MARKET FORCES DETERMINE THE DISTRIBUTION OF A LEAKY FUNCTION IN A SIMPLE
MICROBIAL COMMUNITY

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Supplemental Material

Supplemental Figure 1. Lopsided experimental evolution of a 2-species Black Queen community. 6 replicate populations each were initiated in media with lopsided sugar concentrations, either 90:10 mannose:galactose or 10:90 mannose:galactose. Gray lines track the high galactose populations and black lines track the high mannose populations. Thick lines represent the average of all 6 populations at a given time point. A) Total population density; B) proportion of total cells that are mannose-utilizers (Man); C) proportion of total cells that carry the ampicillin-resistance plasmid (AmpR); D) proportion of AmpR cells that also utilize mannose (Man+).

Supplemental Figure 2. Leakiness of ampicillin resistance demonstrated by disc diffusion assay.

A) Sterile filter paper discs were inoculated with overnight cultures of AmpR strains and then placed in the center of LB agar plates streaked with lawns of an AmpS *E. coli* strain. Growth of the AmpS strain was only possible because of β -lactamase secreted by the AmpR strain at the center of the plate. B) There was no significant difference in the radius of growth facilitated by secretions of the two *E. coli* AmpR strains tested.

Supplemental Figure 3. Protective capacity of supernatants from AmpR *E. coli* strains. Overnight cultures of AmpR varieties of Man and Gal were centrifuged and filtered to produce sterile spent-medium supernatants containing secreted β -lactamase. Serial dilutions of the supernatants were then added as supplements to cultures of AmpS *E. coli* MG1655 treated with an otherwise lethal dose of ampicillin. The growth of AmpS cultures was then assessed by either their area under the curve (AUC, panel A) or the exponential growth rate (panel B) from overnight growth curves. "Gal" or "Man" indicates the strain that produced the supernatant. The strength of the supernatant is represented as the equivalent, based on dilution factor, of the number of cells in the AmpR culture that would have been inoculated if the culture had not been filtered. Curves represent rectangular hyperbola models fit to the data by nonlinear least squares regression.

Supplemental Figure 4. Antibiotic sensitivity on agar media. Gal- and Man- were streaked as lawns on LB agar media and a Kirby-Bauer ampicillin filter paper disc was placed in the center of each plate. Bars indicate the diameter of the zone of clearance around the disc. There was no significant difference between the two strains in susceptibility to ampicillin on agar plates.

A

B

Supplemental Figure 5. Growth yields and rates for Gal and Man. A. Both species produced similar cell densities in their respective media. B. Man grew significantly faster than Gal in both DM-glu and DM-man. Error bars represent the 95% confidence interval of the estimate from a linear model; asterisks indicate significance levels from post-hoc extended marginal means comparisons. ***, $p < 0.001$.

Supplemental Figure 6. Helper Proportion in a Simple 1 Species BQ Toxin System. The helper proportion was determined in a 1 species ecosystem with a toxin. (A) shows the proportion of helpers across detoxification and essentiality parameter space. The black line shows where the helper proportion is 50%. All lines in panels B-F show the helper proportion at 50% with the modified parameter; i.e. they are identical to the black line in panel A. Except when being varied in each subplot, all other parameters remain static ($\mu = 1.0$, $\epsilon = 0.4$, $\delta = 0.15$, $c = 0.15$, $k = 10$, $r = 10$, $S_r = 5 \times 10^7$, $S_t = 1$).

Supplemental Figure 7. Helper Species Proportion Landscape. Datapoints represent the parameter selection for each species in each subplot, while the datapoint color shows the proportion of helpers that consume only mannose. Except when being varied in each subplot, all other parameters remain static ($\mu = 1.0$, $\varepsilon = 0.4$, $\phi=60$, $\lambda=1 \times 10^7$, $\delta = 0.1$, $c = 0.15$, $k=10$, $S_r = 5 \times 10^7$, $St = 1$).

Supplemental Table 1. Primers and PCR conditions used to create the pBQ1 plasmid.

Primer	Sequence	Template	T _a ¹	Extension Time ²
lacZ_fwd	ctccatacccggttttttggATGACCATGATTACGGATTTC	<i>E. coli</i> REL606 <i>lacZ</i> gene	58.6	60s
lacZ_rev	tcttgctagcTTATTTTTTGACACCAGACCAAC	<i>E. coli</i> REL606 <i>lacZ</i> gene	58.6	60s
ampR_F	aaggaagagtATGAGTATTCAACATTTCCG	pBAD24 beta-lactamase gene	56.4	60s
ampR_R	tatgagtaaaacttggtctgaCAGTTACCAATGCTTAATCAG	pBAD24 beta-lactamase gene	56.4	60s
pBAD24_fwd	TCAGACCAAGTTTACTCATATATACTTTAG	pBAD24 backbone	59.2	180s
pBAD24_rev	CCAAAAAACGGGTATGG	pBAD24 backbone	59.2	180s
pBAD_2_F	GCTAGCAGGAGGAATTCAC	pBAD24 backbone	56.3	45s
pBAD_2_R	ACTCTCCTTTTCAATATTG	pBAD24 backbone	56.3	45s

¹ Annealing temperature, in °C

² PCRs were conducted with an initial 5 minute melting step at 95° C followed by 35 cycles of 95° C for 30 seconds, T_a for 30 seconds, and 72° C for the extension time listed in this column.