Appendix B.

Ecological Strategy Methods and Materials

PCR, transformations, and DNA extraction. Integration and deletion constructs were PCR amplified using KOD polymerase as per manufacturer's instructions (Novagen). Standard lithium acetate transformations for homologous recombination were performed as previously described.[28] Integration of the *GAL* promoter was performed by amplifying the *GAL1-10* promoter sequence from pRS314-Gal.[29] This fragment was PCR assembled with the kanamycin resistance gene from pFA6a-ZZ-TEV-S-kanMX6[30] along with flanking homologous regions to the *DAL80* upstream region (506000 - 504030 and 506500 - 506530 on chromosome XI). The construct was transformed, and colonies were selected on 400 ng/mL G418 YPD-agar plates. Integration was confirmed by colony PCR with primers flanking and internal to the integrated construct. Yeast DNA extraction was performed as previously described using the "bust n' grab" method.[31] Primer sequences are available upon request. Similar techniques were employed to integrate the *GAL1-10* promoter upstream of the *GDH1* coding sequence. Gene deletions were performed by amplifying the kanamycin resistance gene from pFA6a-ZZ-TEV-S-kanMX6[30] along with flanking homologous regions for the entire coding region of *GDH1, GDH3*, or *GLT1*. The D150H and C313S catalytic rate mutants were constructed by amplifying genomic DNA from the *GDH1* coding region with primers carrying the appropriate nucleotide substitution, assembled with the *LEU2* gene from pRS315,[29] and transformed as above. The *GLT1* overexpression plasmid was constructed by cloning the *GLT1* coding region in front of an *ADH1* promoter on a 2μm plasmid.

Quantitative RT-PCR. Cells were pelleted and frozen in liquid nitrogen. Pellets were resuspended in a 50 mM NaOAc (pH 5.2), 10 mM EDTA buffer. Cells were lysed by the

addition of SDS to a final concentration of 1.6% and an equal volume of acid phenol. Solutions were kept at 65 °C with intermittent vortexing for 10 min. After cooling on ice, the aqueous phase was extracted, and further extraction was carried out with an equal volume of chloroform. RNA was further isolated and concentrated by use of RNeasy columns (Qiagen) according to manufacturer's instructions. Total RNA was quantified by OD_{260} readings. RNA samples were treated with DNase (Invitrogen) according to manufacturer's instructions. cDNA was synthesized using gene-specific primers and Superscript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. qRT-PCR was carried out on this cDNA using an iCycler iQ system (BioRAD). Samples were prepared using the iQ SYBR green supermix and primer pairs specific for different templates. Data were analyzed using the iCycler iQ software.

Competitions and fitness assays. All competitor strains were derivatives of the S288C background, while the reference strain was derived from the W303 background. These strains showed different electronic volume versus side-scatter distributions that was also used to quantitate population numbers, in good agreement with the values obtained from fluorescent measurements. Equal amounts of competitor and reference strain were mixed and grown in indicated liquid media for 3 generations (approximately 6 hours). The frequency of competitor and reference strain were quantitated before and after the growth period by counting the numbers of GFP expressing cells to non-GFP expressing cells by flow cytometry using a Quanta SC flow cytometer (Beckman Coulter) equipped with the MPL system. Samples were excited with a 488 nm laser, and GFP fluorescence was detected with a 525 nm bandpass filter. A gate was set above the non-GFP expressing

cells in the Quanta analysis software to partition fluorescent from non-fluorescent cells. Samples of only reference or competitor strains and serial dilutions of ratios of competitor to reference strains were run in parallel as quantitation controls. 5,000 events were collected per sample.

Flow cytometry and calculation of noise. Two gates were used to standardize each cell population for analysis using "magnetic gating" in FlowJo flow cytometry analysis software (Tree Star, Inc.). The first gate isolated cells displaying regular morphology based on electronic volume and side-scatter, while the second gate removed non-fluorescent cells from the distribution. This gating method was compared against other methods previously described and the abundance and noise trends observed were consistent between methods.[32,33] Noise was calculated as the square of the coefficient of variation (σ^2/p^2) of the distribution.[26] 50,000 events were analyzed to calculate noise for each sample. Noise trends were similar when calculated as the coefficient of variation (σ/p) and the variance (σ^2).

Mutagenic PCR and construction of promoter libraries. To construct mutant libraries of the *GDH1* promoter, primers flanking 500 nucleotides upstream of the *GDH1* coding region (1043500 - 1043050, chromosome XV) were used to amplify the fragment from yeast genomic DNA using KOD polymerase. The fragment was then diluted into mutagenic PCR buffer[34] (7 mM $MgCl_2$, 0.5 mM $MnCl_2$, 50mM KCl, 10mM Tris pH 8.3 with 1mM dGTP, 0.2 mM dCTP, 0.2 mM dTTP, 0.2 mM dATP) and further amplified using Taq polymerase (Roche). Separately, the kanamycin resistance gene was amplified

from pFA6a-ZZ-TEV-S-kanMX6[30] using KOD polymerase. The kan^r gene fragment and the promoter library were then ethanol precipitated, resuspended in water, and PCR assembled together by virtue of overlapping primer sequences. The resulting large fragment was then transformed into yeast strains using a standard lithium acetate procedure. Transformants were selected in liquid YPD media supplemented with 400 ng/mL G418. The resulting library was grown to stationary phase and frozen in 15% glycerol at –80 °C.

Gillespie simulations. The reactions shown in Supplementary Fig. S6 were simulated using the Gillespie algorithm as described previously.[27] The probability of a reaction occurring in a given amount of time was proportional to the reaction rate. Briefly, birth and death rates of mRNAs are denoted by k_R and δ_R, respectively; enzyme is made with translation rate k_E and degraded with decay rate δ_E; substrate is created with rate k_S and product was allowed to accumulate. Noise was introduced by varying the "burst size" of translation events by modulating the ratio of k_E and δ_R while adjusting k_R to yield similar enzyme abundance between simulations.

Experimental evolution. A 50 μL aliquot from the *GDH1* promoter library freezer stock was inoculated into liquid YPD and allowed to acclimate overnight. 2 μL of the acclimated population was diluted into 2 mL synthetic complete media with two ammonia concentrations at 139 mM ammonia and 556 mM ammonia. Populations were grown in batch culture and diluted 10^3-fold into respective fresh media every 24 hours. Aliquots from the competitions were diluted 4,000-fold, and 50 μL of this dilution was

plated on YPD-agar plates. Single colonies were inoculated in synthetic complete media and grown overnight for further analysis.

Density-dependent fitness assays. Strains were grown overnight in YPD media. Serial 10-fold dilutions of each strain were performed and cell density of the overnight culture was measured using flow cytometry as described above. Competitor and reference strains were mixed in equal ratios in synthetic complete media at the density specified in Figure 4e. Cells were grown for 24 hours and fitness was assayed as described above. 5,000 events were collected for each sample.

Nitrogen utilization efficiency assay. Cells were back diluted and grown to mid-log phase (OD_{600} ~ 0.5), spun down, dried and weighed.[35] The amount of ammonia in the media before and after growth was quantitated via enzymatic assay (Megazyme).

Methods References

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