

# Promoting synthetic symbiosis under environmental disturbances

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By virtue of complex interactions, the behaviour of mutualistic systems is difficult to study and nearly impossible to predict. We have developed a theoretical model of a modifiable experimental yeast system that is amenable to exploring self-organised cooperation while considering the production and use of specific metabolites. Leveraging the simplicity of an artificial yeast system, a simple model of mutualism, we develop and test the assumptions and stability of this theoretical model. We examine how one-off, recurring and permanent changes to an ecological niche affect a cooperative interaction and identify an ecological “Goldilocks zone” in which the mutualism can survive. Moreover, we explore how a factor like the cost of mutualism – the cellular burden of cooperating – influences the stability of mutualism and how environmental changes shape this stability. Our results highlight the fragility of mutualisms and suggest the use of synthetic biology to stave off an ecological collapse.

## Introduction

Life on earth comprises a hierarchy of units of selection. From societies to genes, we find the same patterns of organisation at each of these levels [1]. However, selection is not level-specific. While changes may occur at low levels, such as a single nucleotide or amino acid,

29 selection necessarily operates at a much higher level, such as the organism. Competition  
30 between entities at a specific level of organisation can spell disaster for the higher level. A  
31 clear example of this breakdown of control being cancer [1].

32 Mutualistic interactions, a specialised type of cooperation where replicating components  
33 benefit each other, therefore, can be targeted by selection at a higher level [2]. Due to  
34 conflicts of interest between entities at varying levels of selection, the origin of mutualism  
35 and subsequent selection lack a clear evolutionary explanation – a complete field of research  
36 in itself [3]. Even though it is hard to explain how mutualisms emerge, we see many examples  
37 of them. On a global scale, prominent examples such as coral-*Symbiodinium* symbioses or  
38 plant-rhizobia interactions are well known [4, 5, 6]. Many such mutualisms have evolved over  
39 millions of years, but if mutualisms are fragile and susceptible to collapse, as hypothesised,  
40 then how do they survive for aeons in constantly changing environments?

41 Mutualistic interactions can emerge in numerous ways [3]. Here we focus on how they sur-  
42 vive, because, regardless of how they originate, mutualisms constantly face different threats.  
43 A common challenge for mutualistic communities is exploitation by cheater strains that  
44 benefit from mutualistic interactions, but fail to contribute.

45 This problem of parasitic elements, first noted by Maynard Smith [7], has been extensively  
46 studied. Postulations about compensatory mechanisms that could avoid parasitic exploita-  
47 tion, range from conceptual [8], to mechanistic arguments [9]. Mutualism could also suffer  
48 from insufficient ecological support. That is, if population densities of mutualistic partners  
49 are inadequate, then mutualisms are hard to sustain. This problem highlights the necessity of  
50 a physical structure akin to a “warm little pond” for concentrating the initial mutualists [10].  
51 Without a physical structure or substrate to provide overlapping niches, it would be hard to  
52 kick-start necessary mutualistic reactions [11]. However, what happens to the stability of a  
53 mutualistic system when a threat to mutualism does not involve a parasitic element, but an  
54 unsupportive environment for the system itself? Understanding how cooperative interactions  
55 survive in changing environments that continually alter the basis of mutualistic interactions  
56 is then worthy of investigation.

57 The selective advantage derived from cooperation may be transitory in the face of environ-  
58 mental changes; therefore, in most cases the dynamics of such systems remain in constant  
59 states of flux. Interspecific interactions are essential in determining community stability [12].  
60 Depending on resource availability, interactions between mutualists can change to facultative  
61 mutualism, to competition, and even parasitism [13]. In a synthetic system, interactions are  
62 fixed; therefore, environmental influences on the system can be determined. However, eco-  
63 logical networks are inherently complex [14]. While a complete understanding of a network  
64 provides us with general properties of the interactions, it is often impossible to pin down  
65 the principles that form the building blocks. As such, it is easier to study tightly controlled  
66 systems with a defined number of interactions. Numerous systems adapted from nature allow  
67 us to study evolutionary and population dynamics.

68 Via genetic manipulation, synthetic, cross-feeding, cooperation can be engineered within  
69 microbial communities [15, 16, 17, 13, 18]. Complex population dynamics in response to  
70 temporal, spatial, and environmental factors can thus be dissected by fine-tuning and ma-  
71 nipulating these synthetic systems [19, 18]. We use *Saccharomyces cerevisiae* synthetic  
72 mutualism systems that rely on cross-feeding of amino acids between two strains [15].

73 The system uses feedback resistance (fbr) mutations in adenine and lysine biosynthesis  
74 pathways that results in overproduction of the corresponding amino acid. The strains used are  
75 referred to here as  $LYS\uparrow$  and  $ADE\uparrow$ . The dynamics of this cross-feeding system is comparable  
76 to a simple, but powerful theoretical model of self-organisation – a hypercycle – implicated at  
77 the origin of life as well as the formation of complex communities [20, 21, 11, 22, 18]. Here  
78 we develop a simple, but powerful and easily extendable phenomenological model. We then  
79 use the yeast system to validate the model and finally explore beyond the synthetic system  
80 to understand stability and cost of cooperative interactions in changing environments. While  
81 it is clear that a change in the environment affects the interaction pattern, our aim is to  
82 dissect abiotic factors to under how exactly this occurs. With this aim in mind we begin  
83 with our theoretical and experimental model.

## 84 Model & Results

85 Our model begins with the growth of two yeast strains in an environment that lacks free  
86 adenine and lysine as nutrient sources. The two strains are  $LYS\uparrow$  and  $ADE\uparrow$  whose densities  
87 are denoted by  $x_L$  and  $x_A$ . The strain  $LYS\uparrow$  is deficient in adenine (density  $c_A$ ) while it  
88 overproduces lysine (density  $c_L$ ). The production and requirement for adenine and lysine is  
89 reversed for  $ADE\uparrow$ . Modifying the logistic growth equation gives the dynamical equations for  
90 the growth of the two strains:

$$\dot{x}_L = x_L \left( r_1 \frac{c_A}{c_A + k_{c_A}} \right) \left( 1 - \frac{x_L + x_A}{K} \right) \quad (1)$$

$$\dot{x}_A = x_A \left( r_2 \frac{c_L}{c_L + k_{c_L}} \right) \left( 1 - \frac{x_L + x_A}{K} \right) \quad (2)$$

91 The two strains grow if the required metabolites are present. The strains compete for a  
92 limited amount of space, given by  $K$ . Amino acid concentrations, together with Monod-type  
93 saturation kinetics, control the growth of the strains. Our model is mechanistic regarding  
94 individual amino acid dynamics. Explicitly including metabolite concentrations is crucial, as  
95 pairwise Lotka-Volterra models may not always provide a realistic qualitative picture of the  
96 dynamics [14]. Amino acids are a consumable resource. As they are produced constitutively  
97 by one of the strains, the other strain uses them immediately. The dynamics of metabolite  
98 densities in the culture hence can be captured by:

$$\dot{c}_A = \beta_1 x_A - \frac{\gamma_1 c_A}{c_A + k_{c_A}} x_L \quad (3)$$

$$\dot{c}_L = \beta_2 x_L - \frac{\gamma_2 c_L}{c_L + k_{c_L}} x_A \quad (4)$$

99 The rate at which each metabolite  $i$  can increase in the yeast culture is given by  $\beta_i$ . This  
100 rate determines the amount of metabolite shared by the corresponding overproducing strain.  
101 We assume that use of a metabolite at rate  $\gamma_i$  by strains  $x_i$  also involves formation of  
102 an intermediate; thus being subject to Michaelis-Menten kinetic parameters. The simple  
103 dynamics of such a system are depicted in Fig. 1.

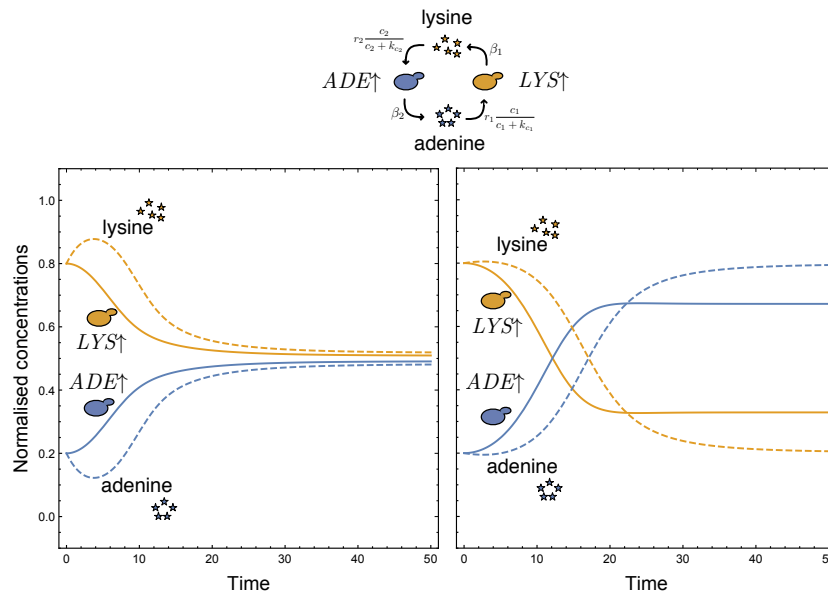


Figure 1: **Unsplemented dynamics (left) the neutral expectation and (right) the expectation informed by experimental growth rates.** We plot the fraction of  $LYS\uparrow$  ( $x_L/(x_L + x_A)$ ),  $ADE\uparrow$  ( $x_A/(x_L + x_A)$ ) and the relative fractions of metabolites adenine ( $c_A/(c_A + c_L)$ ) and lysine ( $c_L/(c_A + c_L)$ ) in a continuous culture. As an initial condition there is no free adenine or lysine in the culture, but nonzero populations of the strains generate the free adenine and lysine in the same relative frequencies. The relative initial fractions for the strains are  $LYS\uparrow = 0.8$  and  $ADE\uparrow = 0.2$  and the rate at which the two strains share the two metabolites is set to  $\beta_1 = \beta_2 = 0.04$ . We set the carrying capacity of the culture vessel to  $K = 5$ . **Left:** The uptake rate of the metabolites as well as the degradation rate are exactly the same ( $r_1 = r_2 = 5$  and  $\gamma_1 = \gamma_2 = 2$ ). Michaelis constant for both metabolites to  $k_{c_A} = k_{c_L} = 1$ . Under these symmetric conditions, normalised densities of the strains, as well as that when the two metabolites reach an equilibrium where normalised concentrations are the same. **Right:** Clearly the assumption of symmetric rates is a simplification. Informed by experiments, we estimate  $r_1 = 11.4906$ ,  $r_2 = 29.3955$  and the Michaelis constants  $k_{c_A} = 26.4326$ ,  $k_{c_L} = 29.0254$ . This asymmetry in uptake rates is reflected in the resulting unequal equilibrium.

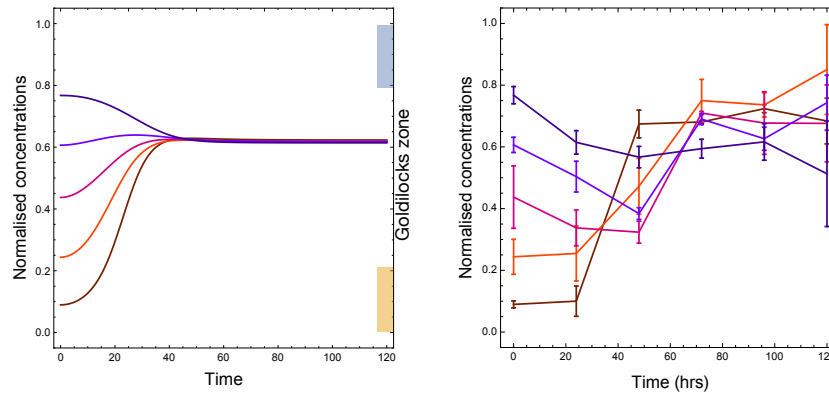


Figure 2: **Theoretical and experimental model dynamics of with different initial  $ADE\uparrow$  concentrations.** Using the theoretical model, in the left panel we predict the dynamics of the normalised fraction of  $ADE\uparrow$  starting at various initial ratios with the parameters set informed by data. The initial concentrations of the amino acids  $c_A$  and  $c_L$  are set to 0.0. Using the experimental model, in the right panel we plot the the normalised concentration of the yeast strain  $ADE\uparrow$  relative to the  $LYS\uparrow$ . Starting at different initial ratios we track dynamics over a 140-hour period in synthetic complete media (without adenine or lysine). Each point is the mean of three replicates and error bars represent a single standard deviation.

104 Adding the required supplement, we calculate the growth rate of the two strains for  
105 different levels of supplementation. The resulting growth rate curves are used to parameterise  
106 the model. The growth parameters are  $r_1 = 11.4906$  and  $r_2 = 29.3955$  and the Michaelis  
107 constants  $k_{c_A} = 26.4326$  and  $k_{c_L} = 29.0254$ . We assume the Michaelis constant estimated  
108 for the uptake rate for adenine and lysine to be the same as the rate at which they degrade  
109 from the pool. Starting at different initial conditions of the  $ADE\uparrow$  to  $LYS\uparrow$  ratio, the final  
110 equilibrium values are close to 0.6, corroborated by experiments Fig. 2.

### 111 **Eco-evolutionary dynamics under environmental disturbance**

112 The mutualistic system rests on the interdependence of the two strains of yeast. If we  
113 undermine this dependency, it will affect the stability of the mutualistic interaction. Typically  
114 the system can be undermined by the introduction of a parasite [18]. We choose to change  
115 the environment itself without altering the genotypes of the interacting strains.

116 If the environment has one of the two required metabolites, then the strain using that  
117 metabolite will not depend on the corresponding strain for survival. A significant amount  
118 of supplementation can drown out the signal of the other strain. In all, we visualise three  
119 different supplementation scenarios that could be used to test the resilience of this mutualistic  
120 system. Specifically, initial supplementation – adenine or lysine added at the beginning  
121 of culturing, continuous supplementation – the two metabolites added steadily throughout  
122 culturing, and intermittent supplementation – the metabolites added at regular time intervals.

123 Depending on the timescale, a single niche can experience all three scenarios, but we analyse  
124 the three scenarios in succession.

### 125 **Initial supplementation.**

126 The initial supplementation regime is a standard batch culture. With nutrients provided  
127 at the start, the culture continues to grow until one or more nutrients become limiting.  
128 We supplemented our experimental cultures with either 0.1, 1, or 10  $\mu\text{g}/\text{mL}$  adenine and  
129 measured the effect on strain ratio at regular time intervals (Materials & Methods, Fig. 3  
130 (top)). The supplementation favoured the *LYS* $\uparrow$  strain, resulting in a lower prevalence of  
131 *ADE* $\uparrow$  when compared to the unsupplemented condition. The mean strain ratio of *ADE* $\uparrow$   
132 to *LYS* $\uparrow$  after 120 hours growth of all starting ratios for 0.1, 1, or 10  $\mu\text{g mL}^{-1}$  adenine  
133 supplementation is 0.5316, 0.5685, 0.0556, respectively.

134 Using the parameterised theoretical model, we explore the final equilibrium value of *ADE* $\uparrow$   
135 as we change the amount of initially supplied metabolites. The model qualitatively mim-  
136 ics the change in the equilibrium condition with the addition of adenine Fig. 3 (bottom).  
137 Experimentally we have tested the addition of only adenine for different initial fractions of  
138 *ADE* $\uparrow$  but theoretically, we explore the consequence of adding both adenine and lysine for  
139 the *ADE* $\uparrow$  starting at 0.5. The results, summarised in Fig. 4 (left panel), reveal that even a  
140 slight asymmetric increase in the amount of initial metabolite present in the environment is  
141 enough to destabilise the equilibrium and push it close to extinction. When the equilibrium  
142 values come close to one of the two edges of the system, the rarer mutualism runs the risk  
143 of going extinct by drift. Close to the edges (0 or 1), stochastic dynamics could lead to  
144 the extinction of either strain. If the equilibrium value of either mutualist falls below 0.2,  
145 we assume that the mutualist system is at high risk of collapse. Thus we define the region  
146 between 0.2 and 0.8 as the “Goldilocks zone” where mutualism can safely exist. The asym-  
147 metry of the graph in Fig. 4 reflects the inherently different uptake rates of metabolites by  
148 the two strains Fig. 2.

### 149 **Intermittent supplementation.**

150 As the initially added supplementation is consumed, it is possible to add metabolites at fixed  
151 intervals. The model is now an example of a hybrid dynamic system [23] where concentrations  
152 of the metabolites are adjusted at regular intervals (see Materials & Methods).

153 Intermittent supplementation acts as a bridge between the other two supplementation  
154 regimes. If the delay between two supplementations is substantial then starting at time  
155  $t = 0$ , the scenario is the same as that of initial supplementation (Fig. 4 middle panel would  
156 resemble the left panel). If the delay between successive supplementations is minimal, then  
157 the concept is similar to continuous supplementation (Fig. 4 middle panel would resemble  
158 the right panel). For a legitimate comparison with initial and continuous supplementation,  
159 we started the intermittent supplementation with a non-zero level of amino acids which was  
160 added at regular intervals.

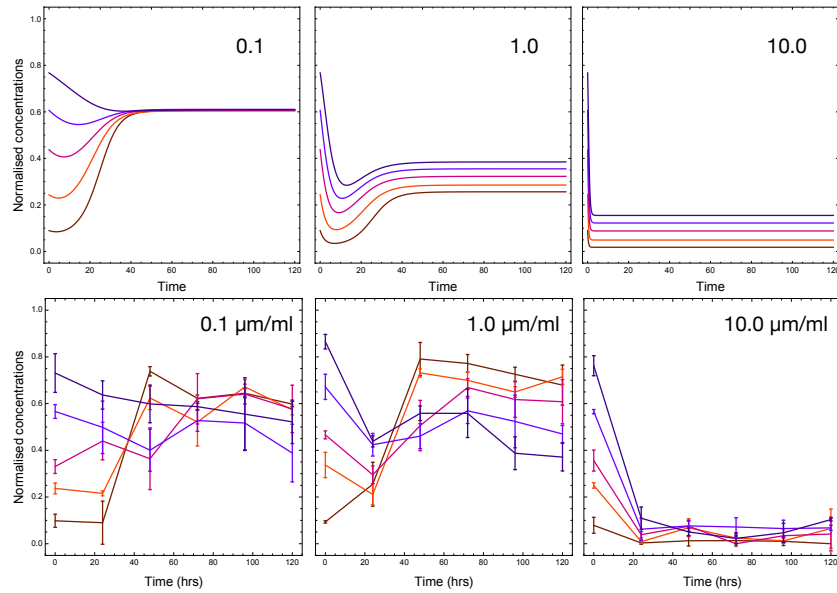


Figure 3: **Theoretical and experimental model dynamics with different initial supplementation levels of adenine.** We explore the theoretical model as depicted in Fig. 2, now with added initial supplementation. The initial concentrations of the metabolites  $c_A$  and  $c_L$  are set to 0.1, 1.0, and 10 as denoted in the panels. Using the modified version of In the bottom row we show the normalised concentration of the yeast strain  $ADE^\uparrow$  relative to the  $LYS^\uparrow$  strain, at varying ratios over a 120-hour period in synthetic complete media supplemented with adenine to a final concentration of either 0.1, 1.0, or 10 µg/mL. Each point is the mean of three replicates and the error bars represent a single standard deviation. The theoretical model qualitatively predicts experimental model dynamics of mutualism. With increasing supplementation, the dependence of  $LYS^\uparrow$  on  $ade^{f^{br}}lys^-$  is reduced and the system exits the Goldilocks zone.

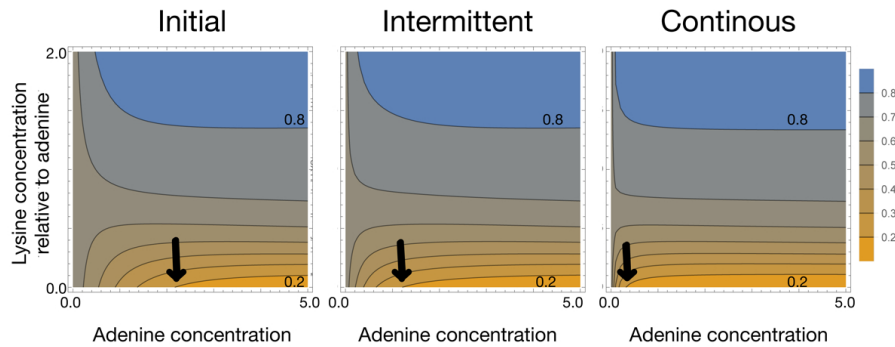


Figure 4: **Equilibrium value of  $ADE\uparrow$  for different supplementation regimes.** Initial normalised concentration of  $ADE\uparrow = 0.5$ . The system is assumed to have equilibrated by 300 time steps. In the plots we show the eventual normalised concentration of  $ADE\uparrow$  going from all  $ADE\uparrow$  (1, blue) to all  $LYS\uparrow$  (0, yellow). The initial concentration of adenine in the system at time 0 is shown on the  $x$ -axis and the amount of lysing relative to adenine is depicted on the  $y$ -axis. For  $y = 1$  the amount of adenine and lysine is the same. The initial supplementation: amino acids provided only at time point  $t = 0$ , intermittent supplementation: starting at  $t = 0$  and then every 5<sup>th</sup> time step and for continuous: at every time step. Going from Initial to Continuous supplementation we see that the Goldilocks zone (region between the contours 0.2 and 0.8) shrinks as the zone for  $LYS\uparrow$  (yellow) increases, and so does the  $ADE\uparrow$  (blue) region.



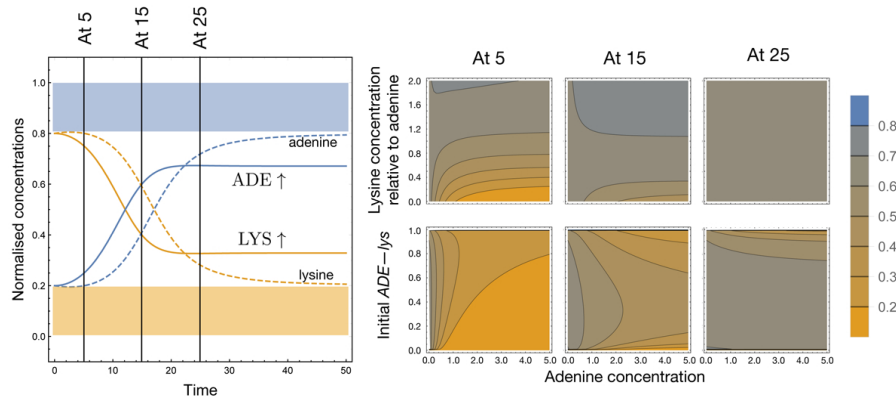


Figure 5: **Disrupting transient mutualism with intermittent supplementation.** Intermittent supplementation (Figure 4) started with the same initial supplementation as the initial supplementation regime, so as to be comparable. If we start with no metabolites in the culture and add them at fixed intervals, then it might be possible to extend the range of the Goldilocks zone, even under supplementation. The exact timing of supplementation provided is crucial in determining the eventual equilibrium. If the pattern of supplementation starts early in the existence of  $ADE\uparrow$  then the resulting equilibrium frequency can be drastically affected. By tuning the timing and dose of supplementation, we can maximise the probability of maintaining the system within the Goldilocks zone (as we move from supplementing every 5 to 15 to 25 time step). If intermittent supplementation starts in the latter phase of the transient where the equilibrium value is already reached then the co-existence seems robust. This will also depend on the initial concentration of  $ADE\uparrow$ . Thus, while the top row is calculated from an initial normalised concentration of  $ADE\uparrow = 0.2$ , the bottom row explores all initial conditions. Equilibrium values are all calculated at the same time point, i.e., the number of cycles are adjusted so that all integrations run until time point  $cycle\ length \times number\ of\ cycles = 300$ .

## 161 Continuous supplementation.

162 Intermittent supplementation, if done at minimal time intervals, represents a continuous  
 163 supply of amino acids. Continuous supplementation competes with the biosynthetic output  
 164 of the strains themselves, drowning out their signal and undermining the basis of cooperation  
 165 between the two strains.

166 As before, we plot equilibrium values of  $ADE\uparrow$  for different amounts of continuously added  
 167 adenine and lysine (relative to adenine concentration) (Fig. 4 right panel). Slight, but  
 168 continuous addition of lysine immediately breaks the mutualism as the  $ADE\uparrow$  strain takes  
 169 over the mixed culture. The Goldilocks zone breaks down almost immediately for continuous  
 170 supplementation as compared to initial supplementation.

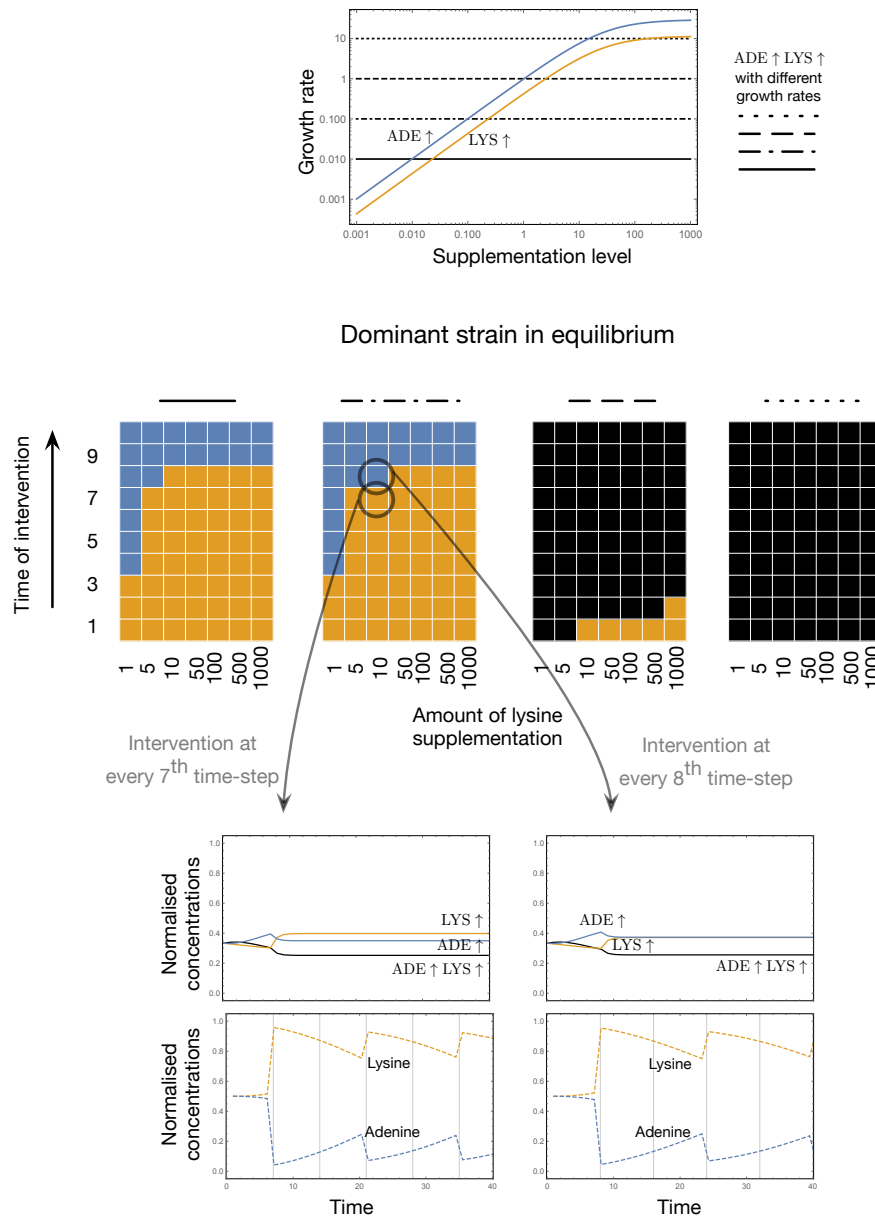
## 171 Intervention measures in the presence of ultimate overproducers

172 A typical threat to a mutualistic system is the evolution of a cheater, which parasitizes the  
173 produced common good [24]. A cheating strain can thrive because it benefits from public  
174 goods without having to pay the cost of contributing. But does mutualism always have to  
175 be extremely costly? If production of the common good entails little cost, then we could  
176 envision mutants that do not require mutualistic interactions, but that contribute to the pool  
177 of common goods, like fictitious  $ADE\uparrow LYS\uparrow$  strains. While not participating in mutualism  
178 might be lethal, taking part in a mutualistic interaction does not need to be costly if it  
179 involves overproduction of a single metabolite. Although specific, yeast can overproduce a  
180 fluorescent protein and only suffer a 1% reduction in cost per copy [25]. Moreover, reliance  
181 on external sources for essential metabolites can have a considerable cost as well. Several  
182 auxotrophic yeast strains, such as those unable to produce their own lysine or adenine, have  
183 up to a 10% reduction in growth, even with environmental supplementation [26, 27].

184 Typically if a strain overproduces a compound, a cost is associated with it. However,  
185 there is potentially also a cost associated with relying on interactions with other organisms.  
186 Our mathematical model is also extensible to include a fictitious strain  $ADE\uparrow LYS\uparrow$  that  
187 overproduces both amino acids and does not require any supplementation. We assume that  
188 any cellular cost incurred will not matter unless it affects the growth rate. In the simplest  
189 case, the growth rate would be largely independent of the environment since the strain can  
190 satisfy its requirements.

191 Freeing itself from any environmental dependence on metabolites, our ultimate overpro-  
192 ducer is defined as having a variable flat growth rate. Compared to the growth rates ex-  
193 trapolated from the experimental data we can envision a number of different scenarios as in  
194 Figure 6 (top). The variable growth rates chosen for the overproducing strain reflect a range  
195 of values within those derived from the other strain experimental data. A clear prediction for  
196 which of the three strains is dominant is derived from our updated model and closely reflects  
197 the underlying growth rates. Even with a comparatively high cost, the  $ADE\uparrow LYS\uparrow$  strain can  
198 quickly become the dominant strain, despite supporting both the  $ADE\uparrow$  and  $LYS\uparrow$  strains. If  
199 we are interested in ensuring mutualism, then we must intervene, but the question is when  
200 and to what degree?

201 The theoretical model shows that we can supplement the culture with one of the metabo-  
202 lites. Since the  $ADE\uparrow$  strain has the higher growth rate, we support it by providing lysine in  
203 the culture (environment). As the amount of supplementation is reduced, it must be provided  
204 earlier so as to maintain  $ADE\uparrow$  as the dominant strain. The later intervention via supple-  
205 mentation occurs, the more likely that predictions of the growth rate for unsupplemented  
206 populations hold true. The same holds for scenarios in which the ultimate overproducer  
207 dominates. We need to provide a high amount of supplement early to offset the fitness  
208 benefit of the ultimate overproducer Fig. 6 (dominant strain panel 3). If supplementation  
209 occurs after the carrying capacity has already been reached, then it has no effect.



**Figure 6: Ultimate overproducers and equilibrium dominance.** The top panel displays experimental growth data for the  $ADE\uparrow$  and  $LYS\uparrow$  strains. A range of constant growth rates for the  $ADE\uparrow LYS\uparrow$  strain that fall inside of the aforementioned experimental rates were selected, shown with the varying black lines. Given these growth rates we can estimate the equilibrium of the system. The colour denotes the dominant strain at equilibrium in each simulation, with yellow representing  $LYS\uparrow$  and blue  $ADE\uparrow$ . For increasing growth rates of the ultimate overproducer, we see that more lysine is required to maintain one of the mutualists as the dominant strain. Furthermore, the exact time of intervention  $k$  (and then every  $k^{\text{th}}$  step) matters. The low fitness strain  $ADE\uparrow LYS\uparrow$  never becomes dominant, but relative dominance of the two mutualists can change (left two panels and the explicit dynamics shown in the insets). For reduced supplementation, the intervention must occur earlier if there is to be a change in dominance. The same is true for higher fitness of  $ADE\uparrow LYS\uparrow$  although in this case, intervention times would have to occur extremely early on.

## 210 Discussion & Conclusion

211 Cooperation is instrumental at all levels of life and on all timescales. On a scale of ecosys-  
212 tems, the biosphere itself can be viewed as interacting networks of varying components.  
213 Although important, the behaviour of these complex interactions is difficult to study and  
214 nearly impossible to predict. Even simple interactions between a small number of coopera-  
215 tors form complex networks, but when these networks are placed in fluctuating environments  
216 their complexity surges. Synthetic biological systems apply engineering principles to an or-  
217 ganism to promote precise control and predictability over natural behaviour. To this end, we  
218 leverage the simplicity of the engineered yeast system and the corresponding mathematical  
219 model. Our work examines both the effects of how initial strain ratios and changes in the  
220 environment alter the dynamics of strain concentrations and influence mutualism stability.  
221 Under stable environmental conditions, as previously shown, the initial strain ratio of the  
222 system is very stable[15]. Stability under changing environmental conditions is an entirely  
223 different matter. Environmental changes have the potential to influence or disrupt mutual-  
224 istic interactions [13]. Here we dissect these influences further with the aim of discerning  
225 how mutualistic interactions may be maintained. Beyond interactions between biotic com-  
226 ponents, we have integrated abiotic factors, such as the amount and timing of environmental  
227 change in our system.

228 The final equilibrium of the mutualistic system depends more on environmental supplementa-  
229 tion shocks than on the starting ratio of the mutualist partners. As initial supplementation  
230 of adenine increases, favouring the *LYS*<sup>↑</sup> strain, the final ratio shifts in both the experi-  
231 mental system and mathematical model (Fig. 3). Confident in the exploratory power of  
232 our mathematical model, we determined the environmental conditions that promote mutu-  
233 alism and provide what we refer to as the Goldilocks zone. The modeled supplementation  
234 regimes are common in microbial culturing, but they also have clear ecological parallels.  
235 The non-supplemented culture represents a niche baseline. Initial supplementation is akin to  
236 an isolated event like the sinking of a whale carcass to the floor of the ocean, resulting in  
237 extreme point-source enrichment [28]. Intermittent supplementation represents seasonal or  
238 periodic change, such as temperature fluctuations or the regular introduction of a nutrient  
239 to the gut of a host animal. Understanding the effect of fluctuating resource availability  
240 is extremely important regarding invasiveness [29]. A temporary reduction in competition  
241 due to nutrient excess can make mutualisms vulnerable to invasion [30]. Finally, continuous  
242 supplementation is a permanent change to an environment, as in a permanent temperature  
243 change or evaporation of a large body of water. In general, we identified a gradually shrinking  
244 Goldilocks zone as one progresses from initial, to intermittent and then to continuous sup-  
245 plementation regimes (Fig. 4). With environmental destabilisation becoming more and more  
246 concrete, the edges of the mutualistic system encroach upon even minimal supplementation  
247 levels. Stochastic effects may dominate these regimes in which mutualism can then collapse  
248 due to chance events. Tracking the size of the zone is thus a good measure of the resilience  
249 of a mutualistic system under changing environments.

250 The mode of intermittent supplementation and in particular, its timing, offers some respite.  
251 These yeast strains have asymmetrical starvation tolerance and do not release their overpro-  
252 duced metabolite until near death [15]. Intermittent supplementation could thus represent

253 death-induced periodic release of nutrients. It is important to note that the frequency of  
254 disturbance in complex communities can severely affect assemblages of microbes [31]. We  
255 explored this by starting intermittent supplementation at different times and by varying sup-  
256 plementation frequency. SI. highlights the importance of supplementation parameters as well  
257 as the starting ratio of the two strains. We observe that when environmental changes are  
258 minute, the initial ratios matter somewhat, but if the change in the environment is drastic,  
259 then the timing of the change is crucial.

260 We include the analysis of a hypothetical ultimate overproducer  $ADE\uparrow LYS\uparrow$  that allows use  
261 to explore both the cost of mutualism generally and to tease apart the influence of the timing  
262 and magnitude of environmental disturbances. Although even modest costs can be rapidly  
263 selected against, it is important to recognise that mutualism need not be expensive from  
264 a cellular point of view. As such, contributions to the common good may persist as what  
265 could be described as genetic anomalies. These individuals are not cheaters, as they support  
266 the existing mutualistic interaction, but could still quickly become dominant members of an  
267 ecosystem if the cost of contribution is lower than the benefits gained from not relying on  
268 other members of the ecosystem (Fig. 6). Moreover, even for a high cost the  $ADE\uparrow LYS\uparrow$   
269 strain can persist in a stable equilibrium with the other strains, in part because they are not  
270 dependent upon cycles in abiotic factors.

271 The disruptive influence of this overproducing strain can be mitigated. Intervention both  
272 early and with considerable supplementation permits continuation of the mutualistic inter-  
273 action between  $ADE\uparrow$  and  $LYS\uparrow$ . In principle, targeted intervention in collapsing ecological  
274 niches that depend on mutualism could save these relationships, or at least forestall their  
275 collapse. Further experimental tests along these lines will provide insight into the role of  
276 abiotic components in the resilience of mutualistic systems.

277 Understanding environmental enrichment (or degradation) is imperative as we face cli-  
278 mate change. The promise of bioengineering together with cooperation is enormous [32].  
279 Beside offering insight into the nature of interactions resulting in community stability [33],  
280 engineered systems have implications for designing complete ecosystems that affect the bio-  
281 sphere in general [34]. With continuing eutrophication of oceans, essential symbiosis may  
282 break down into parasitism [6]. Use of pesticides and synthetic nitrogenous fertilisers can  
283 disrupt the natural nitrogen fixation process – mutualism between leguminous plants and  
284 rhizobacteria [4]. Thus, enriched environments could pose a threat to long evolved mutual-  
285 ism which might lead to further catastrophic events [35]. Since mutualisms bind organisms  
286 together to enhance their survival, they come at the cost of binding the fates of all in-  
287 volved species together [36]. Changing the environment, inadvertently, or purposefully via  
288 anthropogenic activity and climate change can be costly not just to one set of species, but  
289 to the ecosystem at large. Beyond conservation biology, mutualistic interactions also lie at  
290 the heart of translational biology, affecting applied human health and biotechnology. For  
291 example, normal functioning of the human microbiome is decisive for our well-being, and  
292 various pathologies emerge from disruption of that microbiome [37].

293 Microbes often occur as consortia rather than as individual species. Communities of  
294 bacteria are usually the pioneers in harsh environments, from newly formed volcanic rock  
295 to hyper-arid deserts [38, 39, 40]. Some members of the consortia survive by feeding off  
296 inorganic matter, but most of the community survives in a cross-feeding network [41]. In such

327 cases, it would be useful to know how the communities function, and especially their fates  
328 when the environment has changed *due to their presence*. While the diversity of individuals  
329 in a consortium does not always guarantee robustness, engineered microbial consortia might  
330 prove to be more robust in harsh environments [32]. Learning from natural, well-established  
331 communities, we can better design interactions between microbes, making them resilient  
332 against environmental disturbances, and thus useful tools in biotechnology. When developing  
333 techniques to tackle problems such as wastewater treatment, biofuel generation, or oil spill  
334 cleanups, symbiotic communities of microorganisms potentially offer a more efficient pathway  
335 to the breakdown of complex substrates [42].

336 Understanding mutualism can therefore help address questions about the origins, spread,  
337 and diversification of life in inhospitable environments. Synthetic microbiology will help us  
338 take these mutualisms to the next level of rich environments. Competition between multi-  
339 ple, synthetically constructed, cross-feeding systems with different levels of interdependence  
340 would help extract information about successful ensembles of interactions eventually leading  
341 us to the evolution of successful hypercycles – as envisioned by Eigen and Schuster[20]. Our  
342 next steps in synthetic biology will be in this direction.

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## 416 **Author contributions**

417 J.A.D. and C.S.G. conceived the project, analysed the results and wrote the paper. C.S.G.  
418 developed the model. J.A.D. performed the yeast experiments.

## 419 **Competing interests**

420 The authors declare no competing interests.

## 421 **SUPPORTING INFORMATION**

### 422 **Methods and Materials**

#### 423 **Saccharomyces cerevisiae Strains**

424 The synthetic system is composed of two metabolite-overproducing strains previously devel-  
425 oped in the w303 background, provided by Professor W. Shou [15]. The first, WS950, is  
426 an adenine-overproducing strain denominated *ADE*↑ throughout this manuscript. It has the  
427 genotype MATa ste3::kanMX4 lys2Δ0 ade4::ADE4(PUR6) ADHp-DsRed.T4. The second,  
428 WS954, is a lysine-overproducing strain called *LYS*↑ here. This strain has the genotype  
429 MATa ste3::kanMX4 ade8Δ0 lys21::LYS21(fbr) ADHp-venus-YFP.

## 430 **Culturing & Plate Counts**

431 Synthetic complete (SC) media was made from dextrose, FORMEDIUM yeast nitrogen base  
432 and an appropriate synthetic complete dropout supplement (Kaiser). Amino acid free min-  
433 imal media media (SC-aa) was made as above without synthetic complete dropout supple-  
434 ment. Yeast-extract, peptone, dextrose (YPD) media was made using chemicals from BD  
435 Diagnostics or Sigma. All cultures were grown at 30 °C in an orbital shaker at 200RPM.

436 Experimental cultures were generated by selecting individual colonies from a streak plate  
437 and growing each colony in 5 mL SC medium for 48 hours. These cultures were pelleted,  
438 washed twice with SC-aa and resuspended in 5 mL of SC-aa. They were then grown for an  
439 additional 24 hours. These cultures were pelleted, washed and resuspended as above and  
440 then were diluted 1 in 20 with SC-aa. Each experiment was performed in triplicate starting  
441 from a single individual colony. Single-strain growth tests were performed using 5 mL aliquots  
442 of the 1 in 20 dilution and supplementing to a final concentration with either 0, 0.1, 1, 10,  
443 or 100 µg/mL of adenine for the *LYS*<sup>†</sup> strain or lysine for the *ADE*<sup>†</sup> strain. The cultures  
444 were sampled at 0 and 24 hours. Experimental cultures were generated by mixing individual  
445 strain dilutions at volume ratios indicated in each experiment to a final volume of 5 mL. The  
446 ,0,0.1, 1, 10 or 100 µg/mL adenine-supplemented cultures peaked in the number of colony  
447 forming units, suggesting growth saturation, at 144, 120, 72, and 48 hours respectively. In  
448 each case, an equilibrium was reached before saturation. We determined colony forming  
449 units via plate counts on YPD solid media of culture dilutions at time points indicated.  
450 Strain ratios were determined by replicate plating these YPD colonies onto solid media that  
451 permitted growth of only one strain, either SC without lysine or SC without adenine, and  
452 counting the respective colonies.

453 **Estimating growth parameters.** We now parametrize the Michaelis constants ( $k_{c_A}$  and  
454  $k_{c_L}$ ) and the growth parameters ( $r_1$  and  $r_2$ ) using experimental data. Single-strain growth  
455 rates for *ADE*<sup>†</sup> and *LYS*<sup>†</sup> were determined on growth media supplemented with varying con-  
456 centrations of the appropriate required metabolite. The two strains were grown in synthetic  
457 complete media either unsupplemented or supplemented with 0.1, 1, 10, or 100µg/mL of the  
458 corresponding nutrient. Growth after 24 hours was calculated as above. The growth curves  
459 were used to parameterise model growth components.

## 460 **Data Analysis**

461 Colony counts were collated and summary statistics generated using R [43]. A RMarkdown  
462 file containing raw data, summary statistics, all code and preliminary plots is available on  
463 GitHub at <https://github.com/tecoevo/syntheticmutualism> and will be included as a  
464 supplementary file in the final submission. All figures were generated using Mathematica[44]  
465 for consistency.

## 466 **Model extension for supplementation regimes**

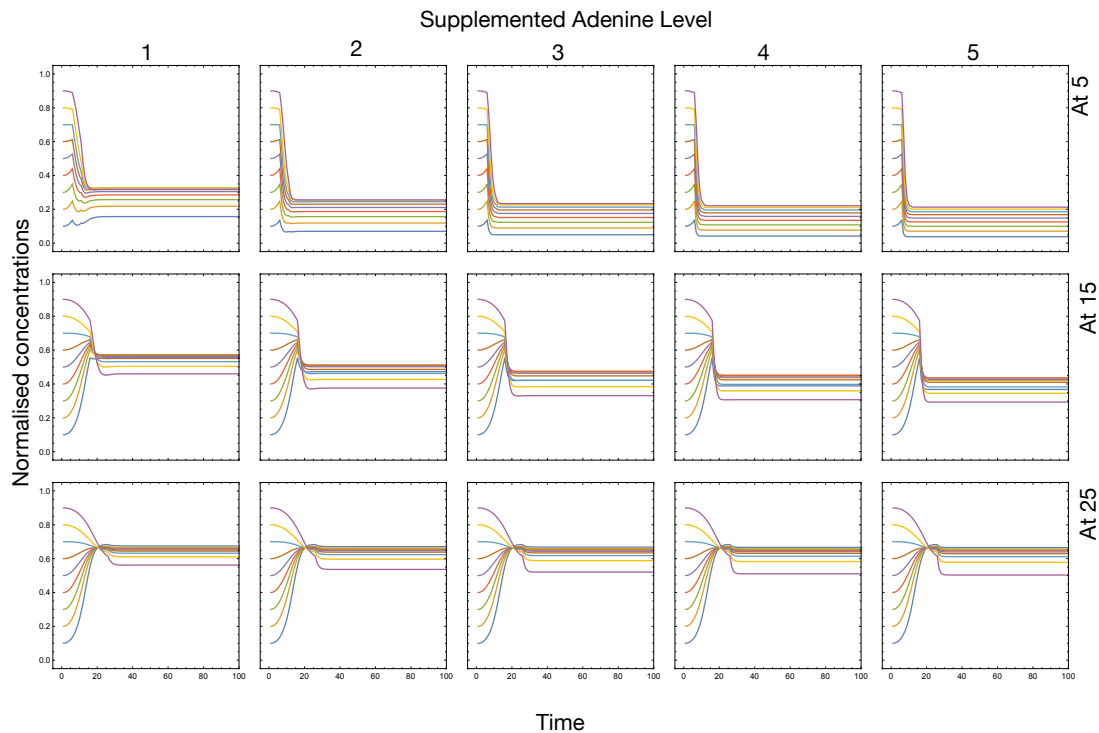
467 For initial supplementation we do not need to modify the model from what its form in Eqs. (2)  
468 and (4). For different values of initial supplementation, we change the initial conditions for

469 the amounts of nutrients. For *continuous supplementation* we modify the Eqs. (4) to,

$$\dot{c}_A = c_{Acont} + \beta_1 x_A - \frac{\gamma_1 c_A}{c_A + k_{c_A}} x_L \quad (5)$$

$$\dot{c}_L = c_{Lcont} + \beta_2 x_L - \frac{\gamma_2 c_L}{c_L + k_{c_L}} x_A \quad (6)$$

470 where the values of  $c_{Acont}$  and  $c_{Lcont}$  determine the amount of nutrient continuously added  
 471 to the culture. For *intermittent supplementation* we make use of a hybrid dynamic system  
 472 [23]. We start with the same initial conditions as for other supplementation regimes, where  
 473 the initial condition of the nutrient matches the amount used for supplementation. Dynamics  
 474 proceed as per equations in the main text. In total, they run for the same amount of time  
 475 as the initial and continuous supplementation experiments before assuming equilibrium, but  
 476 the time is split into many small cycles. Each cycle runs for a short period. Thus we have  
 477  $cycle\ length \times number\ of\ cycles = total\ time\ until\ equilibrium$ . At the end of each cycle,  
 478 we add the predetermined amount of essential metabolites and then allow the next cycle to  
 479 continue. In the absence of external supplementation, the system requires a certain time  
 480 to equilibrate. If supplementation continues to disrupt this process by disturbing transient  
 481 dynamics, then the eventual outcome when evaluated at the same time point as in other  
 482 supplementation regimes can be drastically affected (Fig. 5 and Appendix Fig. 7).



483

Figure 7: As shown in Fig. 5, the frequency and timing of intermittent supplementation changes the equilibrium of the system, but the initial conditions matter as well. In this figure we highlight this dependence. Starting the supplementation regime at time point 5 and then supplementing at every 5<sup>th</sup> time point we get the first row for different levels of supplementation. For the second and third row, the first dose (and subsequent) of supplementation occurs at 15 and 25 time-points. For high supplementation, the number of cycles are immaterial. A single dose of supplementation is enough to shift the equilibrium; however, the exact timing of this dose is crucial. The time at which the first disturbance occurs affects the role of initial conditions. If supplementation begins early (at 5), then the order of initial conditions is not affected as opposed to cases in which supplementation is started later (at 15 and at 25).