Allele surfing promotes microbial adaptation from standing variation

Appendix C: Experimental methods

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Contents

1 Strains

S. cerevisiae – competition experiments

To perform population growth experiments, we used strains yJHK111 ('wild type yellow strain', [1]), yJHK112 ('wild type red strain', [1]), and yMM9 ('red mutant strain', unpublished, courtesy of Melanie J. I. Müller). All three strains have a W303 background (common genotype *MATa bud4Δ::BUD4(S288C) can1-100*, see Table C2 for details). yJHK111 expresses the yellow fluorescent protein ymCitrine, yJHK112 expresses the red fluorescent protein ymCherry. yMM9 expresses ymCherry, but is also resistant to cycloheximide (CHX) via mutation *Q37E* in gene *CYH2* (while yJHK111 and yJHK112 are sensitive). Experiments with tunable selection were performed using the pair yJHK111 and yMM9 with a variable concentration of cycloheximide in the medium. Experiments with neutral standing variation were performed using the pair yJHK111 and yJHK112. Note that, throughout this work, signal in the channel for the fluorescent color of the "mutant" (yMM9 and yJHK112) is pseudo-colored as yellow, while the fluorescent signal of the wild type (yJHK111) is pseudo-colored as blue.

S. cerevisiae – cell tracking at front

To track cells at the front, we used strain yMG10c, a convertant of yMG10, which in turn is based on strain yDM117 (W303 background, *HO::cre-EBD*, courtesy of Jasper Rine), transformed with a cassette (pMG4)

based on pMEW90 (courtesy of Mary Wahl [2]). pMG4 contains a *loxP* cassette followed by *ymCitrine*, linked with *cyh2r* via ubiquitin. yMG10 was incubated with estradiol to induce auto-recombination, and streaked onto plates containing selective amounts of cycloheximide to select for the convertant yMG10c used for the time lapse movie in Fig. 3, which has genotype W303 *HO::cre-EBD SUC2::loxP-ymCitrine-ubq-cyh2r*.

S. pombe

To investigate genetic demixing from neutral standing variation in S. pombe, we used two variants of strain MJ95 (genotype *leu1-*, *ura4-*, *h-*) [3], which were obtained by replacing *mCherry* with the coding region for YFP and CFP from plasmids pOH1 and pOH2 at the *atb2* locus.

Plasmids with fluorescent markers cyan and yellow

pOH1 and pOH2 are based on the vector pTrc99A, with sequences for eCFP and Venus YFP inserted between the *Sac*I and *Xba*I sites, respectively. These plasmid are inducible by IPTG but we found the base level of expression of the fluorescent proteins to be sufficient without inducer. For a more detailed description see Ref. [4].

E. coli – competition experiments

Population growth experiments were performed using three different backgrounds:

1. DH5α transformed with pOH1 and pOH2, resulting in eOH1 and eOH2. These strains are identical to those used in Ref. [4]. For the competition experiments, we transformed eOH2 with the plasmid pACYC184 (New England Biolabs), conferring resistance to tetracycline, resulting in eOH3. Experiments with tunable selection were performed using the pair eOH1 and eOH3 (Fig. 4), adding low concentrations of tetracycline to the growth medium (in addition to ampicillin for plasmid maintenance). Experiments with neutral standing variation were performed using the pair eOH1 and eOH2 (Figs. 4 & 5).

2. Strain MG1655 (not fluorescent) and its derivative SJ102 (genotype MG 1655 *intC::*λ*pR-YFP-Cmr*, courtesy of Ivan Matic), which constitutively expresses YFP and is resistant to chloramphenicol, allowing us to perform experiments with tunable selection (Figs. 4 & 5) by adding low concentrations of chloramphenicol to the growth medium. SJ102 was also used to study the dynamics of *E. coli* cells at the front (Fig. 3, SI movie 2).

3. A pair of JE 5713 [5] (cross between B6 [6] and KL228 [7, 8]), transformed with plasmids pOH1 and pOH2, giving rise to eOH4 and eOH5, were used for competition experiments with neutral standing variation (Fig. 5). These strains have been reported as *rodA* mutants but also carry a point mutation in the gene *mrdA* (Waldemar Vollmer, private communication), causing a round cell shape.

Name	Composition	Used for...	Temperature [°C]
YPD	20g/L peptone, 10g/L yeast extract, 20g/L dextrose	*S. cerevisiae*	30
YES	5g/L yeast extract, 30g/L glucose, 225mg/L adenine, histidine, leucine, uracil, lysine hydrochloride	*S. pombe*	30
LB	10g/L tryptone, 5g/L yeast extract, 10g/L NaCl	*E. coli*	21, 37
M9	200mL/L 5× M9 salts [9], 5g/L dextrose, 2mM MgSO4, 0.1 mM CaCl2	*E. coli* (Fig. 5)	37

Table C1: Media and growth conditions used in this study. For plates, 2% w/v bacto agar was added to the media before autoclaving. Antibiotics were added after autoclaving to cooled media.

Strain	Description	Genotype	Fluor.	Resistance	Reference	Fig(s).
S. cerevisiae						
yJHK111	YFP WT	W303 *MATa bud4Δ::BUD4(S288C) can1-100 his3::prACT1-ymCitrine-tADH1:His3MX6*	yellow	-	[1]	1,4,5
yJHK112	RFP WT	W303 *MATa bud4Δ::BUD4(S288C) can1-100 his3::prACT1-ymCherry-tADH1:His3MX6*	red	-	[1]	1,4,5
yMM9	RFP MT	W303 *MATa bud4Δ::BUD4(S288C) can1-100 his3::prACT1-ymCherry-tADH1:His3MX6 CYH2::cyh2-Q37E*	red	CHX	unpublished	1,4,5
yMG10	converter (RFP)	W303 *HO::cre-EBD SUC2::loxP-RFP-loxP-YFP-ubq-cyh2r*	red	G418, Nat	This study	-
yMG10c	converted (YFP)	W303 *HO::cre-EBD SUC2::loxP-YFP-ubq-cyh2r*	yellow	CHX, Nat	This study	3
S. pombe						
MJ95	RFP WT	*mCherry-atb2::hph, leu1-, ura4-, h-*	red	Hyg	[3]	-
MJ95-CFP	CFP WT	*CFP-atb2::hph, leu1-, ura4-, h-*	cyan	Hyg	This study	5
MJ95-YFP	YFP WT	*YFP-atb2::hph, leu1-, ura4-, h-*	yellow	Hyg	This study	5
E. coli						
eOH1	CFP WT	NEB DH5α *eCFP* (pTrc99A)	cyan	Amp	[4]	4, 5
eOH2	YFP WT	NEB DH5α *YFP* (pTrc99A)	yellow	Amp	[4]	4, 5
eOH3	YFP MT	NEB DH5α *YFP* (pTrc99A) + pACYC184	yellow	Amp, Tet	This study	4, 5
MG1655	WT	-	-	-	Laboratory stock	4, 5
SJ102	YFP MT	MG 1655 intC::λP_R-*YFP*-Cm^R	yellow	Cm	unpublished	3, 4, 5
JE5713	Round cell shape	*mrdA*-mut	-	-	[5]	-
eOH4	CFP round cells	JE5713 *eCFP* (pTrc99A)	cyan	Amp	This study	5
eOH5	YFP round cells	JE5713 *YFP* (pTrc99A)	yellow	Amp	This study	5

Table C2: Strains used in this study.

2 Determining fitness differences as function of drug concentration

Liquid culture (*S. cerevisiae* only)

Single colonies were picked and grown overnight, then diluted 1:10 in fresh media, and grown for another 3 generations to ensure growth in log phase. The resulting cultures were mixed at ratio $P_{\mathrm{i}} = 0.5$ (measured by OD) and about 10000 cells inoculated into the wells of a 96 well plates with fresh YPD containing a range of antibiotics concentrations (3 replicates from the same initial culture per concentration). The plates were sealed and grown at 30°C overnight, then shaken vigorously for at least 1 minute. 10μl of the culture were diluted into PBS for analysis in the flow cytometer (Beckman-Coulter Fortessa X2). Every day, about 10000 cells were re-inoculated into fresh YPD to passage the cells for a total of 5 days, corresponding to about 60 generations. The cultures diluted in PBS were stored at 4°C until they were analyzed using the flow cytometer at a rate of at most 10000 events per second. The resulting ratio of mutants to wild type increased exponentially with the number of generations elapsed, whence the fitness difference could be calculated from the slope of the curve in a semi-logarithmic plot.

On plates

Fitness differences were measured in separate experiments using the colliding colony assay, described briefly in the following, see also Ref. [1]. Two 1μl droplets, each containing one of the two strains in log phase, are placed on agar plates about 5mm apart and incubated for at least 72 hours, until a sizable interface between the resulting colonies is formed. A circle of radius R is manually fitted to the collision interface using the Zeiss *ZEN* software and the distance l between the inoculation centers is measured. The selection coefficient s can be calculated via

$$s = \frac{1 - 2z + \sqrt{1 + 4z^2}}{2z}, \tag{C1}$$

where $z = R/l$. The resulting values of s were found to exhibit an approximately linear dependence on drug concentration (Fig. B1a). We used the values of s given by the linear regression in the figures in the main text (Fig. 1h, i, j, Fig. 4g, h). Following the results from Ref. [1], we assumed the same regression for fitness differences on plates and in liquid culture.

3 Adaptation from standing variation during two types of population expansions

Experiment and quantification

The experimental procedure for competition experiments from standing variation is described briefly in the main text and methods therein, see also Fig. 1a for a cartoon of the experiments. Here, we provide additional details on the experimental procedures.

All competition experiments were performed on one batch of media/plates (per experimental series) and using the same overnight cultures. For each competition experiment on plates we also carried out fitness measurements (via colliding colonies) on the same batch of plates. Final population sizes of the budding yeast colonies were measured by resuspending colonies into PBS, diluting and replating to count colony forming units, or measuring optical density and comparing with a previously obtained calibration.

To measure the frequency of yeast mutants in well-mixed liquid culture, we grew and mixed the strains as described above and grew the mixture overnight in aerated culture tubes at 30°C (2 replicates from the same

[CHX]	0	40	80	120	160	200
Sectors	46	101	178	102	69	35

Table C3: Number of sectors analyzed per cycloheximide concentration.

initial culture per concentration). We separately checked that the competed strains had the same carrying capacity to avoid error due to the cultures entering stationary phase. The next morning, we sampled 20μl from each tube into 180μl of PBS. The mixture was then measured in a Beckman-Coulter Fortessa X20 flow cytometer at a rate of at most 10000 events per second.
To determine the number of cells in the outer rim of the inoculum, N_{mut}, in Fig. 4h, we measured the radius r_0 of the evaporated droplet (5 colonies from the same initial culture), which was easily visible under brightfield illumination. N_{mut} was then calculated as $N_{\text{mut}} = 2\pi r_0 P_{\text{i}}$. The variable $N_{\text{sec}}/N_{\text{mut}}$ in Fig. 4g hence corresponds to the probability of surfing of an individual mutant cells in the very first cell layer, assuming that the droplet rim is perfectly flat. $N_{\text{sec}}/N_{\text{mut}}$ differs from the true surfing probability (of an individual cell in the front) by a numerical factor of order 1 taking into account the irregularities of the droplet perimeter.

Image analysis

To measure the frequency of mutants in colonies, images of the colonies were taken with a Zeiss AxioZoom v16 fluorescence microscope at 3.5x zoom and analyzed using custom routines written in *Mathematica* (Wolfram Research, Inc., Mathematica, Version 10.1, Champaign, IL (2015)). Because the colonies' fluorescence typically becomes weaker near the colony boundary, we employed a local adaptive binarization scheme. Since individual images varied in intensity distribution, it was necessary to set the binarization thresholds by hand for each image such that the binarized shape corresponded well to the observed sector shapes. We expect the error from this "subjective" choice of thresholds to be small. During binarization, the outer radius of the colony, its center, and the radius of the inner ring, stemming from the inoculation droplet, were also measured. The frequency of mutants was then calculated by measuring the area of mutants and dividing by the area of the annulus between the outer and inner radius, i.e., the fraction inside the homeland was neglected, but the emerging bulge (for larger s) was taken into account.
For the frequency per sector in Figs. 1 and B7, we selected only colonies that either only had a single sector, or colonies with few sectors that did not touch. Since the colonies used for Fig. 1 had many sectors at large s, we also used colonies from experiments with smaller P_{i} (0.0025, 0.005, 0.01) to acquire enough "free-standing" sectors. The frequency was then computed as described above. Table C3 gives the number of sectors analyzed for each concentration of cycloheximide.

4 Growth of *S. cerevisiae* colony from single cell

Using a Zeiss AxioZoom v16 upright microscope, we tracked the growth of a colony (strain yMG10c) by taking time-lapse movies of the fluorescence signal detected in a stage-mounted Okolab UNO-PLUS incubator at 30°C and at constant relative humidity. An agar plate in a Petri dish was inoculated with single cells and grown in the stage-top incubator until colonies were visible at the desired magnification. Then, one colony was randomly chosen and the time lapse movie was recorded for 48 hours, taking an image every 30 min. The colony radius was determined by fitting a circle to the circumference of the colony.

5 Cell tracking at the front

Experiment

For single-cell resolution time lapse movies (SI movies 1 and 2) of growing SJ102 and yMG10c, we used a Zeiss LSM700 in confocal mode with a 488nm laser. Agar plates were inoculated with fresh culture droplets (2μl), that were left to dry for several minutes. The agar around the droplet was then cut into a 2cm$\times$2cm pad and inverted onto a clean coverslip, such that the cells touched the coverslip. The coverslip with the cells was then incubated for a day to reach steady-state growth of the colony. After mounting the coverslip in a stage-top incubator, we mounted the incubator on the microscope and let it equilibrate for about 2h. Humidity in the chamber was controlled by the addition of a water reservoir. *E. coli* cells were imaged with a 40x oil objective, *S. cerevisiae* cells with a 20x air objective. Images were taken at 1 frame per minute with a dwelling time of about 6μs/px (31s exposure per frame) for 274/228 minutes, respectively.

Analysis

For cell tracking, all frames were cleaned automatically using a median filter and contrast-adjusted. In SI movie 2, some frames were manually retouched to remove brightness fluctuations. All frames were segmented with a local adaptive binarization algorithm (same parameters for all frames) and objects touching the image boundaries were removed. Because cells far behind the front could usually not be tracked accurately, we only analyzed the first few cells layers by automatically finding the position of the front and removing segmented objects far from it.

To determine the ancestry of cells at the front, we proceeded backwards in time. An individual cell was tracked by creating a mask from its outline, dilating it, and computing the overlap with the previous frame. The cell's position in the previous frame was then determined by finding the cell with maximal overlap.

For Fig. 3, we tracked a total of 692 and 407 cells for 180 minutes in *E. coli* and *S. cerevisiae*, respectively, i.e., we shortened the original time lapse movies to 180 minutes. This was done to maximize the number of tracked cells while still maintaining information over sufficiently long time scales.

To obtain the mean square displacement in Fig. 3f, we proceeded as follows. Each tracked cell in the final frame was traced back to its ancestor 180 minutes ago. Since the front had a defined direction of motion (which we defined as the x-axis), we measured, in each time step, the position of the cell relative to its original position and computed the displacement y from the x-axis, and take the square. These operations are performed for all tracked cells, and averaging is performed over bins of x-displacements to account for cells moving by different amounts in the x-direction per frame. After averaging, the square root was taken in each x-bin, and the curves were fitted using *Mathematica*. In order to compare values for *S. cerevisiae* and *E. coli*, we divided the displacements in x and y by the effective cell sizes d, given by $d = 4.5\mu$m for *S.cerevisiae* and $d = \sqrt{3.5\mu\text{m} \times 0.7\mu\text{m}}$ for *E.coli*. The effective cell size for *E. coli* was determined by the harmonic mean of its semi-axes, which were both measured directly from the time-lapse movie, as was the cell size of *S. cerevisiae*.

Figures

Figures of the cell tracking (Figs. 3a, c and Fig. B8) were created using Adobe Photoshop by overlaying images of the segmented cells at $t = 0$ and $t = 3$h with the computed lineages. For Figs. 3a & c, an outline was added to the tracked lineages and the cells in the lineage to increase visibility.

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