Supporting information, Mutational effects depend on ploidy level: All else is not equal

Aleeza C. Gerstein [1],

1 Zoology Department & Biodiversity Research Center, The University of British Columbia, Vancouver, British Columbia, Canada

∗ **E-mail: gerstein@zoology.ubc.ca**

1 Additional Methods

1.1 Mutation acquisition

Mutations were acquired in haploids of genotype BY4741 in deep well boxes containing 1mL YPD + 4 μM nystatin [1]. Mutation accumulation was initiated by streaking the ancestral strain (BY4741) to single colony from frozen culture onto a YPD plate. A single random colony was then picked and grown for 24 hours in 10 mL YPD at 30 °, shaking at 200 rpm. 10 μL of this ancestral overnight culture was transferred into 240 deep wells. Preliminary experiments had indicated that wildtype culture was unable to grow reliably in this level of nystatin, thus when growth was observed within a well it was indicative of the appearance of a beneficial mutation (I refer to this growth pattern as 'stochastic growth'). Growth was monitored daily by visual examination of the bottom of the 96 well plates. A single colony was isolated from the 64 wells that showed growth within 7 days. Petite mutation lines were discarded, leaving us with 35 beneficial mutation lines [1].

1.2 Sequencing

DNA from all 35 lines was extracted and sequenced in 100 bp single-end fragments using Illumina's HighSeq 2000. The genetic basis of mutations in each line was determined using standard tools and methods (previously reported, [1]). 20 unique mutations in the ergosterol pathway were identified, as described in the main text. When multiple lines were found to contain the same ergosterol mutation only one line was chosen to assay for the current experiments. We identified six nonsynonymous SNPs in non-ergosterol genes (one each in BMN1, BMN22, BMN25, BMN29, BMN30, and BMN31) and three changes in non-coding genomic regions (two in BMN16 and one in BMN35, see Supporting Table S2, [1]); we previously found little to no evidence that these additional mutations affected nystatin tolerance.

Importantly, even if these mutations do have an effect, they would also have been homozygous in our *MATa*/*MATa* diploids, so genotypic differences at these loci could not explain why haploid mutants were consistently more resistant to nystatin than diploid homozygous mutants.

1.3 Nystatin dose-response assay

A maximum likelihood model was fit to the dose-response data to determine the half maximal inhibitory concentration of nystatin (IC_{50}), the slope at IC_{50} (m_{50}) and the density of culture at the asymptote (a). The logistic function

$$y = \frac{y_{max} exp(a(x - IC_{50}))}{1 + exp(a(x - IC_{50}))} + N(0, \sigma) \quad (1)$$

was used as in [1]; x represents the tested concentration of nystatin, y represents the observed OD following 72 hours of growth, N(0, σ) represents a normal deviate with mean zero and standard deviation, σ. The model thus fits the parameters of interest (IC_{50}, m_{50}, and a), as well as y_{max} (the maximal OD under full growth). Nystatin concentrations were ln-transformed prior to model fitting (so that percentage changes, not absolute differences, in nystatin matter), though all reported values of nystatin concentration and IC_{50} are on the original scale. The subplex method of optim in R [2], as implemented in the find.mle routine of the diversitree package [3] was used to identify the maximum likelihood point. Based on the raw data, lower and upper limits were placed on the parameters within the find.mle routine to facilitate convergence to the maximum likelihood point (lower: y_{max}=0.8, IC_{50}=0.0000001, a=-50, σ=0; and upper: y_{max}=1.2, IC_{50}=116, a=0, σ=10).

References

1. Gerstein AC, Lo DL, Otto SP (2012) Parallel genetic changes and non-parallel gene-environment interactions characterize the evolution of drug resistance in yeast. Genetics, advanced online publication. doi:10.1534/genetics.112.142620.

2. R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL `http://www.R-project.org`. ISBN 3-900051-07-0.

3. FitzJohn RG, Maddison WP, Otto SP (2009) Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Syst Biol 58: 595–611.

4. Weiss RL, Kukora JR, Adams J (1975) The Relationship between Enzyme Activity, Cell Geometry, and Fitness in *Saccharomyces cerevisiae*. Proceedings of the National Academy of Sciences 72: 794–798.

Supporting Tables

Table S1. Linear mixed-effects models that account for batch effects in the growth assays show similar statistical results as a two-way ANOVA (presented in the main text).

environment	ploidy	gene	interaction
2μM nystatin	$F_{1,71} = 9.32$, $p < 0.0001$	$F_{3,71} = 121.46$, $p < 0.0001$	$F_{3,71} = 0.49$, $p = 0.69$
4μM nystatin	$F_{1,63} = 6.58$, $p = 0.013$	$F_{3,63} = 30.39$, $p < 0.0001$	$F_{3,63} = 0.36$, $p = 0.78$

Table S2. Line-specific statistical results for dose-response assay parameters between haploids and homozygous diploids. Presented is the difference in log-likehood between full and contrained models. A parameter is significantly different between ploidy levels if the difference is greater than 1.92 (as indicated with *, see Methods))

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BMN line	tolerance (IC_{50})	asymptote (a)	slope (m_{50})
1	7.13*	< 0.01	2.83*
3	0.04	8.62*	2.59*
5	59.44*	13.96*	0.44
6	35.57*	32.38*	< 0.01
9	8.94*	21.36*	0.67
13	42.13*	12.66*	< 0.01
16	0.15	7.85*	0.05
19	39.11*	26.3*	< 0.01
21	16.25*	0.14	4.01*
22	8.01*	0.31	12.17*
23	12.18*	1.21	5.62*
25	6.34*	0.04	4.52*
28	16.19*	< 0.01	0.02
29	4.14*	1.45	0.81
30	5.03*	0.28	8.20*
31	2.80*	0.19	0.48
33	6.61*	0.04	0.66
32	0.59	0.38	3.25*
34	35.23*	0.12	<0.01
35	0.06	0.10	< 0.01

Table S3. Line-specific statistical results for the growth rate assay between haploids and homozygous diploids. Growth rate significance was determined by a t-test between haploids and diploids; * denotes $p < 0.05$, $^+$ denotes $p < 0.10$.

BMN line	2μM nystatin	4μM nystatin	8μM nystatin
1	$t_{9.4}$=3.1 $p = 0.012$*	$t_{14.0}$=1.5, $p = 0.16$	$t_{7.2}$=2.0, $p = 0.078^+$
3	$t_{9.7}$=3.7, $p = 0.004$*	$t_{9.4}$=6.1, $p = 0.0001$*	$t_{14.0}$=2.2, $p = 0.045$*
5	$t_{12.1}$=1.7, $p = 0.11$	$t_{12.9}$=1.3, $p = 0.23$	$t_{13.7}$=4.3, $p = 0.0008$*
6	$t_{13.9}$=6.8, $p < 0.0001$*	$t_{14.1}$=2.8, $p = 0.014$*	$t_{12.0}$=2.3, $p = 0.037$*
9	$t_{8.9}$=2.2, $p = 0.052^+$	$t_{13.5}$=-2.2, $p = 0.045$*	$t_{13.7}$=2.4, $p = 0.032$*
13	$t_{14.0}$=1.8, $p = 0.095^+$	$t_{5.1}$=0.5, $p = 0.63$	$t_{14.0}$=1.9, $p = 0.077^+$
16	$t_{8.1}$=2.3, $p = 0.051^+$	$t_{6.0}$=2.0, $p = 0.090^+$	$t_{13.3}$=11.6, $p < 0.0001$*
19	$t_{13.8}$=5.1, $p = 0.0001$*	$t_{10.6}$=1.4, $p = 0.19$	$t_{12.2}$=2.9, $p = 0.012$*
21	$t_{10.8}$=1.4, $p = 0.18$	$t_{11.0}$=0.9, $p = 0.45$	$t_{8.1}$=0.7, $p = 0.48$
22	$t_{10.7}$=1.2, $p = 0.26$	$t_{12.1}$=0.2, $p = 0.82$	$t_{7.9}$=1.2, $p = 0.28$
23	$t_{12.1}$=0.6, $p = 0.56$	$t_{11.9}$=1.0, $p = 0.32$	$t_{7.1}$=1.4, $p = 0.20$
25	$t_{13.6}$=2.2, $p = 0.049$*	$t_{12.2}$=1.5, $p = 0.17$	$t_{7.1}$=1.5, $p = 0.17$
28	$t_{13.6}$=3.0, $p = 0.011$*	$t_{3.8}$=22.5, $p < 0.0001$*	$t_{7.0}$=3.1, $p = 0.017$*
29	$t_{10.3}$=2.4, $p = 0.034$*	$t_{9.2}$=0.1, $p = 0.92$	$t_{13.8}$=-0.2, $p = 0.84$
30	$t_{13.4}$=2.0, $p = 0.064^+$	$t_{14.8}$=1.9, $p = 0.079^+$	$t_{10.0}$=1.4, $p = 0.19$
31	$t_{8.5}$=0.7, $p = 0.53$	$t_{9.6}$=0.6, $p = 0.55$	$t_{12.2}$=-0.5, $p = 0.65$
33	$t_{13.7}$=1.6, $p = 0.13$	$t_{10.7}$=0.7, $p = 0.47$	$t_{7.0}$=1.6, $p = 0.16$
32	$t_{13.9}$=6.2, $p < 0.0001$*	$t_{13.0}$=1.2, $p = 0.25$	$t_{7.0}$=1.8, $p = 0.11$
34	$t_{7.5}$=-7.1, $p = 0.0002$*	$t_{11.4}$=-1.9, $p = 0.085^+$	$t_{10.3}$=-0.5, $p = 0.63$
35	$t_{9.4}$=0.5, $p = 0.60$	$t_{4.3}$=-0.4, $p = 0.70$	$t_{14.0}$=0.04, $p = 0.97$

Supporting Figures

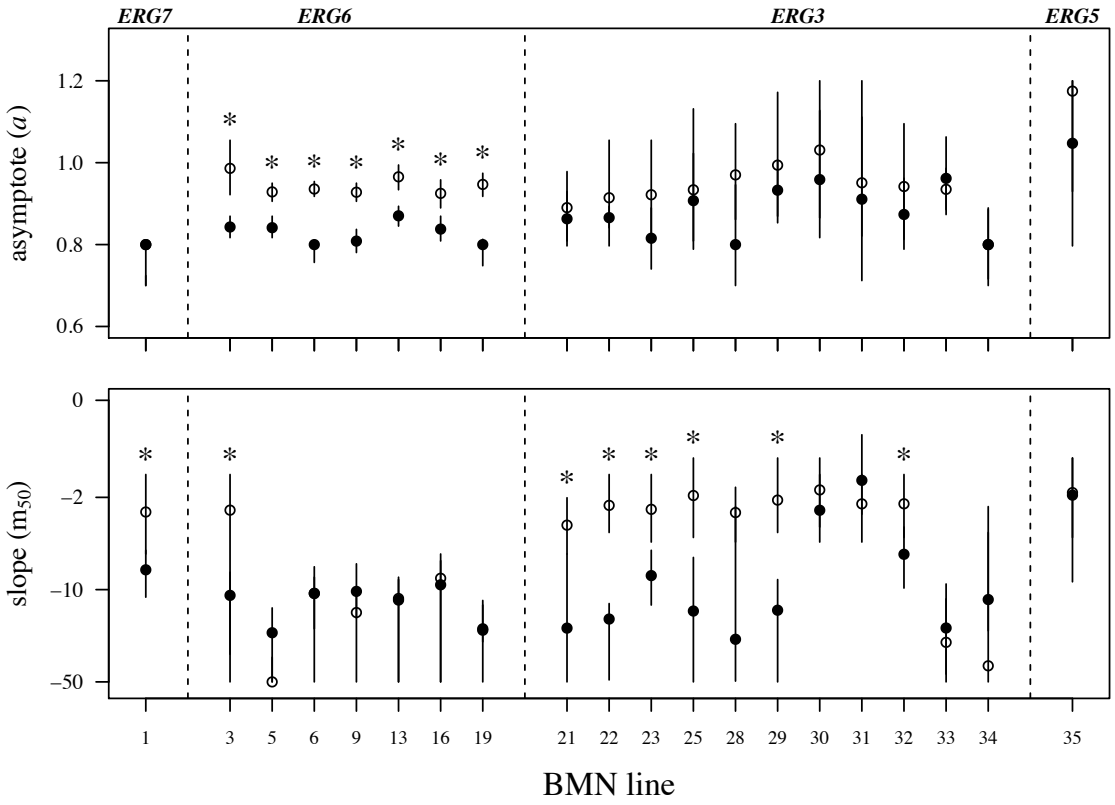

Figure S1. Nystatin adaptive mutations tend to have a higher asymptote (a) at low levels of nystatin (top graph) and higher slope at IC_{50} (m_{50}, bottom) in haploids compared to homozygous diploids. Note that a measures the asymptotic OD of a line when there is little to no nystatin. Because haploid cells are often about half the volume of diploid cells (with about half the opacity per cell, [4]), the asymptotic number of cells is likely much greater for haploids than diploids, even though a is fairly similar.

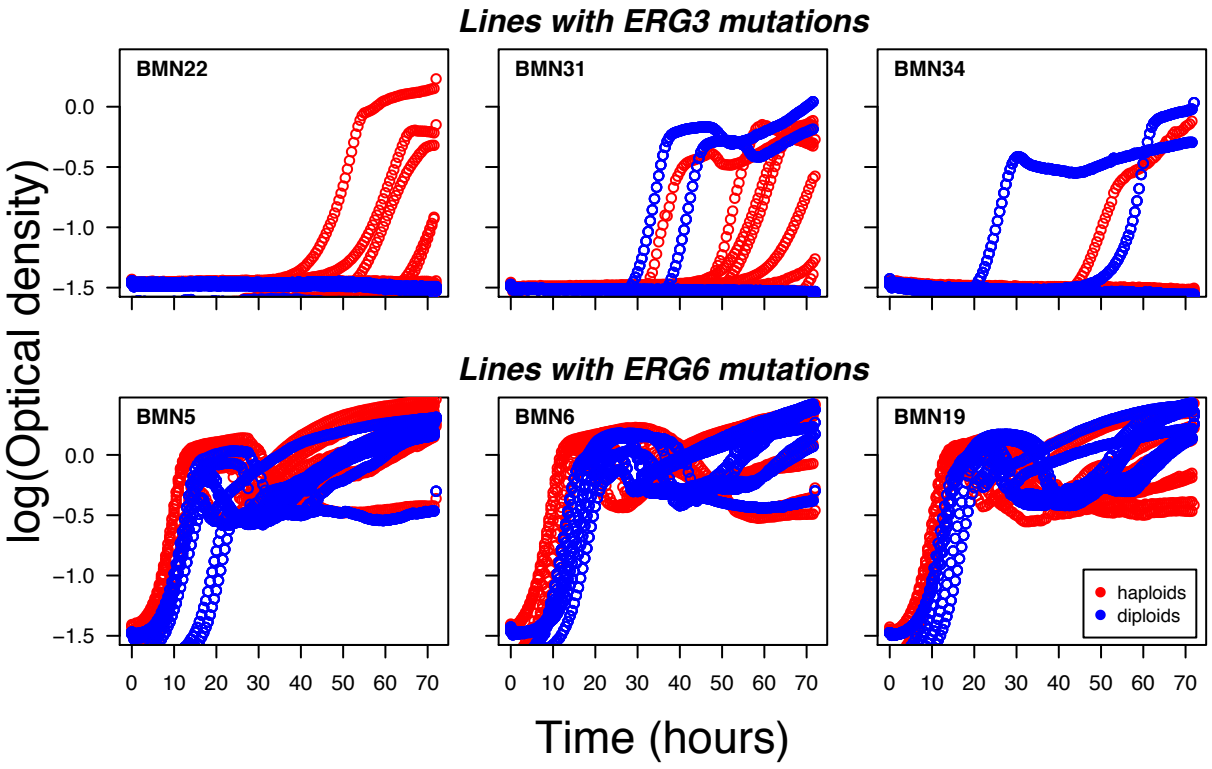

Figure S2. Lines with mutations in *ERG3* grow stochastically in YPD+8 μM nystatin (top three panels), while lines with mutations in *ERG6* continue to grow fairly consistently (bottom three panels). Variation in optical density following the rapid phase of growth likely reflects variation between wells in cell clumping and settling. Note that we use the intrinsic growth rate during rapid growth as a fitness proxy.