Supplementary methods 1: Parameter estimation

A number of estimates of the parameters in model (1) of the main text can be obtained from the literature. However these estimates are not necessarily obtained for the same laboratory yeast strains used in this study. In (Badotti et al. 2008) the authors measured the kinetics of active H+-sucrose symport activity in 1403 – 7A strain and we use this data to define our sucrose uptake rate $J^S$ as

$$J^S(S) = \begin{cases} 
64 \cdot S, & 0 \leq S \leq 0.042 \\
14.8 \cdot S + 2.1, & 0.042 < S \leq 0.43 \\
2.04 \cdot S + 7.57, & 0.43 < S \leq 0.9 \\
1.48 \cdot S + 8.08, & 0.9 < S 
\end{cases}.$$  \hspace{1cm} (1)

Here and throughout the manuscript we use the following conversion 1 g biomass = 0.5 g protein (personal communication with Karin Elbing). In Elbing et al., (2004) the authors estimated the relationship between invertase activity and glucose consumption rate using KOY.PK2-1C83 wild type strain and its various genetically manipulated derivatives. We use this data to estimate the invertase activity $inv(J^G)$ as

$$inv(J^S) = -2.3453 \cdot (J^G)^2 + 32.609 \cdot (J^G) + 77.109.$$  \hspace{1cm} (2)

The estimates

$$V_{max}^G = 40 \text{ mmol g protein } \times \text{ hour},$$  \hspace{1cm} (3)

and

$$K_m^G = \begin{cases} 
1.9 \text{ mmol/agar, for 25 mL agar plate} \\
1.52 \text{ mmol/agar, for 20 mL agar plate}
\end{cases},$$  \hspace{1cm} (4)

are obtained from Otterstedt et al. (2004) which uses CEN.PK2-1C wild-type strain while the estimate

$$K_m^F = \begin{cases} 
3.125 \text{ mmol/agar, for 25 mL agar plate} \\
2.5 \text{ mmol/agar, for 20 mL agar plate}
\end{cases},$$  \hspace{1cm} (5)

was obtained from Reifenberger et al. (1997). The remaining parameters of the model (1) in the main text, for which there are no estimates already available in the literature, are estimated using simple growth experiments described below.

0.1 The growth experiments

Yeast cells (producers or non-producers) were initially distributed in two identical patches per 25mL agar plate, 5cm apart. The patches were initially made up of 5 µl of a liquid culture grown overnight in YFPD broth. The assays were replicated 6 times. The initial and final cell densities were measured in CFU/patch which is converted into g protein/patch in table below using the assumption that 1mg protein = $33 \times 10^6$ cells. This assumption is used throughout the manuscript.
| Experiment | Cell type | Carbon source | Initial cell biomass | Length of experiment | Final cell biomass |
|------------|-----------|---------------|----------------------|----------------------|--------------------|
| 1a         | n-p       | sucrose 0.2%  | $9.737 \times 10^{-6}$ | 4 days               | 0.00215            |
| 1b         | n-p       | sucrose 2%    | $9.737 \times 10^{-6}$ | 4 days               | 0.0083             |
| 2a         | n-p       | glucose 0.2%  | $9.17 \times 10^{-6}$  | 2 days               | 0.0029             |
| 2b         | n-p       | glucose 2%    | $9.17 \times 10^{-6}$  | 2 days               | 0.0047             |
| 3a         | n-p       | fructose 0.2% | $9.17 \times 10^{-6}$  | 2 days               | 0.0025             |
| 3b         | n-p       | fructose 2%   | $9.17 \times 10^{-6}$  | 2 days               | 0.0053             |
| 4a         | p         | fructose 0.2% | $9.4 \times 10^{-6}$   | 2 days               | 0.0023             |
| 4b         | p         | fructose 2%   | $9.4 \times 10^{-6}$   | 2 days               | 0.005              |
| 5a         | n-p       | glucose 0.2%  & fructose 0.2% | $9.17 \times 10^{-6}$ | 2 days | 0.0032 |
| 5b         | n-p       | glucose 2% & fructose 2% | $9.17 \times 10^{-6}$ | 2 days | 0.0043 |
| 6a         | p         | sucrose 0.2%  | $7.663 \times 10^{-6}$ | 4 days               | 0.0071             |
| 6b         | p         | sucrose 2%    | $7.663 \times 10^{-6}$ | 4 days               | 0.011              |

### 0.1.1 Data fitting

Throughout the manuscript data fitting is conducted in the following way. The Experiments 1 – 6 have been set up with two patches on an agar plate each containing the same amount and the same type of cells. The initial and the final cell density in the experiment are recorded per patch. Therefore the initial cell density in the numerical simulation of models (6 – 9) and model (13) is the same as initial cell density in one experimental patch. Mathematical models of growth kinetics (6 – 9) described below are developed at a single patch level. The concentration of carbon sources in the experiment is measured per agar plate (which contains two identical patches) and therefore the initial concentration of carbon resources in the numerical simulation is half of the experimental concentration measured per agar. We run numerical simulations for the same length of time experiments are conducted and find the value of the free parameter that gives rise to the same final cell density in a patch as in the experiment.
0.2 Estimating the rate-efficiency trade-off of $H^+$-symport

The growth of non-producers ($N_n$) on sucrose can be represented by a simple model

\[
\frac{dS}{dt} = -J^S \cdot N_n, \quad (6a)
\]

\[
\frac{dN_n}{dt} = n^S_e \cdot J^S \cdot N_n. \quad (6b)
\]

Having fixed the sucrose uptake rate $J^S$ as in (1), the efficiency of sucrose pathway $n^S_e$ as a function of $J^S$ is estimated from a simple growth experiment of non-producers on sucrose (Experiment 1). We assume the following form of the efficiency function:

\[
n^S_e(x) = \begin{cases} 
  a_1, & 0 \leq x \leq \text{maximal uptake rate at } 0.2\% \text{ sucrose} \\
  b_1, & x > \text{maximal uptake rate at } 0.2\% \text{ sucrose}
\end{cases}
\]

Next we estimate $a_1 = 0.0295 \frac{\text{g protein}}{\text{mM sucrose}}$ by fitting the model (6) to final population data in Experiment 1a, and we estimate $b_1 = 0.0094 \frac{\text{g protein}}{\text{mM sucrose}}$ by fitting the same model to the final population data in Experiment 1b.

0.3 Estimating the rate-efficiency trade-off of hexose transport

The growth of non-producers ($N_n$) on glucose can be represented by a simple model

\[
\frac{dG}{dt} = -J^G \cdot N_n, \quad (7a)
\]

\[
\frac{dN_n}{dt} = n^{Hxt}_e(J^G) \cdot J^G \cdot N_n, \quad (7b)
\]

where $J^G = \frac{V^G_{\text{max}} G}{K_m^G + G}$ which is obtained by setting $F^{G}_{K_C} = 0$ in (10) as there is no fructose in the environment and hence no competition for the hexose transporters.

Since $V^G_{\text{max}}$ and $K_m^G$ are fixed as in (3) and (4) respectively, the efficiency of hexose pathway $n^{Hxt}_e$ as a function of the uptake of hexose resources (in this case glucose only) is estimated from a simple growth experiment of non-producers on glucose. We use data in Weusthuis et al. (1994) to define the following form of the rate-efficiency function illustrated in Figure 1. Note, we do not use the values obtained in (Weusthuis et al., 1994) for the rate-efficiency function as these have been obtained in the chemostat. As pointed out in van Dijken et al. (2000) biomass yield estimates are found to be lower in chemostats than when obtained from agar plates. Instead we rely on (Weusthuis et al., 1994) only for the form of the function $n^{Hxt}_e$ but use agar plate growth experiments (Experiments 2) to estimate the precise values in $n^{Hxt}_e$.

Fitting the model (7) to the final population data in Experiment 2a we estimate $a_2 = 0.048 \frac{\text{g protein}}{\text{mM sucrose}}$ while fitting the same model to the final population data in Experiment 2b we arrive at $b_2 = 0.00632 \ldots$
The parameters $x_1$, $x_2$ and $x_3$ denote the maximal uptake rate at 0.2\%, 2\% and 4\% glucose respectively. The parameter $c_2$ is only needed further on in the manuscript when fitting the model to Experiment 5b, and we take $c_2 = 0.001$ as some small perturbation of $b_2$. Note that given the competition between glucose and fructose for hexose transporters, and the spatial experimental setup (see Methods, main text) the uptake rate of glucose and fructose is never greater than $x_2$ in the fully spatial model (2) in the main text.

### 0.4 Estimating the maximal uptake rate of fructose

The growth of non-producers on fructose can be represented by a simple model

\[
\begin{align*}
\frac{dF}{dt} &= -J^F \cdot N_n, \\
\frac{dN_n}{dt} &= n^{Hxt}_e (J^F) \cdot J^F \cdot N_n,
\end{align*}
\]

where $J^F = \frac{V^{F}_{\text{max}} \cdot F}{K^F_m + F}$ which is obtained by setting $\frac{G}{K^F_e} = 0$ in (11) as there is no glucose in the environment and hence no competition for the hexose transporters. Since $K^F_m$ is fixed in (5) and $n^{Hxt}_e$ has been estimated in the previous section, the maximal fructose uptake rate $V^{F}_{\text{max}}$ is the only free parameter. In this case we have two experimental values for the final cell densities one for growth on 2\% fructose and the other for growth on 0.2\% fructose (Experiments 3a and 3b, respectively). As our model has only one free parameter $V^{F}_{\text{max}}$, we fit the model (8) to Experiment 3b and arrive at $V^{F}_{\text{max}} = 54.93 \, \frac{\text{mmol}}{\text{g protein} \times \text{hour}}$. Next we check whether using the obtained value of $V^{F}_{\text{max}}$ in the model (8) we can correctly predict the outcome of Experiment 3a and find that we can.
0.5 Estimating the cost of invertase production

Experiment 7

We competed producer and non-producer strains in glucose-limited chemostats under conditions that induce the secretion of invertase without any possible benefit of invertase secretion. The two strains were competed against each other for 24 hours in 16 chemostats supplied with glucose-limited culture medium (0.8 g/L) incubated with continuous shaking and aeration. Dilution rate varied between 0.2 and 0.4 per hour. Under these conditions, glucose uptake rate is between 0.2 and 0.4 mmol.gram$^{-1}$.hour$^{-1}$ (Weusthuis et al., 1994), which induces the secretion of invertase in producer cells (Elbing et al., 2004) so that invertase makes up approximately 0.1% of total cell protein. Quantitative PCR on DNA extracted from samples taken from each chemostat before and after competition was used to measure the changes in the abundance of producers and non-producers during competition. Fitness was calculated as the ratio of population doublings during competition ($w$). In this experiment it was found that the non-producers enjoy a small, but detectable, fitness advantage (mean $w = 1.04$, s.e= 0.014, n= 15, $t_{14} = 2.72$, $P = 0.016$).

Remembering that the cost of invertase production is defined as

$$c^{Inv} = inv(J^G)U^{Inv},$$

where $U^{Inv}$ denotes the unit cost of invertase which is a function of invertase activity $inv(J^G)$. Motivated by Dong et al., 1995 and taking into account the results of Experiments 7 we arrive at

$$U^{Inv} = 0.00054e^{0.000001inv(J^G)}.$$

0.6 Estimating the hexose transporters competition functions for glucose ($K_e^G$) and fructose ($K_e^F$)

The competition functions are estimated using the following two models. The growth of non-producers on glucose and fructose can be represented by a model

$$\frac{dG}{dt} = -J^G \cdot N_n,$$

(9a)

$$\frac{dF}{dt} = -J^F \cdot N_n,$$

(9b)

$$\frac{dN_n}{dt} = n_e^{Hxt} (J^G + J^F) \cdot (J^G + J^F) \cdot N_n,$$

(9c)

where $J^G$ and $J^F$ are defined as

$$J^G = \frac{V_{max} \cdot G}{K_m^G \cdot (1 + \frac{F}{K_e^F}) + G}$$

(10)
\[ J^F = \frac{V_{max}^F \cdot F}{K_m^F \cdot \left(1 + \frac{G}{K_F^c}\right) + F}. \] (11)

Similarly, the growth of producers on sucrose can be represented by a model

\[
\begin{align*}
\frac{dS}{dt} &= -J^S \cdot N_p - \text{Inv} \cdot N_p, \\
\frac{dG}{dt} &= -J^G \cdot N_p + \text{Inv} \cdot N_p, \\
\frac{dF}{dt} &= -J^F \cdot N_p + \text{Inv} \cdot N_p, \\
\frac{dN_p}{dt} &= (1 - c^{\text{Inv}}) \cdot n_c^{Hxt} (J^G + J^F) \cdot (J^G + J^F) + n_c^S \cdot J^S \cdot N_p.
\end{align*}
\] (12a, 12b, 12c, 12d)

The free parameters in the above models (9) and (12) are hexose competition functions $K_{c}^G$, $K_{c}^F$. The first one is the function of glucose concentration while the second one is a function of fructose concentration in the environment. We use Experiments 5 and 6 to estimate these functions and in view of the experimental setup we assume the following forms of the competition functions:

\[
K_{c}^G(x) = \begin{cases} 
  a_3, & 0 \leq x \leq y_1^G \\
  b_3, & y_1^G \leq x \leq y_2^G \\
  c_3, & x > y_2^G 
\end{cases}
\]

and

\[
K_{c}^F(x) = \begin{cases} 
  a_4, & 0 \leq x \leq y_1^F \\
  b_4, & y_1^F \leq x \leq y_2^F \\
  c_4, & x > y_2^F 
\end{cases}
\]

The constant $y_1^G$ ($y_1^F$) denotes the 0.2% glucose (fructose) concentration while $y_2^G$ ($y_2^F$) denotes the glucose (fructose) concentration created from degradation of 2% sucrose. Fitting the model (9) and (12) to final population data in Experiment 5a and 6a we estimate $a_3 = 0.67$ mM and $a_4 = 0.05$ mM. Fitting the model (12) to final population data in Experiment 6b we estimate $b_3 = 0.03$ and $b_4 = 0.0088$ mM. Finally fitting the model (9) to the final population data in Experiment 5b we estimate $c_3 = 0.132$ mM and $c_4 = 0.0174$ mM. In each fitting procedure we have two free parameters and one experimental data point so in our choice of the estimates we use the following criteria: glucose is used preferentially over fructose and the sum of the maximal glucose and fructose uptake rate should be such that there is a rate-efficiency trade-off of hexose transporter, in other words $x_1 < max\{J^G + J^F\} \leq x_2$, where $x_1$ and $x_2$ are defined in Figure 1.

0.7 Estimating resource movement parameter $D$
Experiment 8: Yeast cells (producers or non-producers) were initially distributed in two identical patches per 25mL agar plate, 5cm apart. The patches were initially made up of 5 µl of a liquid culture grown overnight in YFPD broth. The assays were replicated 6 times. Note, the initial and final cell densities were measured in CFU/patch which is converted into g protein/patch in table below using the assumption that 1mg protein = $33 \times 10^6$ cells.

| Carbon source | Initial cell biomass producers g protein/patch 1 | Initial cell biomass of non-producers g protein/patch 2 | Length of experiment | Final cell biomass of producers g protein/patch 1 | Final cell biomass of non-producers g protein/patch 2 |
|---------------|--------------------------------------------------|--------------------------------------------------------|----------------------|-----------------------------------------------|-----------------------------------------------|
| 0.2% sucrose  | $1.28 \times 10^{-5}$                           | $9.7 \times 10^{-6}$                                   | 4 days               | 0.0063                                        | 0.0035                                        |

Note that the distance between patches does not alter the outcome (data not shown). Now consider $m = 0$ in the model (2) of the main text which consists of two spatially segregated regions, the first one containing producers only and the second one containing non-producers only. As mentioned before the yeast cells are non-motile but the resources can move between two regions at a diffusion rate $D$. Due to the molecule size we assume that the rate of movement of sucrose is twice as slow as that of glucose or fructose. In this case the growth of producers and non-producers growing on sucrose is modeled by

\[
\begin{align*}
\frac{dS_1}{dt} &= -J^{S_1} \cdot N_{1p} - Inv \cdot N_{1p} + \frac{D}{2} \cdot (S_2 - S_1), \\
\frac{dS_2}{dt} &= -J^{S_2} \cdot N_{2n} + \frac{D}{2} \cdot (S_1 - S_2), \\
\frac{dG_1}{dt} &= -J^{G_1} \cdot N_{1p} + Inv \cdot N_{1p} + D \cdot (G_2 - G_1), \\
\frac{dG_2}{dt} &= -J^{G_2} \cdot N_{2n} + D \cdot (G_1 - G_2), \\
\frac{dF_1}{dt} &= -J^{F_1} \cdot N_{1p} + Inv \cdot N_{1p} + D \cdot (F_2 - F_1), \\
\frac{dF_2}{dt} &= -J^{F_2} \cdot N_{2n} + D \cdot (F_1 - F_2), \\
\frac{dN_{1p}}{dt} &= (1 - c^{Inv}) \cdot (n_e^{Hxt} (J^{G_1} + J^{F_1}) \cdot (J^{G_1} + J^{F_1}) + n_e^{S_1} \cdot J^{S_1}) \cdot N_{1p}, \\
\frac{dN_{2n}}{dt} &= (n_e^{Hxt} (J^{G_2} + J^{F_2}) \cdot (J^{G_2} + J^{F_2}) + n_e^{S_2} \cdot J^{S_2}) \cdot N_{2n},
\end{align*}
\]

where the subscript $i = 1, 2$ denotes patch number. All the parameters in the above model (13) are fixed apart from $D$ and fitting the model to the final population data in Experiment 8 we arrive at $D = 0.007.$
Supplementary results 1: Alternative fitness measure

Rather than using the total titre as a measure of population fitness, we use the population growth rate as defined by Hegreness et al., 2008:

$$\text{growth rate} = \frac{(N_{\text{end}} - N_{\text{start}})}{t_{\text{mid}}},$$

where $N_{\text{start}}$ denotes the initial population size and $N_{\text{end}}$ denotes the population size just after the resources have been exhausted. The parameter $t_{\text{mid}}$ is the time at which the population size reaches $\left(\frac{N_{\text{end}} - N_{\text{start}}}{2}\right)$.

1a

Expected population growth rate as a function of initial producer frequency.

1b

Expected population growth rate as a function of initial producer frequency in the absence of rate-efficiency trade-off.
1c

Expected population growth rate as a function of initial producer frequency when cost of invertase is lowered.

![Graph showing growth rate vs. initial frequency of producers for different values of m.]

1d

Expected population growth rate as a function of initial producer frequency when invertase production matches sucrose levels (perfect information).

![Graph showing growth rate vs. initial frequency of producers for different values of m.]

Legend:
- m=0.1
- m=0.2
- m=0.3
- m=0.4
- m=0.5
- m=0.6
- m=0.7
- m=0.8
Expected population growth rate as a function of initial producer frequency in well-mixed environments.
### Supplementary results 2: Comparison with Gore et al. 2009

#### 2a

| Results                                      | Gore et al. (2009) experiments | Gore et al. (2009) theory | Our experiments | Our theory                                      |
|----------------------------------------------|---------------------------------|---------------------------|-----------------|------------------------------------------------|
| Glucose capture efficiency                  | 1% included as a constant       | not measured             | function of spatial structure, and resource levels (i.e glucose, fructose, sucrose) |
| Cost of invertase production                | 2.5% included as a constant     | 4%                        | function of invertase expression (depends on glucose levels) |
| Growth assumptions                           | power law function of glucose levels | non-linear function of glucose fructose and sucrose levels (with rate-efficiency trade-off) |
| Fraction of producers at equilibrium         | 30% (flask cultures, 5% sucrose) (for parameters estimated experimentally in Gore et al. 2009) | 48% (structured agar plate, 2% sucrose) 36% (unstructured agar plate, 2% sucrose) | [42% – 55%] (low mixing - equiv. to structured agar plate) [31% – 39%] (high mixing - equiv. to unstructured agar plate) |
| Effect of increasing cooperator frequency on population size | not measured | n/a (densities not modeled) | 1. at low frequencies total population increases 2. at high frequencies total population decreases | 1. at low frequencies total population increases 2. at high frequencies total population decreases |
| Effect of increasing cooperator frequencies on growth rate | 1. at low frequencies growth rate increases 2. at high frequencies growth rate decreases | 1. at low frequencies growth rate increases 2. at high frequencies growth rate increases | not measured | 1. at low frequencies growth rate increases 2. at high frequencies growth rate decreases |
| Effects of increasing cost of cooperation   | 1. equilibrium frequency of producers decreases 2. mean growth rate decreases | 1. in agreement with experiments 2. in agreement with experiments | not measured | 1. in agreement with experiments (Suppl. result 2b) 2. in agreement with experiments (Suppl. result 2c) |
| Effects of increasing cell density           | cell grow faster at high than at low cell densities | n/a (densities not modeled) | not measured | in agreement with experiments (Suppl. result 2d) |
Relative producer fitness as a function of initial frequency in theory for the low cost of invertase production described in Figure 4c of the main text. The model predicts that for $m \in [0.5, 0.8]$, an equilibrium frequency is in the range $0.459 - 0.540$ while for $m \in [0.4, 0.1]$ the equilibrium frequency is in the range $0.569 - 0.634$ demonstrating that lowering the cost of invertase production leads to an increase in the equilibrium frequency of producers.

Expected population growth rate as a function of initial producer frequency for varying cost of invertase production. The term "high cost" denotes the experimentally estimated cost used throughout this manuscript while the term "low cost" denotes the reduction in the cost of invertase production described in Figure 4c of the main manuscript. In both cases $m = 0.8$ representing high degree of mixing.
2d

Expected population growth rate as a function of initial producer frequency for varying total initial cell densities. Low density represents $7.27 \times 10^{-6}$ g protein; intermediate density represents $1.454 \times 10^{-5}$ g protein while high density represents $2.181 \times 10^{-5}$ g protein. In all three cases $m = 0.8$ representing high degree of mixing.

Supplementary results 3: Experimental results for low sucrose

3a

Experimental data for final population size as a function of initial producer frequency, derived from the experiment E in the main text with 0.01% initial sucrose.

3b

Experimental data for final population size as a function of initial producer frequency, derived from the experiment E in the main text with 0.1% initial sucrose.
Supplementary results 4: Experimental results for homogeneous environments

Experimental data for final population size as a function of initial producer frequency, derived from the experiment F with 2% initial sucrose.

Theoretical results show that this observation is insensitive to sucrose concentration and that titre plots for 2% - 0.002% initial sucrose always approximate to a flat line.

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