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Haploids adapt faster than diploids across a range of environments

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Abstract

Despite a great deal of theoretical attention, we have limited empirical data about how ploidy influences the rate of adaptation. We evolved isogenic haploid and diploid populations of *Saccharomyces cerevisiae* for 200 generations in seven different environments. We measured the competitive fitness of all ancestral and evolved lines against a common competitor and find that in all seven environments, haploid lines adapted faster than diploids, significantly so in three environments. We apply theory that relates the rates of adaptation and measured effective population sizes to the properties of beneficial mutations. We obtained rough estimates of the average selection coefficients in haploids between 2% and 10% for these first selected mutations. Results were consistent with semi-dominant to dominant mutations in four environments and recessive to additive mutations in two other environments. These results are consistent with theory that predicts haploids should evolve faster than diploids at large population sizes.

Introduction

The rate at which beneficial mutations arise and fix determines how quickly a population can adapt to novel environments. This is particularly important for populations in very stressful environments, where to avoid extinction, novel beneficial alleles must spread fast enough to counter fitness declines owing to external environmental change and internal accumulation of deleterious alleles (Orr & Unckless, 2008; Bell & Collins, 2008). The rate of adaptation is affected by various properties that determine the fixation rate of beneficial alleles: the nature of available mutations (mutational neighbourhood, Burch & Chao, 2000), the mutation rate (μ), the distribution of fitness effects (s) and the dominance of mutant alleles (h). Here, we compare the rate of adaptation between haploid and diploid initially isogenic lines of *Saccharomyces cerevisiae* in seven different environments. Comparing rates of adaptation between ploidy levels and across many environments allows us to make inferences about the genetic properties of the mutations contributing to adaptation in these experiments.

The effect of ploidy on ecology and evolution has long been a question of interest (Adams & Hansche, 1974; Gerstein & Otto, 2009, and references within). Isogenic haploid and diploid populations of *S. cerevisiae* allow direct comparisons between individuals that share a genotype but differ in ploidy. Even with identical genomes, ploidy itself is known to have several direct effects on yeast. Of the proteome, 2.7% was found to change more than 50% in abundance between isogenic haploid and diploid cells (de Godoy *et al.*, 2008). Proteins that differed were in the pheromone pathway (specific to haploid cells), retrotransposon-associated proteins (ten times more abundant in diploids) and cell wall components, which were downregulated by a factor of 0.77 in diploids. Interestingly, this level of reduction in cell wall components is close to the surface area to volume ratio predicted for diploids relative to haploids (0.79) if diploid cells contain twice the volume of haploid cells. The difference in surface area to volume is predicted to directly affect the relative fitness of haploids and diploids in some environments. Under nutrient stress, for example, where the limiting nutrient diffuses across the cell membrane, haploids are expected to have an advantage

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(Weiss *et al.*, 1975; Lewis, 1985). Support for this hypothesis has been found in some studies (Adams & Hansche, 1974), but not others (Mable, 2001).

At present, we have only fragmentary knowledge about how the frequency and properties of novel beneficial mutations are affected by ploidy levels. Some evidence suggests that the availability of particular types of beneficial mutations may differ by ploidy. Gresham *et al.* (2008) found that diploids were more likely than haploids to select large amplification and deletion mutations during a 200 generation chemostat experiment. Similarly, Thompson *et al.* (2006) found a class of mutations selected among diploid mutator strains that conferred an advantage across a range of environmental conditions; these mutations did not appear in haploid mutator strains or in nonmutator lines of either ploidy. Further investigation revealed that the specific mutation may have been a chromosomal rearrangement, which was potentially beneficial to heterozygous diploids but deleterious or neutral to haploid cells. The mutation rate could also differ between haploids and diploids. One experiment found that although the point mutation rate was the same (1.06×10^{-6}), canavanine-resistant mutations arose 100-fold more frequently in heterozygous diploids compared with haploids, owing to an increase in types of mutations available to diploids (i.e. gene conversion, chromosome rearrangement, allelic crossover; Ohnishi *et al.*, 2004). In contrast, a second experiment found that microsatellites in the mitochondrial genome had a 100-fold higher mutation rate in haploids (Sia *et al.*, 2003). The most comprehensive studies to estimate the base substitution rate using next generation sequencing technology have, however, found very similar per basepair mutation rates for haploids (3.3×10^{-10}, Lynch *et al.*, 2008) and diploids (2.9×10^{-10}, Nishant *et al.*, 2010). Nevertheless, the number of sites that could carry a beneficial mutation (target size) might well depend on ploidy.

Regardless of the rate and nature of mutations, the efficacy of selection is predicted to differ between haploids and diploids. The fixation probability of a beneficial mutation in a diploid is approximately $2hs$ (Haldane, 1927), where h denotes the dominance of the mutation, i.e. how much of its homozygous fitness benefit (s) is experienced in a heterozygote. If beneficial mutations are on average recessive to additive (a single-mutated allele is masked by the wildtype, $h < 0.5$), diploids are always expected to evolve slower than haploids, despite having twice the number of mutational targets (Orr & Otto, 1994). Even if beneficial mutations are partially dominant, diploids will not necessarily evolve faster than haploids, because of the lower probability of fixing a beneficial mutation, as well as the fact that, in asexual diploids, beneficial alleles arise and fix in the heterozygous state, providing a reduced fitness benefit by a factor h. As a consequence, in large asexual populations, we expect haploids to evolve faster than diploids unless the dominance of beneficial mutations is very high (Otto & Whitton, 2000). Paquin & Adams (1983) showed that diploids adapted faster than haploids using a fluctuation assay with five haploid and six diploid lines evolved for up to 300 generations in glucose-limited chemostats, although it has been shown that the inferred number of mutations was larger than could have fixed during the course of these experiments (Dykhuizen, 1990).

The distribution of dominance of beneficial mutations remains largely unknown, although empirical results have shown that the dominance of mutations does significantly affect the relative rate of adaptation of haploids and diploids. Zeyl *et al.* (2003) evolved haploid and diploid *S. cerevisiae* populations asexually for 2000 generations in minimal medium at large population sizes (where selection was the primary evolutionary force acting). They found that haploid populations adapted significantly faster than the diploids; consistent with theory (Orr & Otto, 1994), the average dominance of the beneficial mutations selected in one of the haploid lines was 0.20. Anderson *et al.* (2004) also demonstrated the potentially critical role of dominance in determining the relative rates of adaptation by adapting *S. cerevisiae* to the drug fluconazole. At low concentrations of fluconazole, resistance is primarily achieved through dominant mutations in the *PDR1* gene, whereas predominantly recessive mutations in the *ERG3* gene are fixed at high concentrations (Anderson *et al.*, 2004). When haploid and diploid populations were evolved for 100 generations to low concentrations of fluconazole, diploids, with double the mutational targets, evolved faster. In contrast, at high concentrations of fluconazole, haploids were able to fix the required recessive mutations and adapted faster than diploids.

In this paper, we have compared the rate of adaptation between haploids and diploids evolved at large population sizes in seven different environments for 200 generations. We find the broad pattern to be identical across environments – haploids adapted faster than diploids – although the magnitude of this difference varied across environments, with significant differences in three environments. We measured haploid and diploid effective population size (N_e) and found that haploid populations are significantly larger than diploids in almost all environments. Previous theory relating the rate of adaptation of haploids and diploids to dominance coefficients was adjusted to allow for differences in population sizes and mutation rates. Applying this theory predicts the average dominance of the beneficial mutations selected during these early adaptive steps to be additive to dominant in the four environments where diploid adaptation was observed and recessive to additive in two environments were diploids did not adapt significantly. Our results indicate that haploid microbes are likely to evolve faster than diploids across an array of environmental challenges.

Methods

Haploid and diploid lines

Initially isogenic haploids and diploids of haplotype *MATa-a1 ste6△8-694 ura3 leu2 his4 trp1 can1* were created as previously described (Mable, 2001). Results obtained after the experiments reported here were initiated showed that both the haploid and diploid ancestral clones are aneuploid for chromosome 9 (haploid one extra copy, diploid two extra copies, Gerstein *et al.*, 2008). This is not expected to affect our results strongly, as chromosome 9 aneuploids have similar cell volumes and doubling times as wildtype (Torres *et al.*, 2007).

Environments and experimental evolution

In addition to a standard rich medium (YPD), six stressful environments were used in these experiments. Moderately high levels of the following stressors were added to YPD: ethanol, salt (NaCl), caffeine, nystatin, potassium hydroxide (KOH) and hydrochloric acid (HCl). A brief description of the stressful environments and their major cell targets is provided in Table 1, and more complete methods are provided in the Supporting Information. These stressors affect yeast cells in a variety of ways, although (as with many stressors of single-celled organisms) all affect some aspect of the cell membrane or cell wall, which, as noted above, represents a primary phenotypic difference between isogenic haploids and diploids. The specific stressors were not chosen with any *a priori* expectation about their differential effect on haploids or diploids. The level of each stressor was chosen so that the initial growth rate was reduced by approximately 20% relative to that in YPD (unpublished results).

The isogenic haploid and diploid cultures were streaked from freezer stocks maintained at −80 °C and grown on YPD plates for 48 h. A single colony for each ploidy level was picked randomly and grown for 48 h in 10 mL YPD. Each ancestral ploidy culture was used to inoculate five replicate lines in the seven different environments (six stressors plus a YPD control) for a total of 70 lines (2 ploidy × 5 replicates × 7 environments). For all 70 lines, 100 μL stationary phase culture was transferred into 10-mL fresh medium (i.e. 1 : 100 dilution) every 24 h (± 1 h); under this regime, there are approximately 6.7 generations of evolution between transfers ($2^{6.67} = 101$). Cultures were maintained at 30 °C with continual shaking (200 rpm). Each line was evolved for a total of approximately 187 generations with aliquots taken and frozen at −80 °C in 15% glycerol every 47 generations. The length of the experiment was chosen because previous experiments had demonstrated that these haploid lines tend to diploidize under stressful conditions over longer time periods (Gerstein *et al.*, 2006). The ploidy of all evolved lines at four time points (47, 94, 140 and 187 generations) was checked at the conclusion of the experiment using flow cytometry (methods described in Gerstein *et al.*, 2006), and no changes were observed.

Contamination by other microorganisms was checked under a microscope for all cultures every 24 h. In addition, culture was periodically plated onto synthetic-complete plates lacking leucine; any growth on these plates indicated a contaminant (or possibly a revertant). Thirteen different experimental lines did show contamination at different points during the experiment with a variety of other microorganisms, and in each case, we returned to the tube prior to the contamination and restarted the experiment from that time point (all evolved cultures were kept in tubes at 4 °C for approximately

Table 1 Evolutionary environments.

Stressor	Stress level	Major cell targets
Caffeine	4.23 mM	Mutagen (acts as a purine analogue)[1]; inhibits repair of double strand breaks[2] and/or overrides DNA damage checkpoints[3]; affects metabolite transport across the cell membrane and protein translocation[3]
Ethanol	4%	Increases membrane fluidity; inhibits glycolytic enzymes; increases protein denaturation; affects transport systems such as general amino acid permease and glucose uptake; induces mutations in mitochondrial DNA[4]
HCl	pH ~2.8*	Initiates yeast general stress response pathway; increases ROS production[5]; induces HOG-1 dependent cell wall organization changes[6]
KOH	pH ~7.6*	Disrupts membrane proton gradients and uptake of solutes from the medium[7]; decreases nutrient and ion limitation[8]; can lead to cell wall damage; source of oxidative stress[8]
NaCl	0.6 M	Decreases cell volume and turgor pressure[9]; causes hyperosmotic and ionic stress[10]; can decrease ATP hydrolysis[11]
Nystatin	0.6 μM	Fungicide that causes membrane leakage[12]; increases permeability to protons[13]; alters vacuolar membrane and vacuolar morphology[14]

*A constant amount of HCl and KOH was added to YPD each time new medium was autoclaved; the pH varied slightly for each medium batch.

[1]Kuranda *et al.* (2006), [2]Hannan & Nasim (1977), [3]Blasina *et al.* (1999), [4]Aguilera *et al.* (2006), [5]Giannattasio *et al.* (2005), [6]Kapteyn *et al.* (2001), [7]Lamb *et al.* (2001), [8]Serrano *et al.* (2006), [9]Nevoigt & Stahl (1997), [10]Matsumoto *et al.* (2002), [11]Nass *et al.* (1997), [12]Bard *et al.* (1980), [13]Palacios & Serrano (1978), [14]Bhiyan *et al.* (1999).

4 days). The contaminants detected appeared haphazardly among lines. Although cross-contamination among lines within our experiment could not be detected by these methods, the fact that changes in ploidy level, which would be expected in 50% of cross-contamination events, were not observed by flow cytometry, suggests that cross-contamination was absent or rare.

Measuring competitive fitness

Competitive fitness against a reference strain was used as a proxy for total fitness. The reference strain was constructed from BY74741 (*MATa his3△1 leu2△0 met15△0 ura3△0*) obtained from Open Biosystems. We inserted a 3320-bp region of the pJHK043 plasmid containing YFP under control of the *ACT1* promoter linked to a histidine marker, generously provided by John Koschwanez (FAS Center for Systems Biology, Harvard University). The region was isolated and amplified with primers TTCTTCGAAGAATATACTAAAAA ATGAGCAGGCAAGATAAACGAAGGCAAAGATG CGTA CGCTGCAGGTCGACGG and TACACATGTATATATAT CGTATGCTGCAGCTTTAAATAATCGGTGTCACTACATACA GATCCGCGGCCGCATAGG following J. Koschwanez (pers. comm.). This cassette was then inserted into BY4741 at the *HIS* locus, and successfully transformed cells were selected on -his plates.

To quantify the rate of adaptation, we determined the early (generation 47) and late (generation 187) competitive fitness for all 70 strains in their evolutionary environments. To eliminate any potential differences owing to acclimation (i.e. nongenetic changes) between haploids and diploids, we used experimental strains after 47 generations of initial adaptation to assess the ancestral competitive fitness. Competition assays were done separately for each of the seven environments; each competition assay involved 80 tubes (2 ploidy × 2 time points × 5 lines × 4 replicate competitions). The reference strain and experimental strains were streaked onto YPD plates from freezer stocks maintained at −80 °C and grown for 48 h at 30 °C. Culture was then inoculated from plates into tubes containing 10 mL of their experimental environment (YPD for the reference strain) and grown overnight at 30 °C, shaken at 200 rpm.

All competition assays except nystatin were initiated by inoculating 50 μL from both the reference and experimental strains into 10 mL of the experimental environment. The nystatin competitions were initiated with 75 μL reference and 25 μL experimental strains (see Supporting Information for justification). Transfers were performed every 24 h for 4 days in a manner that exactly mimicked the evolution experiment (100 μL stationary phase culture was transferred into 10-mL fresh medium with growth maintained at 30 °C with continual shaking at 200 rpm). The ratio of fluorescing to nonfluorescing cells was measured on days 0, 2 and 4 after initiation (days 0, 2, 3 and 4 for nystatin). On each measurement day, we placed 300-μL aliquots into a 96-well plate exactly 2 h after transfer. Plates were spun down for 3 min at 2500 rpm. The supernatant was removed, and pellets were re-suspended in 300 μL sodium citrate.

Ninety-six well plates were read on an LSRII flow cytometer with the High Throughput Sampler attachment. Ten thousand cells were measured for each well. The raw data (.fcs files) were exported into FLOWJO version 8.7 (Tree Star Inc., Ashland, OR, USA). An initial gate was set by looking at the forward scatter (FSC-W) and side scatter (SSC-W) data to exclude small debris; this gate included between 95% and 99% of total events recorded. The data were plotted on FITC-A (*x*-axis) and AmCyan-A (*y*-axis), which provided maximal separation of fluorescing and nonfluorescing cells. Gates were drawn around the two distinct clusters of nonfluorescing and fluorescing cells (Fig. S1). All gates were set at the beginning of the experiment and were not subsequently altered.

For each line of interest, we thus have four replicate competitions at three time points, day 0, 2 and 4 of competition, which correspond to 0, 13.4 and 26.8 generations. The competitive fitness (m) was determined for each line using the formula for evolutionary change:

$$\text{NonFluor} = \frac{p_0 e^{mT}}{1 - p_0 + p_0 e^{mT}} \qquad (1)$$

where NonFluor is the fraction of nonfluorescing cells, p_0 is the initial fraction of nonfluorescing cells at the start of the competition, T is the generations of competition, and m is the Malthusian parameter of the experimental strain minus that for the YFP-marked reference strain (relative growth rate). We use the nls function in the R programming language (R Development Core Team, 2008) to determine the best fitting p_0 and m for each competition assay. We measured the rate of adaptation as the rate of change in competitive fitness (m) for each of the 70 strains evolved in this experiment by calculating the slope over time (from generation 47 and 187) using the lm function in R (R Development Core Team, 2008). We compared the five haploid slopes to the five diploid slopes in each environment using a two-sample *t*-test with the Welch modification for degrees of freedom, which does not assume equal variance between groups.

Effective population sizes

The number of cells produced after 24 h of growth for all ancestral (generation 47) and evolved (generation 187) lines was determined by a plating experiment. All lines were streaked onto YPD plates from freezer stocks maintained at −80 °C and grown for 48 h at 30 °C. Culture was inoculated from plates into tubes containing 10 mL of their experimental environment and grown for another 48 h at 30 °C, shaken at 200 rpm. We then mimicked the evolution experiment exactly by transferring 100 μL overnight culture into fresh medium and allowing cells to grow for exactly 24 h. After 24 h we diluted culture and plated three different dilutions onto three plates each.

We use theory developed by Campos *et al.* (2008) to calculate the effective population size with periodic bottlenecks as $N_e = r^2 \tau N_0$, where τ equals the number of generations between bottlenecks (6.7 in our experiment) and r is the growth rate. We can use the equation $N_f = N_0 e^{r\tau}$ to isolate r as $\ln(N_f/N_0)/\tau$. Since N_f/N_0 equals the dilution rate (101 in our experiment) and τ is 6.7, $r = 0.689$. Thus, we multiply our final number of cells (N_f) by 0.031 to obtain the effective population size (N_e).

Results

Haploids were found to adapt faster than diploids in all environments (Fig. 1), significantly so in three of the seven environments. We first conducted a two-way ANOVA comparing all haploid and diploid slopes (change in competitive fitness) across all environments. There was a significant difference between ploidy levels ($F_{1,54} = 22.1$, $P < 0.0001$) and across environments ($F_{6,54} = 17.03$, $P < 0.00001$), but no significant interaction ($F_{6,54} = 1.09$, $P = 0.38$). Haploid slopes were found to be significantly higher than diploid slopes within YPD ($t_7 = 2.6$, $P = 0.033$), YPD + ethanol ($t_{5.93} = 4.28$, $P = 0.005$) and YPD + NaCl ($t_{7.99} = 6.73$, $P = 0.0001$). The rate of adaptation, although higher in haploids, did not differ significantly between ploidy levels in the remaining four environments (YPD + KOH: $t_6 = 1.24$, $P = 0.261$, YPD + HCl: $t_{7.3} = 1.85$, $P = 0.104$, YPD + nystatin: $t_{5.28} = 1.62$, $P = 0.163$, YPD + caffeine: $t_{7.18} = 1.37$, $P = 0.210$).

We next measured the effective population size for all ancestral and evolved populations. As can be seen in Fig. 2, the effective haploid population (open symbols) is generally greater than the effective diploid population (closed symbols) in all environments. We conducted a two-way ANOVA for each environment with time, ploidy and the interaction between them as predictors of population size. In all but one environment, ploidy was the only significant factor (YPD: $F_{1,16} = 46.1$, $P < 0.0001$; YPD + HCl: $F_{1,16} = 11.1$, $P = 0.004$; YPD + ethanol: $F_{1,16} = 62.8$, $P < 0.0001$; YPD + nystatin: $F_{1,16} = 13.2$, $P = 0.002$; YPD + KOH: $F_{1,16} = 143.6$, $P < 0.0001$; YPD + caffeine: $F_{1,16} = 5.5$, $P = 0.03$; see Table S1 for full statistical results). In YPD + NaCl, the evolved number of haploids cells decreased to that of the diploid lines, and all three predictors were significant (ploidy: $F_{1,15} = 25.7$, $P = 0.0001$; time: $F_{1,15} = 38.2$, $P < 0.0001$; ploidy × time: $F_{1,15} = 12.27$, $P = 0.003$). As ancestral and evolved population sizes were generally similar, the remainder of analyses combine the measured population sizes across timepoints (results using ancestral or evolved N_e were not detectably different in any case). Even though haploids had larger effective population sizes, diploids, with two gene copies per individual, still had more total gene copies (with the exception of YPD + KOH using the evolved population sizes). Thus, diploids generally had more targets for beneficial mutations than haploids in the environments tested, though not a two-fold difference as previously predicted.

The design of this experiment was aimed at measuring the relative rates of adaptation in haploids and diploids. Without knowing the number and frequency of underlying beneficial mutations, we cannot estimate their selection and dominance coefficients with any certainty. That said, we can gain some sense of their likely magnitudes by applying theory (Otto & Whitton, 2000) predicting the rate of adaptation for haploid and diploid asexuals, given their effective population sizes (see Supporting Information). This theory assumes that the

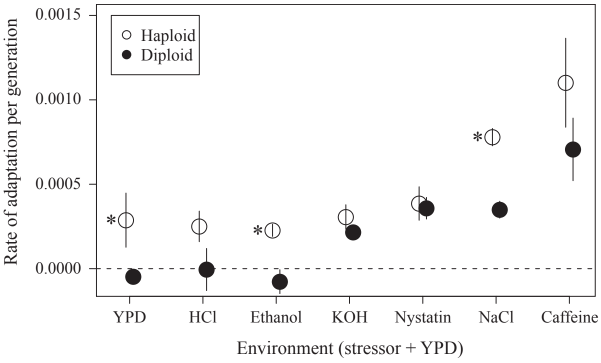

Fig. 1 Haploids adapted faster than diploids in all environments. The rate of adaptation for haploids (open circles) and diploids (closed circles) was calculated as the change in m (Malthusian parameter) per generation over 140 generations. Each dot is the mean ± SE of five lines evolved independently. Stars (*) indicate a significant difference ($P < 0.05$) between haploid and diploid lines (Welchs t-test).

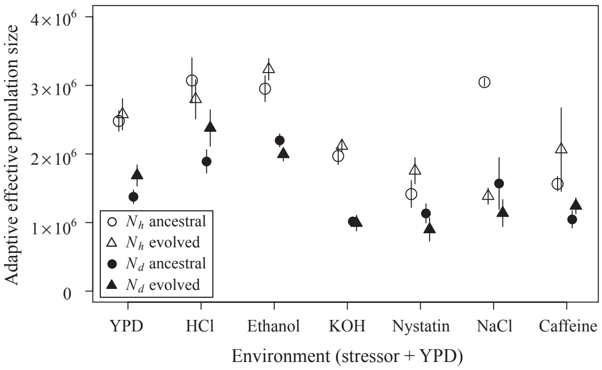

Fig. 2 The effective population size of haploid (open symbols) and diploid (closed symbols) populations. With the exception of haploids in NaCl, there was little difference between ancestral (circles) and evolved (triangles) population sizes; haploid populations were nearly always larger than diploids.

rate of adaptation is set by the time between the appearance of mutations destined to fix within the population, accounting for the fact that for two mutations to fix in an asexual population, the second must occur within an individual bearing the first. The observed rate of adaptation can then be used to estimate the selection coefficient in haploids, *s*, and in diploids, *hs* (Table 2). Doing so requires that we have observed the rate of adaptation (Fig. 1) with enough replication and for sufficient time to obtain an accurate measure of it. It does not require that the mutations necessarily be at high frequency at the end of the experiment (see Supporting Information), because the calculations account for the frequency dynamics of the beneficial mutations given their effect sizes. It should also be emphasized that the estimated effect sizes are not representative of all beneficial mutations, only those that were likely to fix early on in the adaptive process.

Bearing in mind the above caveats, we can estimate the dominance coefficients if we further assume that the selection coefficient (*s*) is the same in haploids and diploids. We can then use the ratio of the rate of haploid adaptation over the rate of diploid adaptation (Fig. 1) and the measured effective population sizes (Fig. 2) to estimate the dominance of beneficial mutations (eqn S4). The results are illustrated in Fig. 3. Points give the dominance coefficient from eqn (S4) using the mean rate of adaptation and the mean effective population size across the five replicates within an environment for haploids and diploids; confidence intervals are based on parametric bootstrapping of the rates of adaptation as well as the effective population sizes of haploids and diploids, again with five replicates each as in the original experiment. To ensure that real-valued estimates of dominance were obtained, we constrained the bootstrapped values of the rate of adaptation to be slightly positive (see Supporting Information).

Low dominance coefficients could be excluded in four environments: YPD + KOH, YPD + nystatin, YPD + NaCl and YPD + caffeine, which were the four environments within which diploids showed significant evidence of adaptation (one-sample *t*-test; YPD + KOH: $t_4 = 6.40$, $P = 0.003$; YPD + nystatin: $t_4 = 6.47$, $P = 0.003$; YPD + NaCl: $t_4 = 7.84$, $P = 0.0014$; YPD + caffeine: $t_4 = -4.26$, $P = 0.013$). In the remaining three environments, diploids did not show significant evidence of adaptation (one-sample *t*-test, YPD: $t_3 = -1.07$, $P = 0.36$; YPD + ethanol: $t_4 = -1.22$, $P = 0.29$; YPD + HCl: $t_4 = -0.05$, $P = 0.97$). In YPD + HCl, the lack of diploid adaptation is largely driven by a single lineage (see Table S2), and the data are too variable to allow us to estimate a dominance with any precision. In YPD and YPD + ethanol, however, the data are consistent with recessive to near-additive mutations.

Because the population sizes were so large, mutations were not strongly limiting the rate of adaptation. Thus, the above estimates of dominance were virtually unaffected by the mutation rates assumed in haploids and diploids (see Fig. S4; the rate used in the text was 10^{-7} beneficial mutations per genome per generation in both haploids and diploids). The estimated dominance coefficients were also unaffected by differences in haploid and diploid mutation rates, unless the haploid mutation rates were orders of magnitude lower than the diploid rates (Fig. S5), in which case we would not expect to see such similar rates of adaptation among replicate lineages (Fig. 1; Table S2). As described in the introduction, previous results suggest that the per base mutation rate is very similar in haploid and diploid *S. cerevisiae* (Ohnishi

Table 2. Mutation effect sizes in haploids and diploids. Equations (S2 and S3) were used to estimate *s* in haploids and *hs* in diploids, respectively, from the mean rate of adaptation in each environment. The table reports values based on the mean of ancestral N_e and evolved N_e measurements combined

	Haploid lines (*s*)	Diploid lines (*hs*)	Dominance estimate (*h*)
YPD	0.022	0*	0*
YPD + HCl	0.045	0*	0*
YPD + ethanol	0.046	0*	0*
YPD + KOH	0.053	0.044	0.84
YPD + nystatin	0.069	0.053	0.78
YPD + NaCl	0.083	0.056	0.67
YPD + caffeine	0.100	0.080	0.80

*The average measured rate of adaptation of diploids lines was slightly negative in these environments.

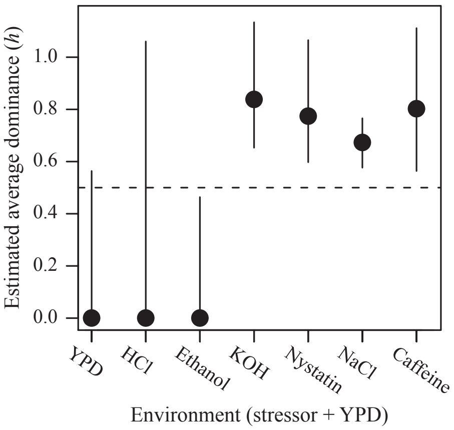

Fig. 3 Dominance estimates based on the relative rate of haploid and diploid rates of evolution. Recessive mutations can be excluded in the four environments where diploid adaptation was observed. Points are based on eqn (S4), using the mean rate of adaptation and mean effective population size in haploids and in diploids in each environment. Error bars indicate 95% confidence intervals obtained by simultaneous parametric bootstrapping haploid and diploid rates of adaptation as well as haploid and diploid mean effective population sizes. Negative rates of adaptation obtained by bootstrapping were forced to be very small but positive. See Supporting Information for details.

et al., 2004; Lynch *et al.*, 2008; Nishant *et al.*, 2010). Similarly, the inferred dominance coefficients were also insensitive to changes in the population sizes of haploids and diploids, unless they are orders of magnitude too large. Finally, results obtained using theory based on travelling waves (Rouzine *et al.*, 2008, eqn 52) instead of eqns (S1–S4) gave very consistent results (Table S3).

Discussion

Consistent with theoretical expectations (Orr & Otto, 1994), we found that haploids adapted faster than diploids in seven different environments when evolved at large population sizes, significantly so in three environments. We expect that single mutations largely contributed to the improvement in fitness; as shown in Fig. S2, a fully dominant mutation that confers a 10% fitness advantage will only reach approximately 50% frequency within 200 generations (see Supporting Information). That said, we cannot exclude the possibility that multiple mutations contributed to the observed rate of adaptation. Previous microbial evolution experiments have found support for the presence of multiple mutations of moderate effect within populations (de Visser & Rozen, 2006; Desai *et al.*, 2007; Kao & Sherlock, 2008).

The results presented here describe a short-term (< 200 generation) evolution experiment. When we compare these to a previous study over a much longer scale (approximately 1800 generations, Gerstein *et al.*, 2006), we find a surprising disconnect between the rate of adaptation in the short-term and long-term shifts in ploidy. In this study, we found that haploids adapted faster in both YPD and YPD + NaCl, yet in our previous study, we saw that diploid mutants arose and took over all replicate lines within 1800 generations in YPD and 800 generations in YPD + NaCl. This contrast emphasizes the fact that simply accruing beneficial mutations at a faster rate does not protect haploid populations from invasion by diploid mutants. Because *S. cerevisiae* is predominantly diploid in nature, these yeast may be better adapted to the cell geometry and gene expression patterns of diploid cells. Consequently, diploid mutations that arise over the longer term might combine the adaptive mutations accumulated haploid with the cellular advantages of a history of diploid evolution. Alternatively, there might be certain beneficial mutations that are accessible to diploids alone, either because of rearrangements involving homologous chromosomes or because one allele can diverge in function while retaining the original function via the second allele. Under either explanation, the results from these two studies provide an interesting contrast, with haploid yeast consistently evolving faster and yet remaining susceptible to invasion by diploids.

Using theory that relates the rate of adaptation to the selective effects of mutations, we obtained rough estimates of the average selection coefficients in haploids (Table 2). Keeping in mind that these are likely to be the best available mutations, our s estimates are consistent with other experiments performed in *S. cerevisiae* (Dickinson, 2008: average s of beneficial mutations after 4800 generations of bottlenecks on YPD $= 0.08$, maximum of 0.12; Gresham *et al.*, 2008: $s \approx 0.05$–0.1 for beneficial mutations in carbon and phosphorus limitation; Desai *et al.*, 2007: mean $s = 0.02$). A recent paper estimated the average s of first mutations fixed in a different asexual microbe (*Aspergillus nidulans*, Schoustra *et al.*, 2009) as slightly higher at 0.2. We acknowledge that our experiments were not designed to specifically measure the effect size of single mutations, and we recognize the limitation of our inferences.

In five of the seven environments, the rates of adaptation in diploids versus haploids were consistent with additive to dominant beneficial mutations, although in one of these environments we could not exclude the possibility that mutations were additive to recessive. In two of the environments, we found less evidence of diploid adaptation (only 1 diploid line increased in competitive fitness in YPD and 2 lines in YPD + ethanol, Table S2), and dominance estimates were consistent with recessivity to additivity. These estimates assume that the effect size of beneficial mutations in haploids is equal to that in diploids. Because we use the haploid selection coefficient (s) to tease out the dominance coefficient from the effect size in heterozygous diploids (hs), any increase in the effect size in diploids relative to haploids would cause a proportional decrease in the estimated dominance coefficient. Korona (1999) found no significant difference for the effect size of deleterious mutations when isogenic haploids and homozygous diploids were compared, although this need not be true for beneficial mutations. Moreover, our calculations of dominance assume that beneficial mutations remain heterozygous, but mitotic recombination and gene conversion could yield homozygous diploids bearing the beneficial mutation at high enough rates to affect the process of adaptation (Mandegar & Otto, 2007). To the extent that such homozygotes have formed, the inferred dominance coefficients would be biased towards one. An additional caveat is that the measured selection and dominance coefficients might reflect deleterious alleles hitchhiking along with the beneficial alleles, although we expect this to be minor given that all lines were initially bottlenecked and the genome-wide mutation rate is low (Lynch *et al.*, 2008; Nishant *et al.*, 2010). Stronger inferences on the dominance of beneficial mutations await future work using genomic sequencing technology to pinpoint mutations and to measure their selective effects directly.

Although we know from previous studies that the majority of deleterious mutations are partially recessive (Mukai *et al.*, 1972, Ohnishi, 1977, Mable, 2001, Szafraniec *et al.*, 2003, and references within), we currently have few empirical measurements for the dominance of beneficial mutations. The most comprehensive study examined the dominance of beneficial mutations in pesticide and herbicide resistance genes. In a survey of more than 70 different studies, Bourguet & Raymond (1998) found that alleles that confer resistance via target site mutations varied from complete recessivity to complete dominance. The picture that emerges from that study and the results presented here is that the average dominance of beneficial mutations is highly environment specific.

Yeast launched the genomics era of eukaryotes with the first published genome sequence in 1996 (Goffeau *et al.*, 1996), and yeast studies have continued to lead the charge in understanding the genomic basis of evolution (Dujon, 2010). Experiments such as these can be used not only to study population genetic questions but also to obtain testable predictions about the number and type of mutations that we may find as we move forward with broad-scale sequencing experiments. In particular, our results suggest that future sequencing studies should find mutations of larger effect size in haploid lines evolved in YPD + caffeine and YPD + NaCl compared to the other five environments. Similarly, we expect less dominant mutations in lines evolved in YPD or YPD + ethanol. These experiments have demonstrated that haploids consistently evolve faster than diploids and suggest further experiments to confirm our estimates for the effect size and dominance of the first selected mutations in these environments.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Two way ANOVA results for effective population sizes.

Table S2 Rate of adaptation between generations 47 and 187 within each lineage.

Table S3 Selection and dominance coefficients in haploids and diploids based on travelling wave theory of Rouzine *et al.* (2008).

Figure S1 10 000 cells from each culture of interest were read in 96 well plates on an LSRII.

Figure S2 Time required for a beneficial mutation to reach 50% frequency in our experiments with a starting population size of 595 067 (the measured average population size transfered daily across all environments; N_o as in Campos & Wahl, 2009 eqn 6) and with periodic bottlenecks every 6.7 generations.

Figure S3 Dominance estimates are not sensitive to changing *v*, keeping the haploid mutation rate equal to the diploid mutation rate.

Figure S4 The sensitivity of dominance estimates to decreasing the haploid mutation rate.

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Supporting Information

Experimental Environments

The six stressful environments were created by the addition of stressor to a YPD base. NaCl, HCl (1M), and KOH (5M) were added directly to YPD before autoclaving while the appropriate amount of stock solutions of caffeine (0.103M), ethanol (95%), and nystatin (1mM) were added to YPD after autoclaving. To ensure there was no difference in the concentration of nutrients (YPD) or stressors, the volume of all bottles was kept constant through the addition of sterilized water as required, post-autoclave. All medium was prepared in batches weekly. Midway through the experiment nystatin was added individually to each YPD+nystatin 10mL test tube to minimize the risk of contamination (as a preventive measure).

Nystatin Competitions

Using the same protocol as with the other environments (50μL reference to 50μL competing strains), we initially found that the fraction of non-fluorescing cells (experimental strains) in many cases reached 95% by day 2 (the second measurement), even in the strains isolated from generation 49. To give us more power to detect selection, we thus decreased their starting volume (to 25μL experimental strain and 75μL reference strain) and added an extra measurement day (measured on days 0, 2, 3 and 4).

Comparing the rate of haploid to diploid adaptation

We use equation A2 from Otto & Whitton (2000) to interpret the rate of adaptation in asexual populations in terms of the selection coefficients underlying the adaptation. This method is based on early work by Kimura & Crow (1964), who noted that for a mutation to fix in an asexual population, it must occur within a lineage already carrying any other beneficial mutations that are destined to fix. As described by Otto & Whitton (2000), this logic can be used to determine the rate at which fitness rises over time in an asexual population with ploidy level c as the inverse of the number of generations that pass on average between the appearance of two successful beneficial mutations (σ_c and σ_c'), where success is defined as the mutation ultimately becoming fixed within the population.

$$\triangle W_{asexual} = \frac{\sigma_c \sigma_c'}{ln[cN(Exp[\frac{\sigma_c'}{2c\nu N\sigma_c}] - 1)(\frac{\sigma_c'}{\sigma_c' + \sigma_c})]} \tag{S1}$$

(equation S1 corrects typographical errors in the original Otto & Whitton 2000 paper).

Equation S1 describes the long-term average rate of fitness increase; we assume here that the fitness changes over the 140 generations of our experimental treatments can be used as a proxy for $\triangle$W, in the absence of more detailed information about the genetic changes that have occurred.

To estimate selection, we assume that the beneficial alleles that are destined to fix have a roughly constant advantageous effect size over the time course of these experiments (σ_c = σ_c' = s for haploids, sh for diploids). We allow haploid and diploid populations to have different effective population sizes ($N_{e,h}$ and $N_{e,d}$, respectively) and different beneficial mutation rates (ν_h and ν_d). We can then describe both haploid (equation S2, with c=1) and diploid (equation S3, with c=2) rates of adaptation:

$$\triangle W_h = \frac{s^2}{ln[N_{e,h}(Exp[\frac{s}{2\nu_h N_{e,h} s}] - 1)(\frac{1}{2})]} \tag{S2}$$

$$\triangle W_d = \frac{s^2 h^2}{ln[2N_{e,d}(Exp[\frac{sh}{4\nu_d N_{e,d} sh}] - 1)(\frac{1}{2})]} \tag{S3}$$

Assuming that s in haploids equals that in diploids and taking the ratio of the rate of haploid adaptation (equation S2) and diploid adaptation (equation S3), the rate of change in fitness can be used to obtain a dominance coefficient of beneficial mutations equal to

$$h = \sqrt{\frac{ln[2N_{e,d}(Exp[\frac{1}{4\nu_d N_{e,d}}] - 1]) \cdot \triangle W_d}{ln[N_{e,h}(Exp[\frac{1}{2\nu_h N_{e,h}}] - 1) \cdot \triangle W_h}} \tag{S4}$$

In the text, we assumed an equal mutation rate of (10^{-7}) for haploids and diploids. The inferred dominance coefficients were not, however, sensitive to the mutation rate across a broad range of potential values (Figure S4). The inferences were also unaffected if the measured genomic mutation rates were used to scale the relative rate of mutations in haploids and diploids to $\nu_h/\nu_d = 3.3/2.9$ (Lynch et al., 2008; Nishant et al., 2010). Only if the haploid mutation rate were orders of magnitude smaller than assumed in the text would our dominance estimates have been overestimated (Figure S5).

Equations (S2) – (S4) do not account for there being a distribution of selective effects or for the fact that only the best of the beneficial mutations that arise are likely to fix within the population. That is, competition among beneficial mutations for fixation (clonal interference) will lead to the fixation of mutations with a higher selective advantage s (high hs in diploids) than expected based on the average of all possible beneficial mutations (Gerrish & Lenski, 1998; Rozen et al., 2002). These equations also assume that beneficial mutations destined to fix are nested within the previous lineage destined to fix. With high enough mutation rates and population sizes, however, leap-frogging becomes possible, such that multiple beneficial mutations can arise and change the fate of a previously doomed lineage. To investigate the impact of this possibility, we also applied equation (52) from Rouzine et al. (2008), which calculates the speed of a travelling wave of adaptation and accounts for stochasticity at the wave front; this theory allows for multiple mutations to rescue genotypes of lower fitness. Similar selection and dominance coefficients were estimated by this method (Table S3).

Confidence intervals on *h*

To obtain 95% confidence intervals for the dominance coefficient, we bootstrapped 10000 sets of five haploid and five diploid rates of adaptation from a normal distribution (with means and standard deviations equal to the means and standard deviations of our measured results for haploids and diploids in each environment). Effective population sizes here are very large, and mutations are not limiting; there is virtually no difference in dominance estimate whether ancestral or evolved population sizes are used, even in YPD+NaCl where the population size significantly decreased over the experiment (results not shown). We thus bootstraped 10000 sets of five haploid and five diploid effective population sizes with mean and standard deviation equal to the mean and standard deviation of the effective population sizes in the original experiment (after averaging ancestral and evolved measurements). The bootstrapped datasets were then used to calculate dominance 10000 times for a particular environment. The upper and lower bounds were set to the 97.5 and 2.5 quantiles from the bootstrap distribution of dominance coefficients and represent confidence intervals.

In a number of cases the bootstrap procedure led to negative estimates of the rate of adaptation (primarily in estimating diploid rates of adaptation in YPD, YPD+HCl and YPD+ethanol, though also a small number of times for both ploidy levels in other environments); in these situation the inferred h value from equation (S4) would be complex.

Because the population sizes were large, we assumed that negative rates of adaptation were due to sampling error, and we forced the rate of adaptation to be very small but positive (10^{-6}, though results were insensitive to forced rates between 10^{-4} – 10^{-9}).

Only single mutations are likely present at high frequency in most lines

Here, we ask what the minimum time to reach 50% would be for mutations of varying beneficial effects (s) and dominance coefficients (h). To do so, we use theoretical results from Campos & Wahl (2009) developed for these types of evolutionary experiments with periodic bottlenecks, calculating $T_{50\%} \approx \frac{1}{2}\frac{ln(N_0)}{hs_b}$ (Campos &Wahl 2009, equation 6 and Supplementary material). For a dominant mutation (h=1) to reach 50% in 200 generations, the selective advantage (s) must be at least 0.13 (Figure S2). As the dominance of the beneficial mutation decreases, the effect size of the mutations must correspondingly increase to reach 50% within 200 generations (Figure S2). The results presented use the average population size transfered daily (N_0) across all environments, though population size did not greatly affect the rate at which the beneficial mutations are predicted to reach 50% frequency. Populations an order of magnitude larger of smaller than our measured population sizes (upper and lower bounds on Figure S2) show nearly identical results. We thus believe that it is likely only single mutations are present at high frequency in any of our lines.

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Table S1: Two Way ANOVA results for effective population sizes. Significant factors are shown in bold.

	Ploidy	Time	Ploidy * Time
YPD	$F_{1,16} = 46.1$ $\mathbf{p < 0.0001}$	$F_{1,16} = 1.9$ $p > 0.05$	$F_{1,16} = 0.053$ $p > 0.05$
YPD + HCl	$F_{1,16} = 11.1$ $\mathbf{p = 0.004}$	$F_{1,16} = 0.2$ $p > 0.05$	$F_{1,16} = 2.5$ $p > 0.05$
YPD + Ethanol	$F_{1,16} = 62.8$ $\mathbf{p < 0.001}$	$F_{1,16} = 0.1$ $p > 0.05$	$F_{1,16} = 3.7$ $p > 0.05$
YPD + KOH	$F_{1,16} = 143.6$ $\mathbf{p < 0.0001}$	$F_{1,16} = 0.5$ $p > 0.05$	$F_{1,16} = 1.0$ $p > 0.05$
YPD + Nystatin	$F_{1,16} = 13.2$ $\mathbf{p = 0.002}$	$F_{1,16} = 0.1$ $p > 0.05$	$F_{1,16} = 3.3$ $p > 0.05$
YPD + NaCl	$F_{1,15} = 25.7$ $\mathbf{p < 0.0001}$	$F_{1,15} = 38.2$ $\mathbf{p < 0.0001}$	$F_{1,15} = 12.3$ $\mathbf{p = 0.003}$
YPD + Caffeine	$F_{1,16} = 5.5$ $\mathbf{p = 0.03}$	$F_{1,16} = 0.2$ $p > 0.05$	$F_{1,16} = 0.6$ $p > 0.05$

Table S2: Rate of adaptation between generations 47 and 187 within each lineage. These data were divided by 140 generations to obtain the per generation rate used in Figure 1.

	Haploid lines ($140\times\Delta W_h$)	Diploid lines ($140\times\Delta W_d$)
YPD	0.098	-0.015
	0.022	-0.011
	0.021	-0.003
	0.018	0.008
	NA*	-0.012
YPD + HCl	-0.003	0.021
	0.026	-0.06
	0.047	0.014
	0.063	0.010
	0.042	0.014
YPD + Ethanol	0.028	0.001
	0.029	-0.041
	0.045	-0.019
	0.038	-0.005
	0.019	0.009
YPD + KOH	0.043	0.023
	0.028	0.023
	0.024	0.048
	0.043	0.025
	0.075	0.033
YPD + Nystatin	0.048	0.030
	0.070	0.077
	0.064	0.051
	0.079	0.053
	0.0089	0.040
YPD + NaCl	0.117	0.036
	0.117	0.069
	0.123	0.051
	0.088	0.035
	0.100	0.054
YPD + Caffeine	0.176	0.109
	0.177	0.071
	0.255	0.069
	0.086	0.060
	0.077	0.186

* One haploid line in YPD became contaminated over the course of the experiment, and data was not collected.

Table S3: Selection and dominance coefficients in haploids and diploids based on travelling wave theory of Rouzine et al. (2008). Estimates of s and hs are based on equation (52), with ν set to 10^{-7} using the averaged N_e values. Estimates of s and hs are somewhat sensitive to the mutation rate assumed, but the dominance coefficient h is robust and similar to that shown in Figure 3.

	Haploid lines (s)	Diploid lines (hs)	Dominance (h)
YPD s	0.022	0^*	0^*
YPD + HCl	0.048	0^*	0^*
YPD + Ethanol	0.046	0^*	0^*
YPD + KOH	0.055	0.046	0.83
YPD + Nystatin	0.074	0.057	0.76
YPD + NaCl	0.091	0.059	0.65
YPD + Caffeine	0.108	0.085	0.79

* The average measured rate of adaptation of diploids lines was negative in these environments.

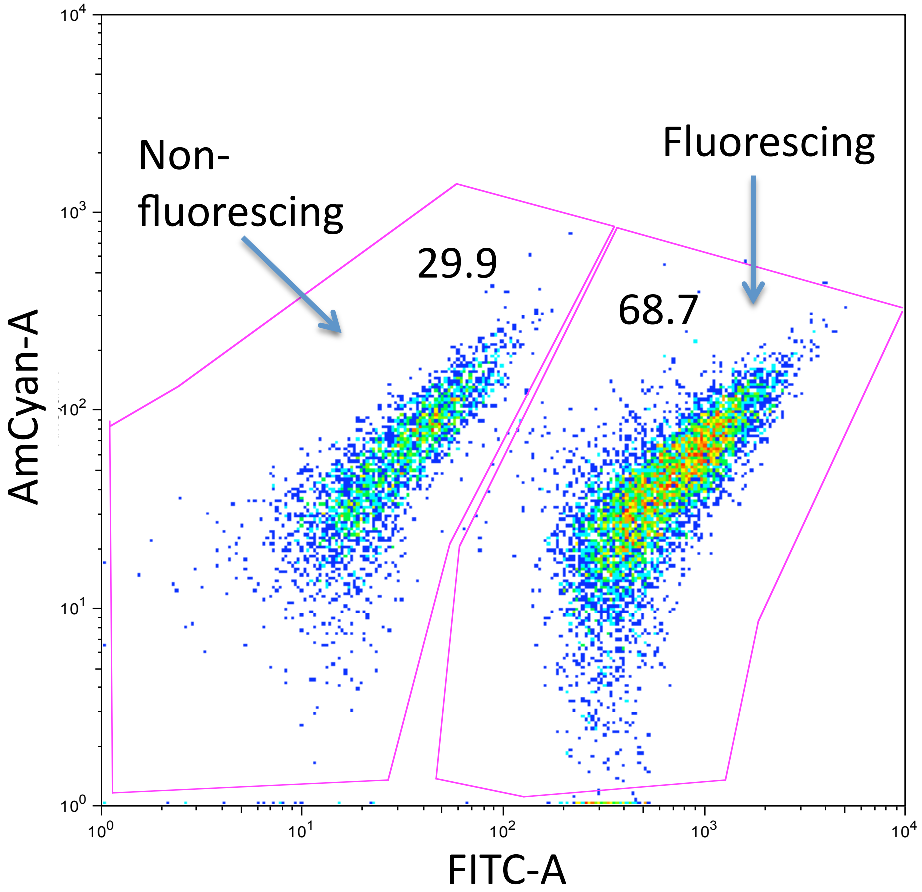

Figure S1: 10000 cells from each culture of interest were read in 96 well plates on an LSRII. Each cell is plotted on AmCyan-A and FITC-A axes which separates out non-fluorescing (left gate) from fluorescing cells (right gate). Numbers in each gate indicate the proportion of cells; this number is used to determine the fraction of non-fluorescing cells for further analysis (e.g., $NonFluor = \frac{29.9}{29.9+68.7}$ in this case).

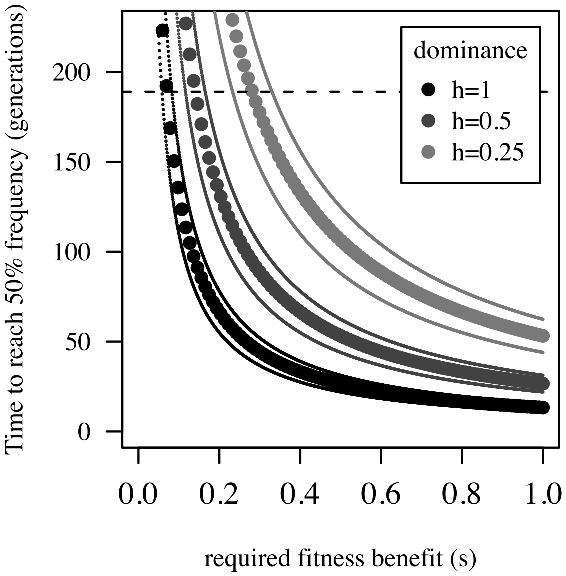

Figure S2: Time required for a beneficial mutation to reach 50% frequency in our experiments with a starting population size of 595067 (the measured average population size transfered daily across all environments; N_0 as in Campos & Wahl 2009 equation 6) and with periodic bottlenecks every 6.7 generations. Lines around the main points indicate the result found when effective population sizes are an order of magnitude larger (upper bound) or smaller (lower bound).

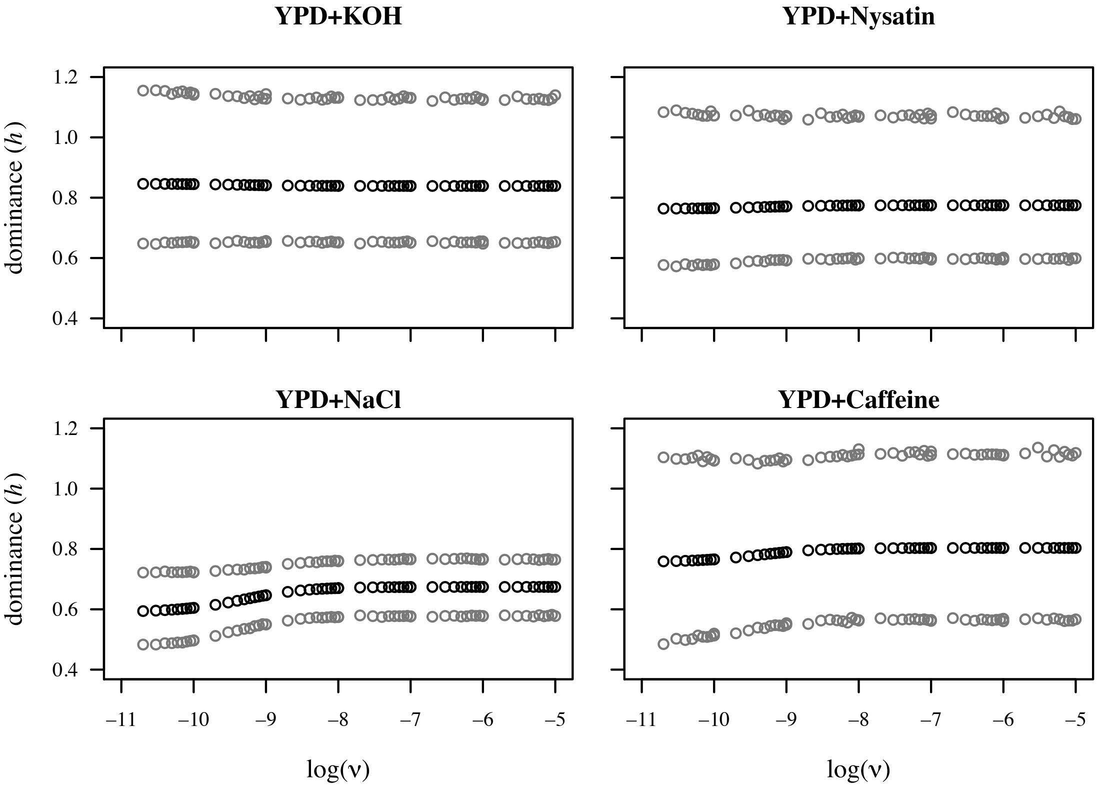

Figure S3: Dominance estimates are not sensitive to changing ν, keeping the haploid mutation rate equal to the diploid mutation rate. Black dots are based on equation (S4), using the mean rate of adaptation and mean effective population sizes observed in haploids and in diploids. Grey dots indicate 95% confidence intervals obtained by simultaneous parametric bootstrapping haploid and diploid rates of adaptation and haploid and diploid effective population sizes (as in Figure 3) while changing the mutation rate (ν).

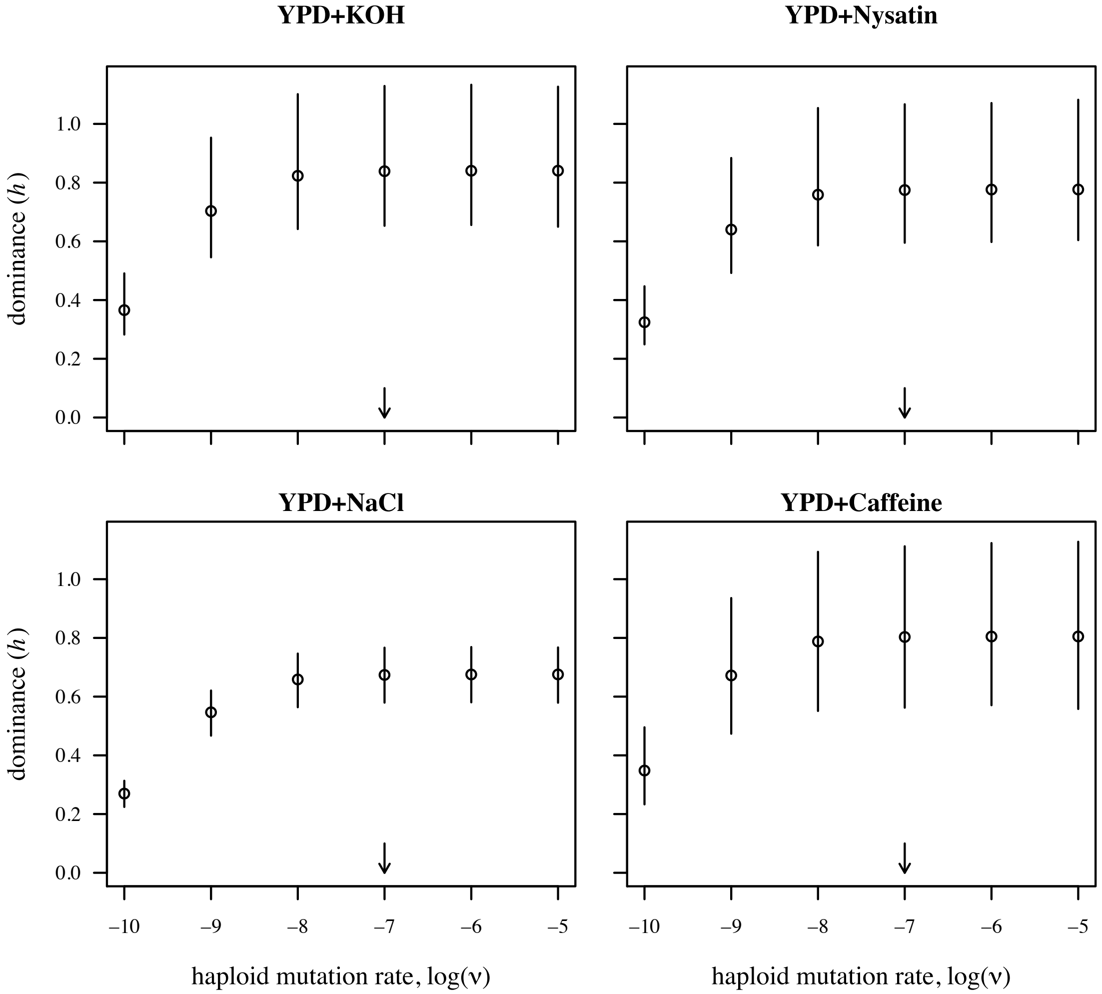

Figure S4: The sensitivity of dominance estimates to decreasing the haploid mutation rate. The diploid mutation rate was 10^{-7} (arrow) as in the simulations presented above, while the haploid mutation rate was changed. If the haploid mutation rate was three orders of magnitude lower than for diploids, the dominance estimates presented in Figure 3 would be overestimated.