EnvA and EnvB. First, we observed that the
334 mutagenized genotypes had significantly lower growth rates than non-mutagenized genotypes,
335 averaging over adaptive history and testing environments (bootstrap $P < 2 \times 10^{-4}$),

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336 demonstrating that mutagenesis was effective in generating (predominantly deleterious)
337 mutations. The average magnitude of fitness reduction caused by mutagenesis was equivalent
338 to approximately 6458 generations of spontaneous mutation accumulation, using data from the
339 wild-type diploid MA lines of Sharp et al. (26) as a standard. For the A-evolved populations,
340 mutagenesis did not significantly reduce average growth rate in EnvA (bootstrap $P = 0.63$) but
341 did so in EnvB (bootstrap $P = 0.0124$). For the B-evolved population, mutagenesis resulted in
342 reduced average growth rates in both EnvA (bootstrap $P < 2 \times 10^{-4}$) and EnvB (bootstrap $P < 2$
343 $\times 10^{-4}$) (Fig. 5).
344
345 Our primary interest was in whether the average effect of mutations depended on the degree of
346 adaptation to the testing environment. If the mean fitness effect of mutations were to become
347 more beneficial in maladapted genotypes, we would expect to find a higher mean relative fitness
348 in mutants assessed in “away” environments than in “home” environments. Our results do not
349 show this pattern. Indeed, mutations were more deleterious on average in the away
350 environment than in the home environment for A-evolved population mutants (bootstrap $P =$
351 0.0466). For the B-evolved case, mutational effects did not differ between environments
352 (bootstrap $P = 0.86$). Averaging across evolutionary environments, there was no evidence that
353 adaptedness influenced the average effect of mutations (bootstrap $P = 0.30$). Given this lack of
354 a genotype-by-environment interaction effect, we can test for main effects of each factor. On
355 average, the fitness effects of mutations were more deleterious in the B-evolved genetic
356 background than the A-evolved genetic background (bootstrap $P = 0.0132$), but we do not find
357 evidence for a difference in mutational effects between EnvA and EnvB ($P = 0.172$). In other
358 words, we detect a main effect of genotype but not environment.
359
360 Fitness landscape theory (17) predicts that the variance of fitness effects of mutations should be
361 higher for genotypes further from the fitness optimum. In our experiment, each mutant genotype

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362 was only measured once in each environment, so we cannot estimate genetic variances.
363 However, if we assume a constant value for error variance in all treatment groups, then the
364 following comparisons would be informative. We detected no difference in the phenotypic
365 variance between the two environments for the mutants derived from A-evolved populations (P
366 = 0.81). Mutants derived from B-evolved populations did show a significant difference in fitness
367 variance, but it was higher in the home environment than in the away environment ($P < 2 \times 10^{-4}$)
368 (See Fig. S2). On its face, this is inconsistent with the prediction that variance should be higher
369 for maladapted genotypes, but we cannot rule out differences in error variance among groups.
370

371 **Discussion**

372 We investigated ideas about mutational fitness effects in relation to degree of adaptedness by
373 conducting experimental evolution and mutagenesis. By using a fast-growing model organism,
374 we were able to allow for >1000 generations of adaptation to complex, novel environments,
375 while maintaining a high degree of control over environmental conditions. We also allowed for
376 frequent genetic mixing within populations, which may have facilitated the response to selection.
377 We surmise that adaptation to the novel environments most likely involved multiple genetic loci,
378 given the chemical complexity of the environments and the relatively gradual adaptation we
379 observed (Fig. 3). We think this scenario is a better match to what natural populations might
380 experience than, e.g., the presence of a single stressful drug. We did not attempt to identify
381 adaptive alleles in our experimental populations, which could serve to verify that adaptation was
382 multifaceted. However, the theory we set out to test requires only differences in fitness between
383 environments, for which we found strong evidence (Fig. 4). Similarly, our mutagenesis protocol
384 had clear effects on fitness (Fig. 5), but we did not attempt to identify the molecular nature of
385 induced mutations.
386

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387 We found that random mutations tended to reduce mean fitness, regardless of whether the
388 genetic background on which they arose was well-adapted to the testing environment or not.
389 This finding is not consistent with the idea that the net effect of mutations might be more
390 beneficial in poorly-adapted genotypes. Formal fitness landscape theory also rejects this notion,
391 predicting instead that the increased availability of beneficial mutations in poorly-adapted
392 genotypes is counteracted by an increase in the severity of deleterious mutations, resulting in
393 no effect of adaptedness on the average effect of new mutations. Our results are not perfectly
394 consistent with this prediction either, as we find that mutations in the A-evolved genetic
395 background were more deleterious on average when tested in EnvB versus EnvA. We also do
396 not see greater variance in mutational effects in novel environments, which is predicted by the
397 theory (Fig. S3), though we can examine only phenotypic and not genetic variances.
398
399 Our experiment was partly motivated by the finding that MA studies don't always show a pattern
400 of fitness decline (13,14). We set out to determine if variation in the adaptedness of the initial
401 genotype could help explain this phenomenon. Theoretical investigations of Gaussian fitness
402 landscapes suggest that such an effect should not be expected (17), and an effect of
403 adaptedness has now been rejected by our study of yeast, along with similar studies of new
404 mutations in *Drosophila* (21) and *Arabidopsis* (22); the fact that emprical studies using diverse
405 model organisms and methods for generating mutations all reject a role of adaptedness
406 suggests that this result may be general. At the same time, these experiments also indicate that
407 a simple application of the fitness landscape model may not be sufficient to predict the fitness
408 effects of new mutations, as there are several cases where mutations appear to be more
409 deleterious in maladapted genotypes.
410
411 There are limitations to our experiment that could affect our conclusions. While we confirmed
412 that fitness increased over time in our experimental populations (Fig. 3), resulting in a pattern of

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413 "local adaptation" (Fig. 4), fitness might have continued to improve if we had allowed even more
414 time for evolution in the novel environments. If the mean effect of new mutations depends on
415 adaptedness, this would presumably be easier to detect when the difference in fitness between
416 adapted and non-adapted genotypes in a given environment is large. One indication that further
417 adaptation may have been possible in our experiment is that the evolved populations still had
418 lower absolute growth rates than the ancestral genotype growing in YPD (compare Fig. 1 and
419 Fig. 3B). Nevertheless, even if the experimental populations had not yet reached new fitness
420 "peaks", we would still expect the predictions of the fitness landscape model to bear out, just to
421 a lesser degree (36). Given the substantial difference in fitness between our adapted and non-
422 adapted populations, particularly in EnvB (Fig. 4), we should be able to detect an effect of
423 adaptation on mutational fitness effects, if present.
424
425 We expect our mutagenesis procedure to generally result in heterozygous mutations, since
426 mutagenized cells did not undergo sex and there was little time for loss-of-heterozygosity events
427 to occur; while this could make it harder to detect the effects of partially recessive mutations
428 (14), selection on heterozygotes is relevant in many natural populations. Additionally, UV
429 mutagenesis is known to create a molecular spectrum of mutations that is somewhat distinct
430 from the spontaneous spectrum (31). Prior experiments of this kind examined X-linked gene
431 dispruption alleles in flies that were hemizygous in males and heterozygous in females (21), or
432 spontaneous mutations in selfing lineages of *Arabidopsis*, which would be largely but not
433 exclusively homozygous by the time of the fitness assays (22).
434
435 As in prior studies, we did not find evidence for a role of adaptedness—the interaction between
436 genotype and environment—in determining the average effect of new mutations. Instead, we
437 found that mutations were more deleterious, on average, in B-adapted yeast, regardless of the
438 testing environment. This is in contrast to the prior studies, which both identify the environment,

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439 rather than the genetic background, as a main effect (21, 22). A main effect of genetic
440 background could in principle be due to a reduced susceptibility of the A-evolved populations to
441 the mutagenesis.
442
443 In conclusion, there appears to be no theoretical or empirical support for the idea that the
444 degree of adaptedness to a given environment has any predictable impact on the average
445 fitness effect of new mutations. On the other hand, attempts to test this idea have identified
446 effects of environment or genotype *per se*, along with other studies (e.g., 37). We can potentially
447 quantify relative adaptedness (or “stress”) in any system, and so it would be convenient if this
448 unifying metric had predictive value for the DFE. Unfortunately, there is more evidence that
449 environment and genetic background, of which there are innumerable potential states, can
450 independently determine how new mutations affect fitness, and so predicting the DFE, or even
451 its average, remains a significant challenge.
452
453

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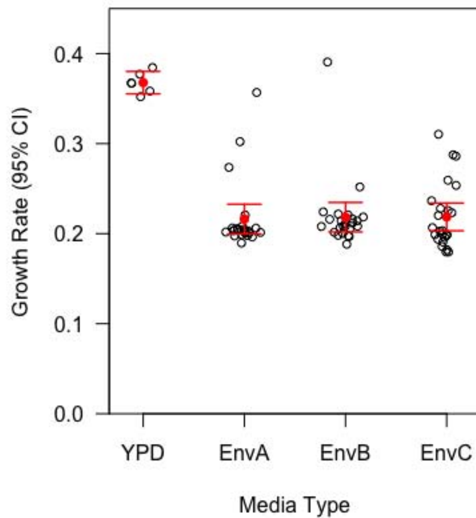
454 **Figures**



455

456 **Figure 1. Initial media environments reduced the growth rate of laboratory yeast.** All three
457 of the newly formulated environments had a significant impact on growth rate when compared to
458 standard laboratory YPD (t-tests; EnvA: $P = 2.566 \times 10^{-15}$; EnvB: $P = 3.387 \times 10^{-15}$; EnvC: $P =$
459 2.473×10^{-15}). The three novel environments all reduced growth to a similar extent. Growth rate
460 refers to the rate of change of OD per hour.

461

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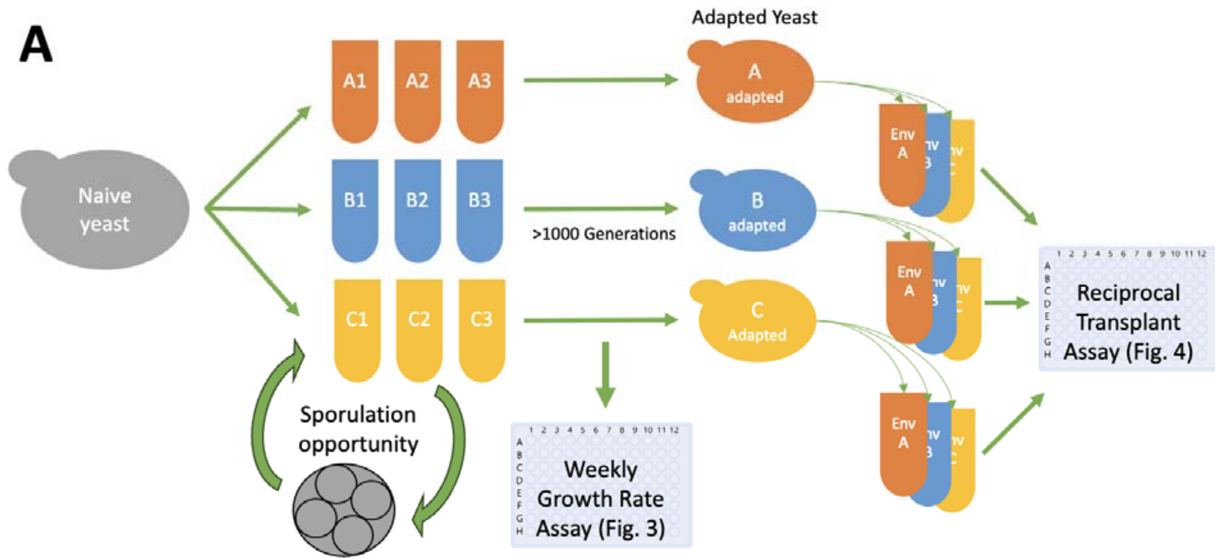

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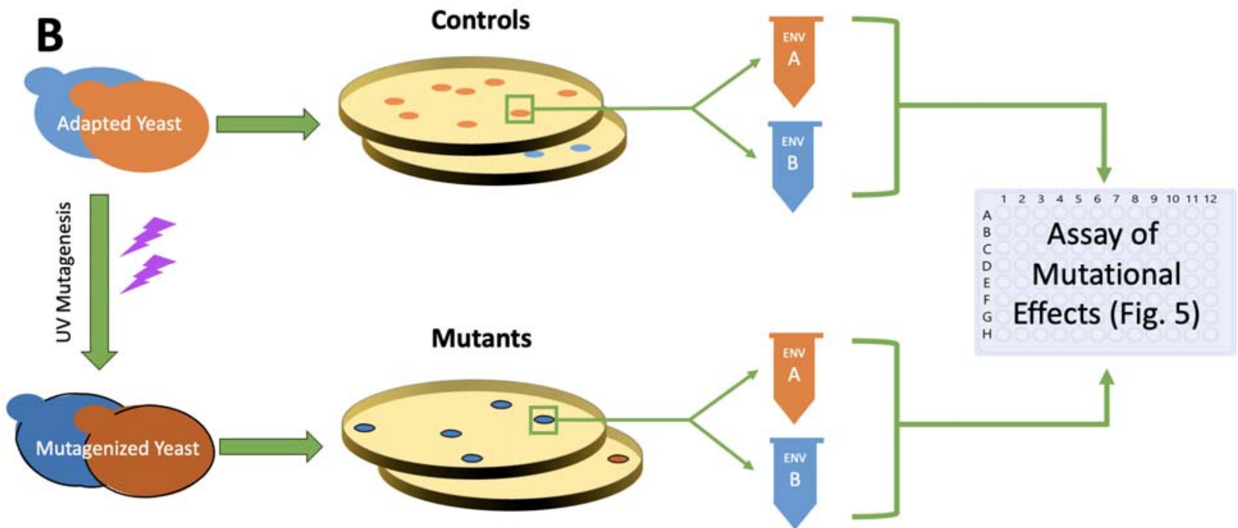

464

465

466 **Figure 2: Diagrammatic representation of the experimental protocol.** Panel A depicts the
467 experimental steps that were undertaken during the “adaptation” phase of the experiment where
468 we took populations of yeast and subjected them to challenging environments for many
469 generations, with periodic opportunities for sexual reproduction (sporulation). The results of this
470 part of the experiment are shown in Figures 2 and 3. Panel B depicts the steps taken to

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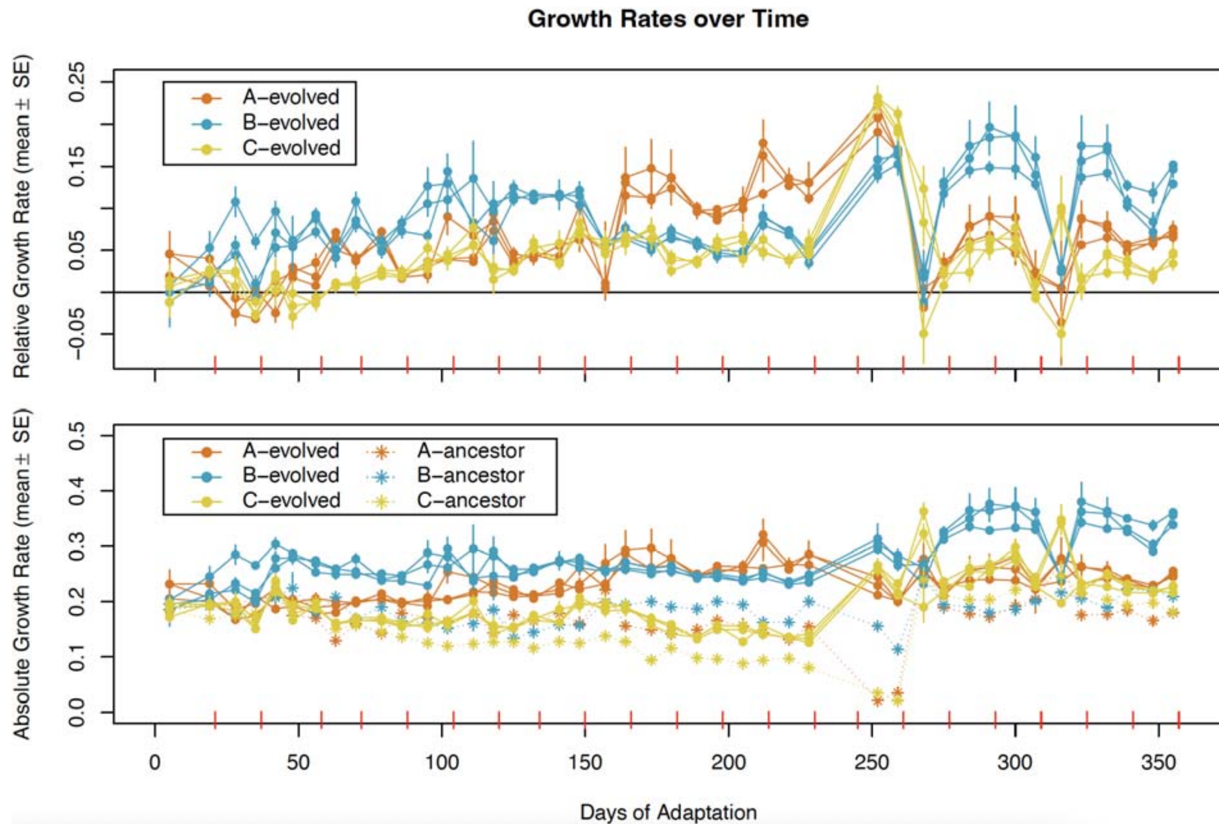
471 mutagenize yeast and then measure their fitness in both familiar and novel environments. The
472 results of these experiments are shown in Figure 5.



473

474 **Figure 3. Growth in novel environments improved over time.** The top panel shows growth
475 rate of the evolving populations relative to ancestors while the bottom shows the absolute
476 growth rate of ancestors and evolving lines. Red tick marks on the x-axes indicate sporulation
477 opportunities. These were not counted toward total days of adaptation because sporulation
478 medium was permissive. By the end of the experiment, all evolving populations showed
479 substantially improved growth when compared to the ancestor.

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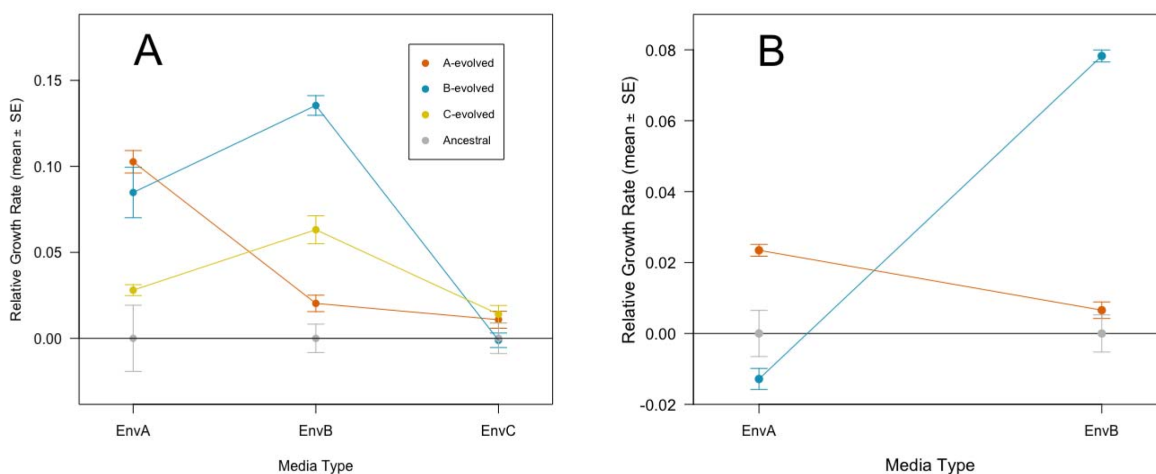
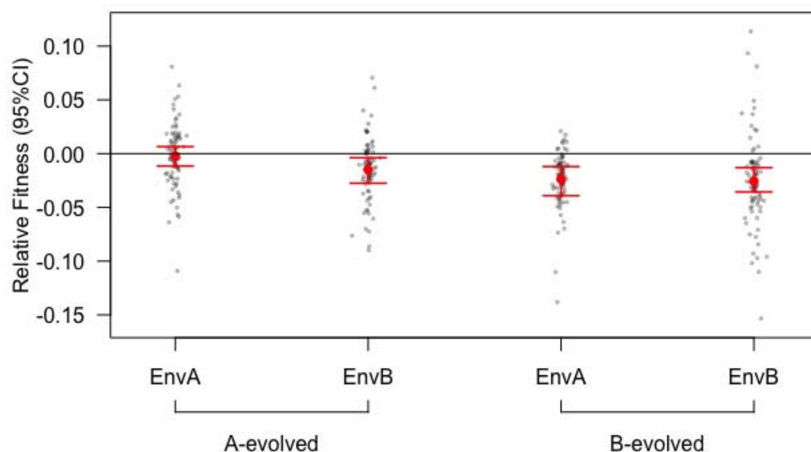


480

481 **Figure 4. Transplantation assays reveal signatures of local adaptation.** Shown here are the
482 relative growth rates compared to ancestors in our first (A) and second (B) transplantation
483 assays. The initial assay (A) showed the expected signature of adaptation was absent for C-
484 evolved lines, so we repeated the assay (B) with only A-evolved and B-evolved lines and their
485 respective environments. In panel (B) we can see that yeast perform best in the environments to
486 which they had been adapting. Ancestors are included to show the standard error for unadapted
487 lines. The data plotted here are also available in Table S2.



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489 **Figure 5. The effect of new mutations given alternative histories of adaptation**. Red dots
490 indicate the mean relative fitness of the group of mutants in a given environment while the
491 smaller black dots indicate the individual mutants. Relative fitness here represents the
492 difference in growth rates between a given mutant in a given environment and a non-
493 mutagneized control with the same adaptive history (originally from the same evolving
494 population) in the same environment. 95% confidence intervals were obtained from 10 000
495 bootstrap replicates. Mutations were consistently deleterious on average regardless of adaptive
496 history. Exact values can be found in Table S3.
497
498 **Supplementary Information**
499
500

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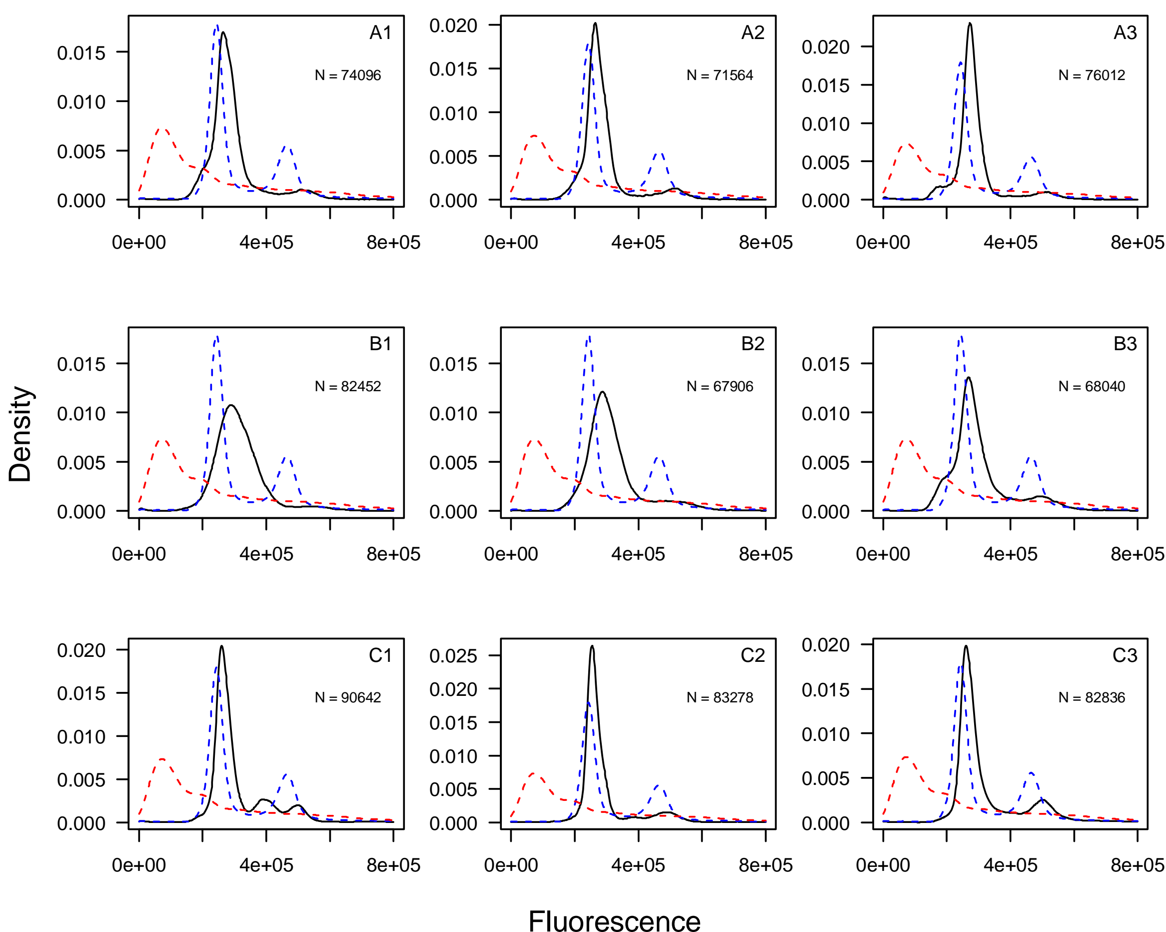

501

502 **Figure S1. Diploidy was maintained in all experimental populations.** Each panel shows the
503 standardized fluorescence density profile for cells from a given experimental population, with the
504 population ID indicated in the top right. Black lines represent the experimental population of
505 interest; dashed lines represent known haploid and diploid strains for comparison (red and blue
506 lines respectively, the same in each panel). Particle numbers are shown in each panel for the
507 experimental populations; known haploids and diploids the plots reflect 44880 and 77041
508 particles, respectively. In each case, the experimental populations resemble the diploid
509 standard.

510

511

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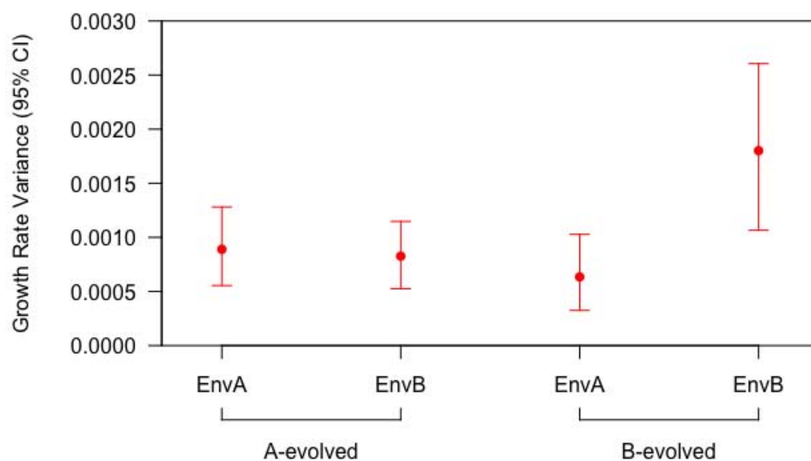

513

514 **Figure S2. The phenotypic variance of growth rate given alternative histories of**
515 **adaptation.** Dots indicate the variance of each set of mutants in a given environment. The bars
516 indicate the 95% confidence intervals calculated from the same bootstrapped data in Figure 4. It
517 is predicted that mutational variance would be higher when populations were in environments
518 they had not adapted to. Variance did not differ significantly across environments for A-evolved
519 mutants but B-evolved mutants generally had higher variance in EnvB—the opposite of what we
520 would predict. Exact values can be found in Table S2.

521

522 **Table S1: Media recipes.**

Environment	Compound	Molarity (mol/L)**	Stress
EnvA	$NiCl_2 \bullet 6H_2O$	1.00×10^{-3}	Metal (Nickel)
EnvA	Caffeine	4.00×10^{-3}	Organic
EnvA	Boric Acid, $B(OH)_3$	1.00×10^{-2}	Other
EnvA	NaF	1.00×10^{-2}	Flouride
EnvB	Acetic Acid, CH_3COOH*	3.00×10^{-1}	pH (acidity)
EnvB	LiCl	4.00×10^{-2}	Lithium
EnvB	Acetominophen	6.62×10^{-2}	Organic
EnvB	NaCl	1.00×10^{-1}	Osmotic
EnvC	$CrK(SO_4)_2 \bullet 12H_2O$	2.50×10^{-3}	Metal (Chromium)
EnvC	$CuSO_4 \bullet 5H_2O$	2.53×10^{-2}	Metal (Copper)
EnvC	Sodium Benzoate, C_6H_5COONa	3.75×10^{-3}	Benzoate
EnvC	$ZnCl_2$ Anhydrous	1.25×10^{-3}	Metal (Zinc)

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523 We formulated our environments in a haphazard fashion, aiming to create distinct environments
524 without overlap of stressors between environments or overemphasis of a single stressor type in
525 any given environment. Each environment contains a variety of stressors. We wanted to keep
526 the environments somewhat similar so each one was formulated with 4 unique compounds.
527 Environments were all made by mixing autoclave-sterilized, standard YPD with powdered
528 chemicals and mixed with a magnetic stir bar and gentle heating until fully dissolved. The
529 finalized media with all compounds added was filter sterilized through a sterile 0.2 μM aPES
530 membrane and aliquoted into sterilized glass bottles.
531 *Acetic acid was added as a concentrated 3M solution. This added a negligible amount of water
532 to the final solution.
533 **Molarity was calculated using manufacturer provided formula weights which includes hydration
534 in the case of metal salt compounds.
535
536
537
538 **Table S2. Table of values from Figure 3.** These values show the mean relative growth rates
539 for the adapting populations in each environment; Assay 1 corresponds to Fig. 4A and Assay 2
540 corresponds to Fig. 4B.

Treatment	Env A	EnvB	EnvC
A-adapted (Assay 1)	0.103	0.0203	0.0108
B-adapted (Assay 1)	0.0848	0.135	-0.00114
C-adapted (Assay 1)	0.0280	0.0631	0.0140

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A-adapted (Assay 2)	0.0235	0.00655	NA
B-adapted (Assay 2)	-0.0128	0.0783	NA

541

542

543 **Table S3. Table of the values from Figure 4.** These values represent means and variances of
544 relative growth rate of mutagenized genotypes, corresponding to Figure 5.

	A-evolved in EnvA	A-evolved in EnvB	B-evolved in EnvA	B-evolved in EnvB
Mean	-2.22×10^{-3}	-1.50×10^{-2}	-2.55×10^{-2}	-2.34×10^{-2}
Variance	8.89×10^{-4}	8.26×10^{-4}	6.34×10^{-4}	1.80×10^{-3}

545

546

547 **Acknowledgements**
548 Thanks to C. Hittinger for providing yeast strains and to Denise Smith for assistance in the lab.

549

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