# 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 (50$\mu$L reference to 50$\mu$L 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 25$\mu$L experimental strain and 75$\mu$L 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 ($\sigma_c$ and $\sigma_c'$), 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 ($\sigma_c$ = $\sigma_c'$ = $s$ 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 ($\nu_h$ and $\nu_d$). 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 possi-

ble, 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.

# References

Campos, P.R.A. & Wahl, L.M. 2009. The effects of population bottlenecks on clonal interference, and the adaptation effective population size. *Evolution* **63**: 950–958.

Gerrish, P. & Lenski, R. 1998. The fate of competing beneficial mutations in an asexual population. *Genetica* **102-3**: 127–144.

Kimura, M. & Crow, J.F. 1964. The number of alleles that can be maintained in a finite population. *Genetics* **49**: 725–38.

Lynch, M., Sung, W., Morris, K., Coffey, N. & Landry, C. 2008. A genome-wide view of the spectrum of spontaneous mutations in yeast. *PNAS* **105**: 9272–9277.

Nishant, K., Wei, W., Mancera, E., Argueso, J., Schlattl, A., Delhomme, N., Ma, X., Bustamante, C., Korbel, J. & Gu, Z. 2010. The baker's yeast diploid genome is remarkably stable in vegetative growth and meiosis. *PLoS. Genet.* **6**: 5–10.

Otto, S.P. & Whitton, J. 2000. Polyploid incidence and evolution. *Annu. Rev. Genet.* **34**: 401–437.

Rouzine, I.M., Brunet, E. & Wilke, C.O. 2008. The traveling-wave approach to asexual evolution: Muller's ratchet and speed of adaptation. *Theor. Pop. Biol.* **73**: 24–46.

Rozen, D., de Visser, J. & Gerrish, P. 2002. Fitness effects of fixed beneficial mutations in microbial populations. *Curr. Biol.* **12**: 1040–1045.

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

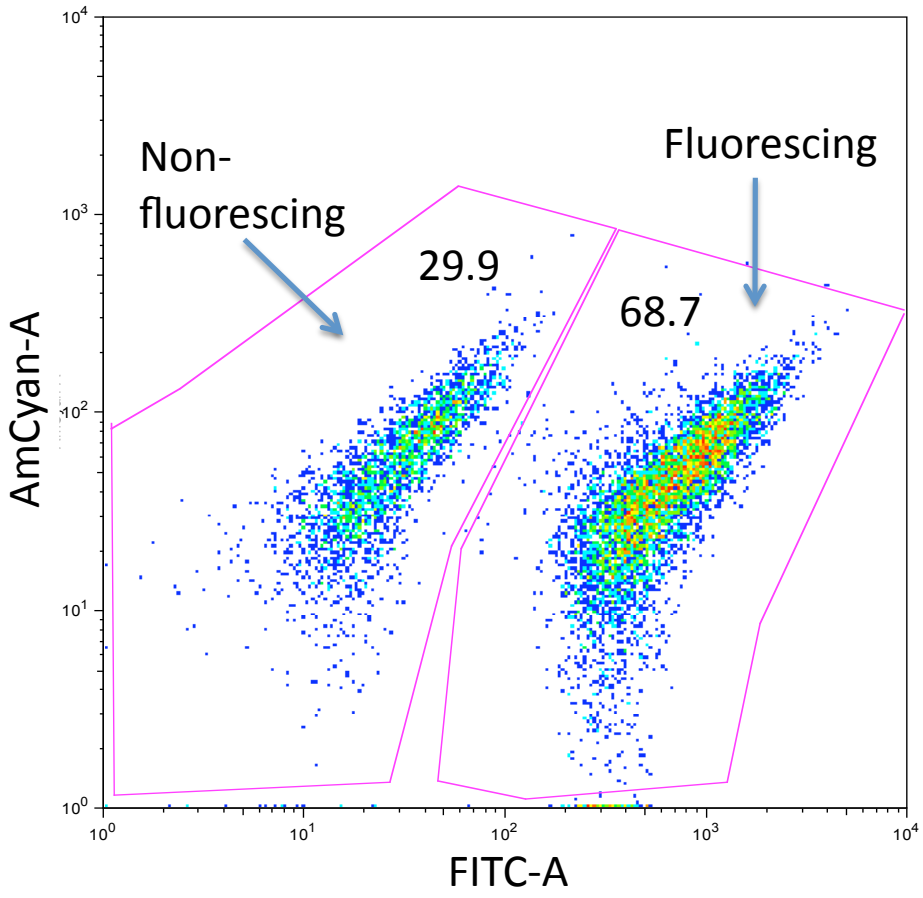


Figure S1: 10000 cells from each culture of interest were read in 96 well plates on an LSRII. Each cell is plotted on AmCyan-A and FITC-A axes which separates out non-fluorescing (left gate) from fluorescing cells (right gate). Numbers in each gate indicate the proportion of cells; this number is used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

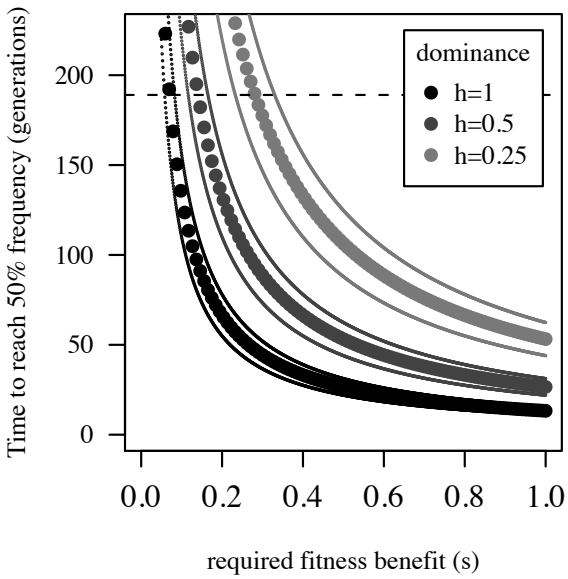


Figure S2: Time required for a beneficial mutation to reach 50% frequency in our experiments with a starting population size of 595067 (the measured average population size transfered daily across all environments; $N_o$ as in Campos & Wahl 2009 equation 6) and with periodic bottlenecks every 6.7 generations. 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).

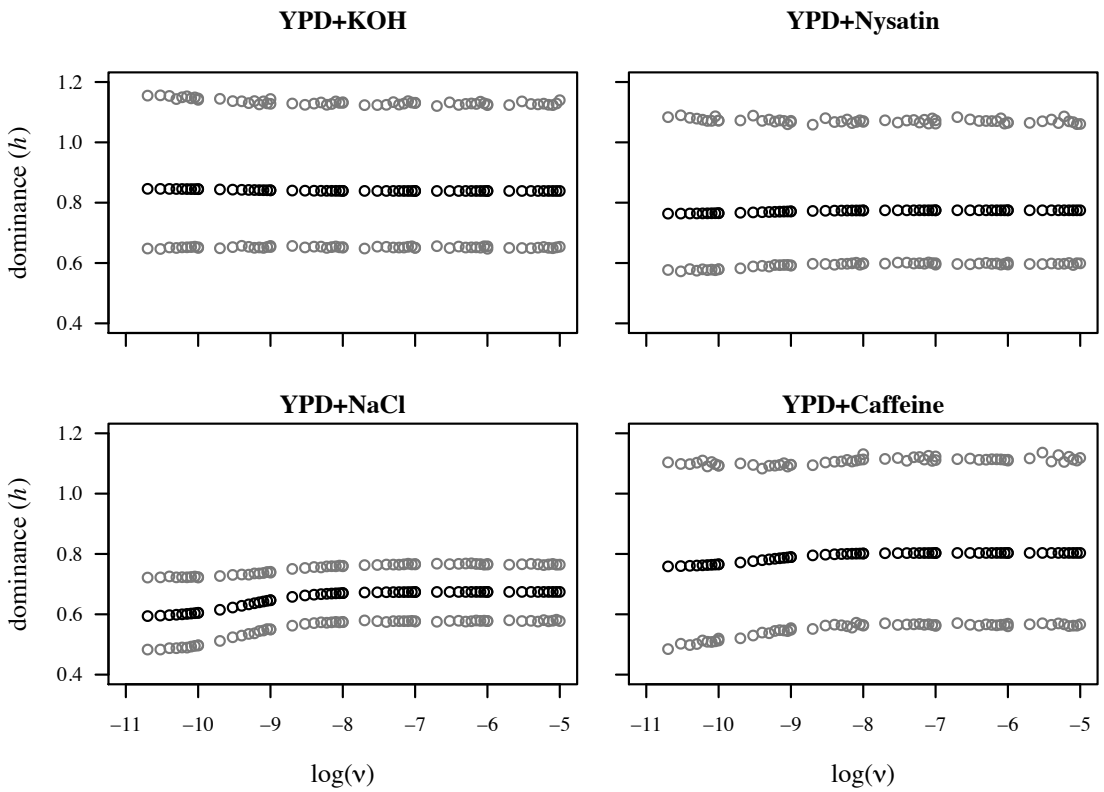


Figure S3: Dominance estimates are not sensitive to changing $\nu$, keeping the haploid mutation rate equal to the diploid mutation rate. Black dots are based on equation (S4), using the mean rate of adaptation and mean effective population sizes observed in haploids and in diploids. Grey dots indicate 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 ($\nu$).

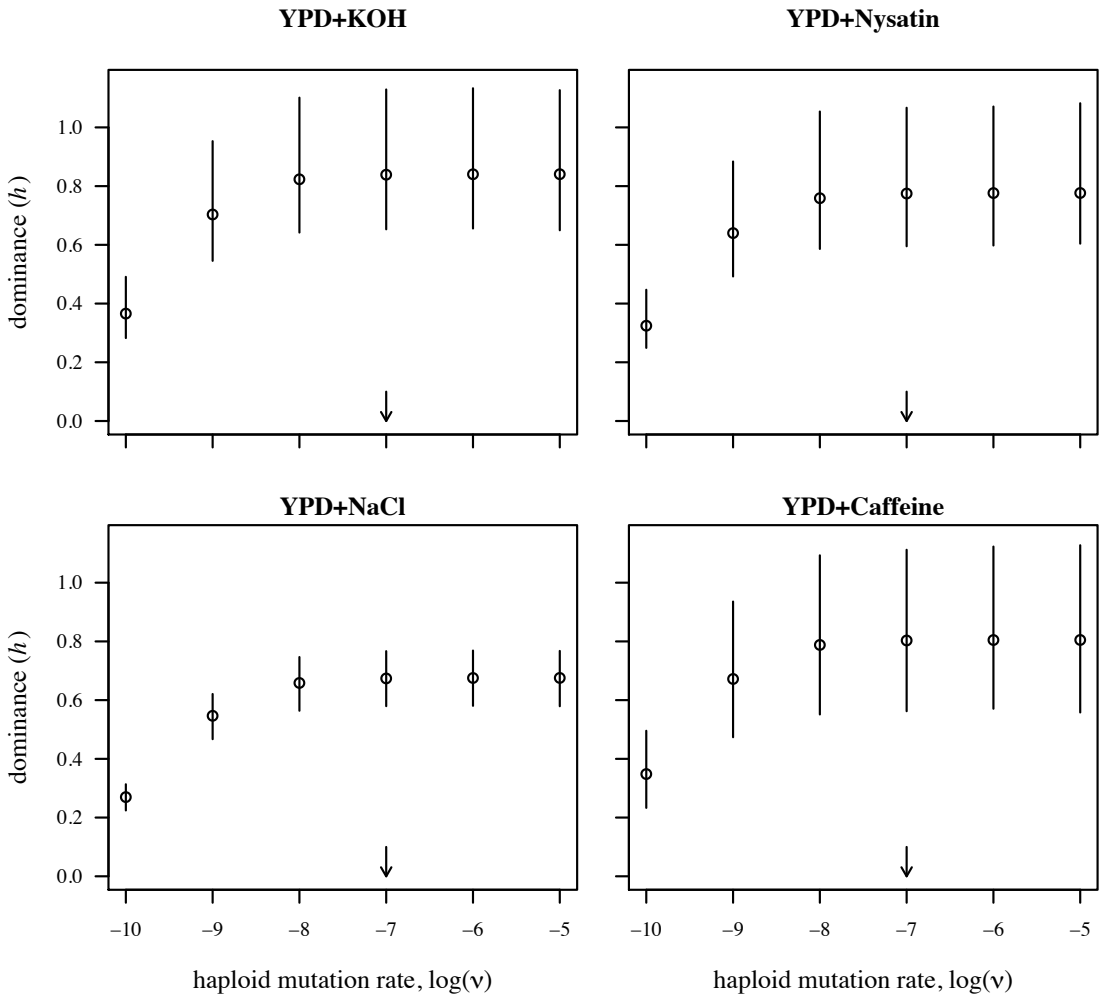


Figure S4: The sensitivity of dominance estimates to decreasing the haploid mutation rate. The diploid mutation rate was $10^{-7}$ (arrow) as in the simulations presented above, while the haploid mutation rate was changed. If the haploid mutation rate was three orders of magnitude lower than for diploids,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