Environmental disturbances compromise synthetic symbiosis

By virtue of complex interactions, the behaviour of mutualistic systems is difficult to study and nearly impossible to predict. We have developed an easily amendable theoretical model of an equally modifiable experimental yeast system to explore 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, reoccurring 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 such mutualisms and suggest the use of synthetic biology to stave off an ecological collapse.

Introduction

All life on earth is made up of smaller units of selection. From societies to genes, we find the same patterns inset within each of these levels [1]. However, selection is not level specific. A change at a lower level, like a single amino acid, can be selected for at a much higher level, like a whole organism. Competition between entities at a specific level of organisation can spell disaster for the higher level, a clear example of this breakdown of control being cancer [1]. A mutualistic interaction, a specialised type of cooperation where replicating components benefit each other, therefore, can be the target of selection at a higher level [2]. Due to the conflict of interest between entities at varying levels of selection, the origin of mutualism and subsequent selection lacks clear evolutionary explanations – a complete field of research in itself [3]. Even though it is hard to explain how mutualism emerges, we see them all around. On a global scale, prominent examples such as the coral-fungi symbiosis or plant-rhizobia mutualism stand out [4, 5, 6]. Many such mutualisms have evolved over millions of years and now form an intricate part of the biosphere. If mutualisms are fragile and susceptible to collapse, as hypothesised, then how do they survive over aeons in constantly changing environments?

There can be numerous ways that mutualistic interactions initially emerged [3]. We focus on how they survive, because, however they originate, mutualisms are constantly under different threats. A commonly challenge for a mutualistic community is the exploitation by a cheater strain that derives benefit from the mutualistic interaction but fails to contribute. This problem of parasitic like elements, since first pointed out by Maynard Smith [7] has been extensively studied. Postulation of compensatory mechanisms which could avoid the parasitic exploitation range from conceptual [8], to mechanistic arguments [9]. Mutualism could also suffer from insufficient ecological support: if the population densities of the mutualistic partners are not adequate, then the mutualism is hard to sustain. This problem highlights the necessity of a physical structure akin to a "warm little pond" for concentrating the initial mutualists together [10]. Without a physical structure, such as a vesicle or a membrane to hold the mutualists in or a physical substrate providing a common ecological niche, it would be hard for the population to aggregate to kick-start the necessary mutualistic reactions [11]. However, what happens to the stability of a mutualistic system when the threat to mutualism is not via a parasitic element or an unsupportive ecology but the environment of the system itself? Understanding how cooperative interactions can survive in a changing environment that continually challenges the mutualistic interaction is then imperative.

The selective advantage derived from cooperation may be fleeting if the environment changes and therefore in most cases the dynamics of a system are in a constant state of flux. Interactions between individual species are then essential in determining community stability [12]. Depending on the resource availability, the (apparent) interactions between mutualists can change to facultative mutualism, competition and even parasitism [13]. In a synthetic system, the interactions are set and therefore environmental influences on the system can be determined. However, these ecological networks are inherently complex [14]. While a complete understanding of the network provides us with general properties of the interactions, it is often impossible to pin down the principles which form the building blocks. As such, it is easier to study tightly controlled systems with a defined nature and number of interactions. Numerous systems adapted from nature allow us to study their evolutionary and population dynamics.

Via genetic manipulation, synthetic, cross-feeding, cooperation can be engineered within microbial communities [15, 16, 17, 13, 18]. Complex population dynamics in response to temporal, spatial and environmental factors can be thus be dissected via the fine-tuning and manipulating of these synthetic systems [19, 18]. We use the *Saccharomyces cerevisiae* synthetic mutualism systems that relies on the cross-feeding of amino acids between two strains [15]. The system uses feedback resistance (fbr) mutations in adenine and lysine biosynthesis pathways that results in an overproduction of the corresponding amino acid. The strains used are referred to here as $LYS\uparrow$ and $ADE\uparrow$The dynamics of this cross-feeding system is comparable to a simple but powerful theoretical model of self-organisation – a hypercycle – implicated at the origin of life as well as the formation of complex communities [20, 21, 11, 22, 18]. Informed by experiments, we develop a simple but powerful and easily extendable phenomenological model. We then use the yeast system to validate the model and finally explore beyond the synthetic system to understand the stability and cost of cooperative interactions in changing environments. While it is clear that a change in the environment affects the interaction pattern, our aim is to tease apart the abiotic factors to

under how exactly this takes place. With this aim in mind we begin with our theoretical and experimental model.

Model & Results

Our model begins with the growth of two yeast strains in an environment which does not have any free amino acids to be consumed. The two strains are *LYS*↑ and *ADE*↑ whose densities are denoted by x_L and x_A. The strain *LYS*↑ is deficient in adenine (density c_A) while it overproduces lysine (density c_L). The production and requirement of amino acids is vice versa for *ADE*↑Modifying the logistic growth equation gives the dynamical equations for the growth of the two strains,

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

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

The two strains grow if the required amino acids are present. The strains compete for a limited amount of space given by K. The amino acid concentrations together with Monod type saturation kinetics controls the growth of the strains. Our model is mechanistic regarding individual amino acid dynamics. Explicitly including the amino acid concentrations is crucial as pairwise Lotka-Volterra models might not always provide a realistic qualitative picture of the dynamics [14]. The amino acids are a consumable resource – as they are being produced constitutively by one of the strains, the other type is using them immediately. The dynamics of the amino acid densities in the culture hence can be captured by,

$$\dot{c_A} = \beta_1 x_A - \frac{\gamma_1 c_A}{c_A + k_{c_A}} x_L \tag{3}$$

$$\dot{c_L} = \beta_2 x_L - \frac{\gamma_2 c_L}{c_L + k_{c_L}} x_A \tag{4}$$

The rate at which each of the two amino acids i can increase in the yeast culture is given by β_i. This rate determines the amount of amino acid shared by the corresponding feedback-resistant strain. We assume that the use of the amino acid with rate γ_i by the strains x_i also goes through the process of forming an intermediate and is thus subject to the same Michaelis-Menten kinetic parameters. The simple dynamics of such a system is depicted in Fig. 1.

Adding the required supplement, we calculate the growth rate of the two strain for different levels of supplementation. The resulting growth rates curves are used to parametrize the model. The growth parameters are $r_1 = 11.4906$ and $r_2 = 29.3955$ and the Michaelis constants $k_{c_A} = 26.4326$ and $k_{c_L} = 29.0254$. We assume the Michaelis constant estimated for the uptake rate to amino acids to be the same as the rate at which they degrade from the pool. Starting at different initial conditions of the *ADE*↑ to *LYS*↑ ratio, the final equilibrium values are close to 0.6, corroborated by experiments Fig. 2.

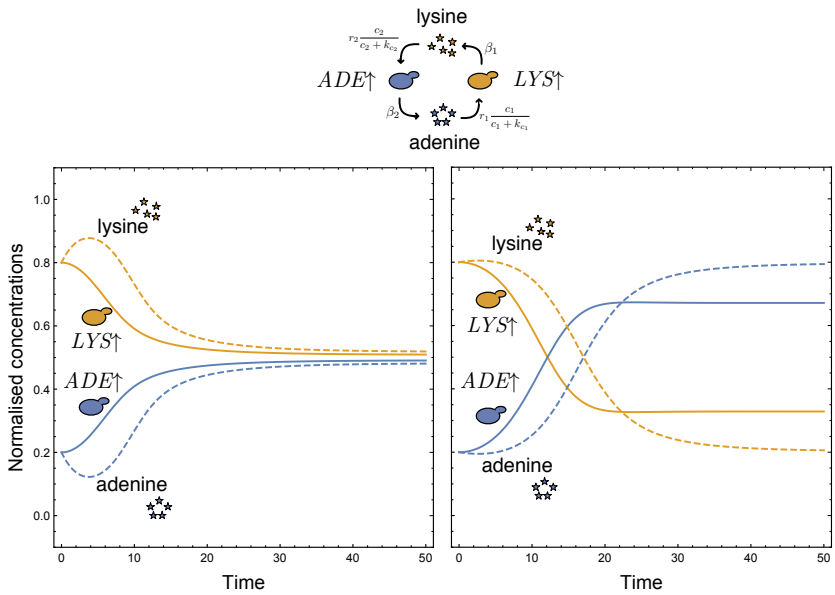

Figure 1: **Unsupplemented dynamics (left) neutral expectation and (right) expectation informed by experimental growth rates).** We plot the fraction of *LYS*↑ $(x_L/(x_L + x_A))$, *ADE*↑ $(x_A/(x_L + x_A))$ and the relative fractions of amino acids adenine $(c_A/(c_A+c_L))$ and lysine $(c_L/(c_A+c_L))$ in a continuous culture. As an initial condition there are no free amino acids in the culture but the nonzero amounts of the strains generates the free amino acids in the same relative frequencies. The relative initial fraction for the strains are *LYS*↑ $= 0.8$ and *ADE*↑ $= 0.2$ and the rate at which the two strains share the amino acids 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 amino acids 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 the amino acids to $k_{c_A} = k_{c_L} = 1$. Under these symmetric conditions the normalised densities of the strains as well as that on the free amino acids reach an equilibrium where the 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 the uptake rates is reflected in the resulting unequal equilibrium.

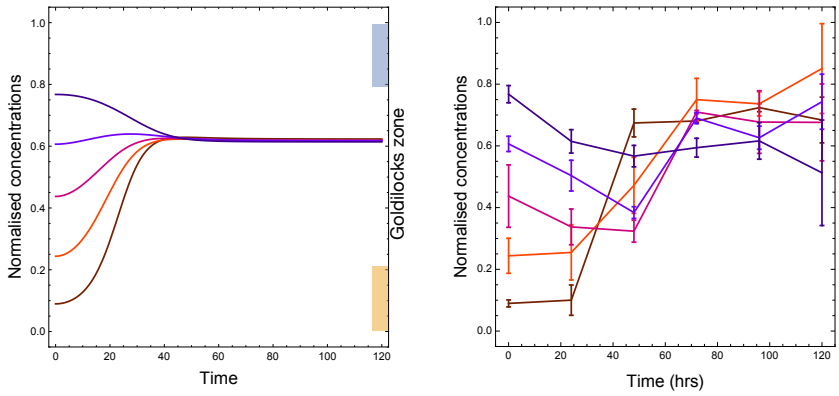

Figure 2: **Theoretical and experimental model dynamics of with different initial ADE↑ concentrations.** Using the theoretical model, in the left panel we predict the dynamics of the normalised fraction of *ADE*↑ starting at various initial ratios with the parameters set informed by data. The initial 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*↑ relative to the *LYS*↑ . Starting at different initial ratios we track the dynamics over a 140 hour period in synthetic complete media (without amino acids). Each point is the mean of three replicates and the error bars are a single standard deviation.

0.1 Eco-evolutionary dynamics under environmental disturbance

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

If the environment has one of the two required amino acids, then the strain using that amino acid will not depend on the corresponding strain for survival. A significant amount of supplementation can drown out the signal of the other strain. In all, we visualise three different supplementation scenarios which could be used to test the resilience of this mutualistic system. Specifically, initial supplementation – amino acid added at the beginning of culturing, continuous supplementation – amino acid added steadily throughout culturing, and intermittent supplementation – amino acid added at regular time intervals. Depending on the timescale a single niche can undergo all three scenarios, but we analyse the three scenarios in turn.

Initial supplementation.

The initial supplementation regime is a standard batch culture. With nutrients provided at the start, the culture continues to grow until one or more nutrients become limiting. We supplemented our experimental cultures with either 0.1, 1 or 10 µg/ml adenine and measured

the effect on strain ratio at regular time intervals (Materials & Methods, Fig. 3 (top)). The supplementation favoured the *LYS*↑ strain resulting in a lower prevalence of *ADE*↑ when compared to the unsupplemented condition. The mean strain ratio of *ADE*↑ to *LYS*↑ after 120 hours growth of all starting ratios for 0.1, 1 or 10 μg ml^{-1} adenine supplementation is 0.5316, 0.5685, 0.0556, respectively.

Using the parameterised theoretical model we explore the final equilibrium value of *ADE*↑ as we change the amount of initially supplied amino acids. The model qualitatively mimics the change in the equilibrium condition with the addition of adenine Fig. 3 (bottom). Experimentally we have tested the addition of only adenine for different initial fractions of *ADE*↑ but theoretically, we explore the consequence of adding both adenine and lysine for the *ADE*↑ starting at 0.5. The results, summarised in Fig. 4 (left panel), reveal that even a slight asymmetric increase in the amount of initial amino acid present in the environment is enough to destabilise the equilibrium and push it close to extinction. When the equilibrium values come close to one of the two edges of the system, the rarer mutualism runs the risk of going extinct by drift. Too close to the edges (0 or 1) and stochastic dynamics could play an essential role in leading to the extinction of either of the strains. If the equilibrium value of one of the mutualist partner falls below 0.2 we assume that the mutualist system is under high risk of collapse. Thus we define the region between 0.2 and 0.8 as the "Goldilocks zone" where mutualism can safely exist. The asymmetry of the graph in Fig. 4 is a reflection of the inherently different uptake rates of amino acids by the two strains Fig. 2.

Intermittent supplementation.

As the initially added supplementation is used up, it is possible to add amino acids at certain fixed intervals. The model is now an example of a hybrid dynamical system [23] where the concentrations of the amino acids are adjusted at regular intervals (see Materials & Methods).

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

Continuous supplementation.

The intermittent supplementation if done at minimal time intervals represents a continuous supply of amino acids. Continuous supplementation competes with the supplementation provided by the strains themselves, drowning out their signal and thus undermining the basis of cooperation between the two strains.

As before, we plot the equilibrium values of *ADE*↑ for different amounts of continuously added adenine and lysine (relative to adenine concentration) (Fig. 4 right panel). Slight but

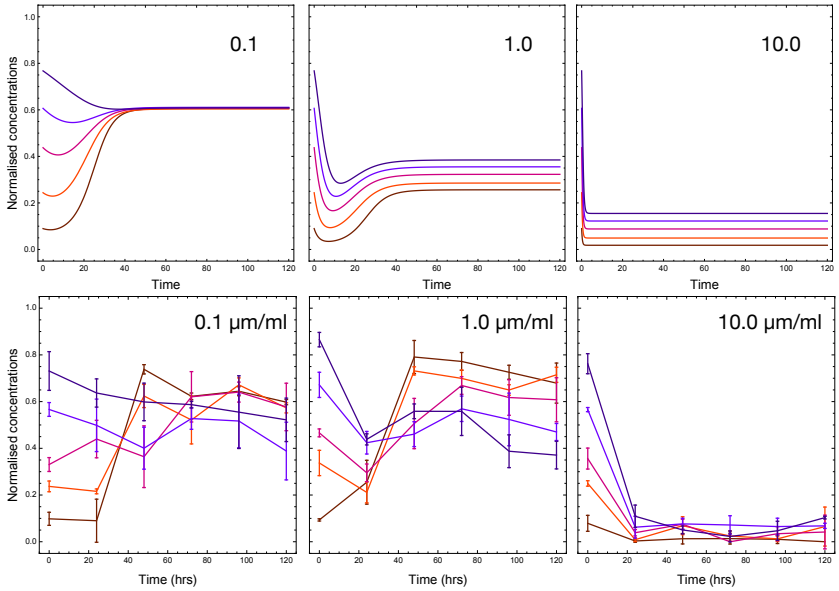

Figure 3: **Theoretical and experimental model dynamics with different initial supplementation levels of adenine.** We explore the theoretical model as depicted in Fig. 2, now with added initial supplementation. The initial concentrations of the amino acids c_A and c_L are set to 0.1, 1.0 and 10 as denoted in the panels. Using the modified version of In the bottom row we show the normalised concentration of the yeast strain *ADE*↑ relative to the *LYS*↑ strain, at varying starting 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 are a single standard deviation. The theoretical model qualitatively predicts the experimental model dynamics of mutualism, With increasing supplementation, the dependence of *LYS*↑ on $ade^{fbr}lys^{-}$ is reduced and it the system exits the Goldilocks zone.

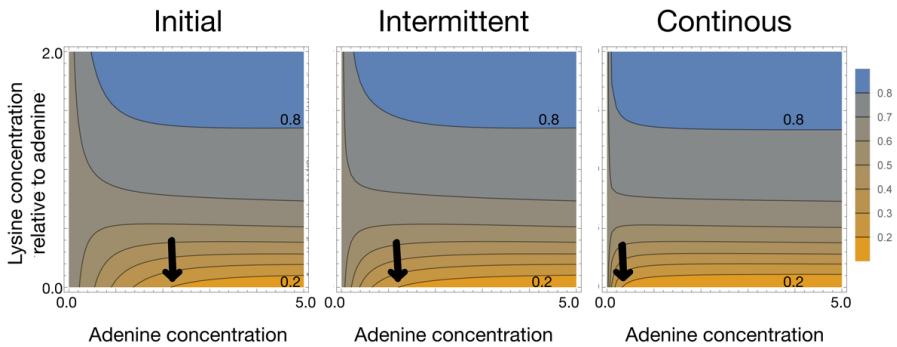

Figure 4: **Equilibrium value of ADE↑ for different supplementation regimes.** Initial normalised concentration of *ADE*↑ $= 0.5$. The system is assumed to have equilibrated by 300 time steps. In the plots we show the eventual normalised concentration of *ADE*↑ going from all *ADE*↑ (1, blue) to all *LYS*↑ (0, yellow). The initial concentration of adenine in the system at time 0 is as per the value on the x-axis and the amount of lysing relative to adenine is denoted 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 5th time step and for continuous: at ever 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*↑ (yellow) increases and so does the *ADE*↑ (blue) region.

continuous addition of lysine immediately breaks the mutualism as the $ADE\uparrow$ strain takes over the mixed culture. The Goldilocks zone breaks down almost immediately for continuous supplementation as compared to the initial supplementation.

Intervention measures in presence of ultimate overproducers

A typical threat to a mutualistic system is the evolution of a cheater which takes unfairly of the produced public good [24]. A cheating strain can thrive because it benefits from public goods without having to pay the cost of contributing. But does mutualism always have to be extremely costly? If the production of the public good itself is incredibly cheap, then we could envision a mutant that does not require a mutualistic interaction but contributes to the pool of public goods, like a fictitious $ADE\uparrow LYS\uparrow$ strain. While not taking part in mutualism might be lethal, taking part in a mutualistic interaction does not need to be costly if it is via the overproduction of a single protein. Although specific, yeast can overproduce a fluorescent protein and only suffer a 1% reduction in cost per copy [25]. Moreover, reliance on external sources for esentially metabolites can have a considerable cost as well. Several of the auxotrophic yeast strains, such as those unable to produce their own lysine or adenine, have up to a 10% reduction in growth even with environmental supplementation [26, 27].

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

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

The theoretical model shows that we can supplement the culture with one of the amino acids. Since the $ADE\uparrow$ strain has the higher growth rate we support it by providing lysine in the environment. As the amount of supplementation is lowered, we need to provide it earlier so as to maintain $ADE\uparrow$ as the dominant strain. The later intervention via supplementation occurs, the more likely previous unsupplemented predictions of the growth rate hold true. The same holds for scenarios where the ultimate overproducer dominates, we need to provide a high amount of supplement early on to offset the fitness benefit of the ultimate overproducer Fig. 6 (dominant strain panel 3). If supplementation occurs after the carrying capacity has already been reached then it is useless.

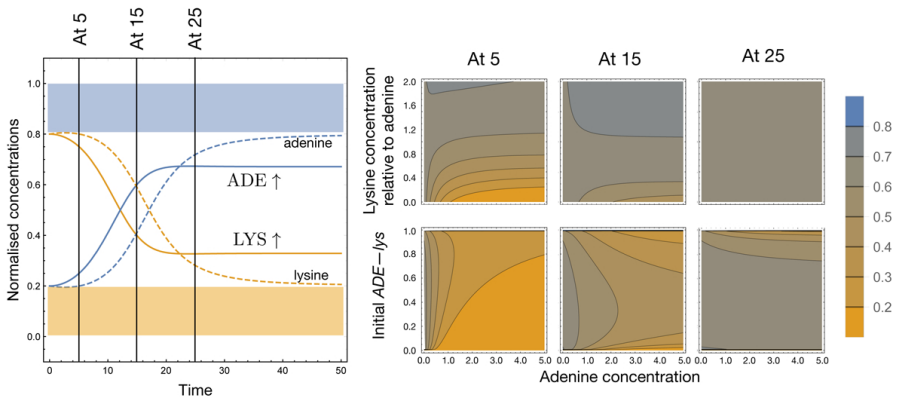

Figure 5: **Disrupting the transient by intermittent supplementations.** Intermittent supplementation as shown in Figure 4 started with the same initial supplementation as the initial supplementation regime so as to be comparable. If we start with no initial amino acids in the culture and add the amino acids at fixed time intervals then it might be possible to extend the range of the Goldilocks zone even under supplementation. The exact timing of when the supplementation is provided is crucial in determining the eventual equilibrium. If the pattern of supplementation starts early on in the population dynamics of the *ADE*↑ then the resulting equilibrium frequency can be drastically affected. Tuning the timing and dose of the 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). If the 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 the *ADE*↑. Thus while the top row is calculated from an initial normalised concentration of *ADE*↑ $= 0.2$ the bottom row explores all initial conditions. The equilibrium values are all calculated at the same time point i.e. the number of cycles are adjusted so that all numerical integrations run till time point *cycle length* $\times$ *number of cycles* $= 300$.

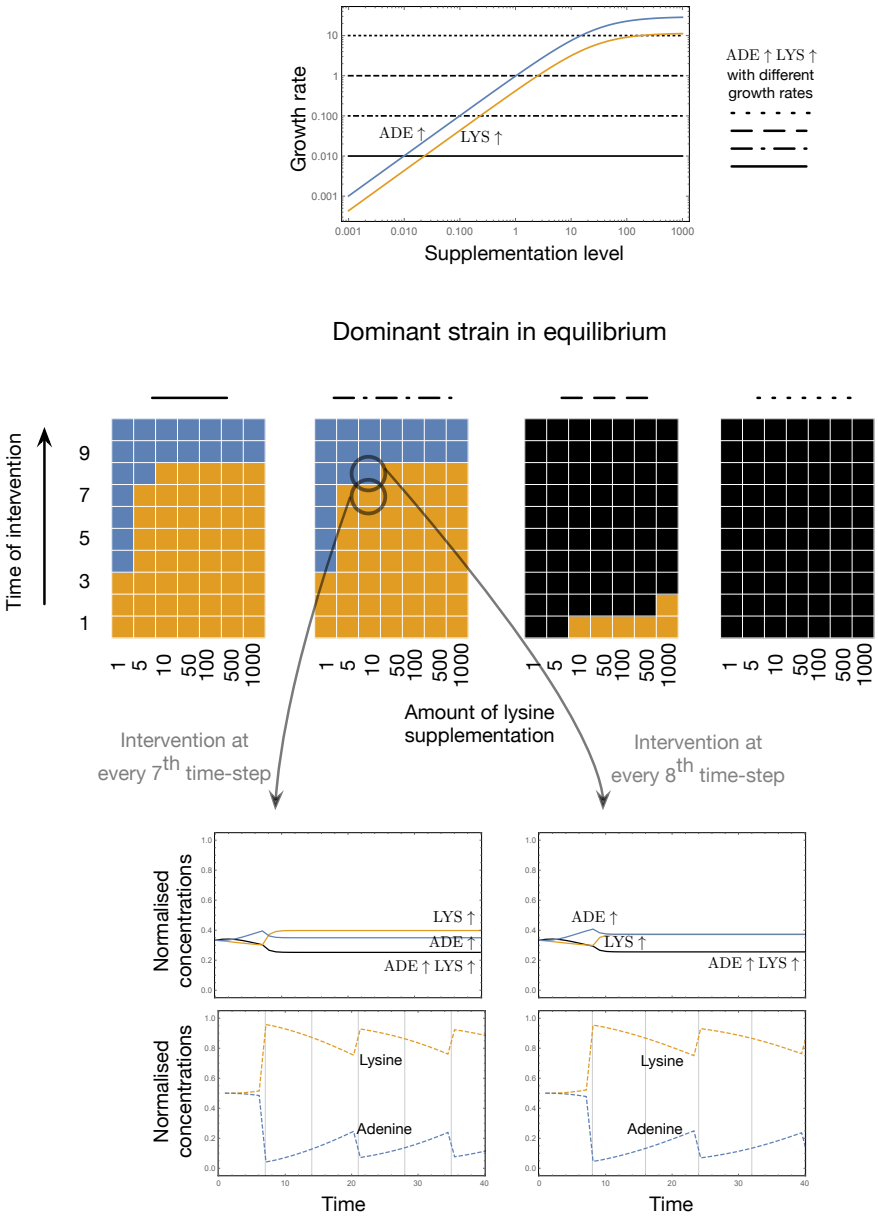

Figure 6: **Ultimate overproducers and equilibrium dominance.** The top panel contains the experimental growth data for the *ADE*↑ and *LYS*↑ strains. A range of constant growth rates for the *ADE*↑*LYS*↑ strain that fall inside of the aforementioned experimental rates were selected, shown with the varying black lines. Given these growth rates we numerically estimate the equilibrium of the system. The colour denotes the dominant strain at equilibrium in each simulation, with yellow denoting *LYS*↑ and blue *ADE*↑ . For an increasing growth rates of the ultimate overproducer we see that more amount of lysine is required to maintain one of the mutualists as dominant strain. Furthermore, also the exact time of intervention k (and then every k^{th} step) matters. The low fitness *ADE*↑*LYS*↑ never makes it to the top but the dominance between the two mutualists can change (left two panels and the explicit dynamics shown in the insets). For lower amounts of supplementation, the intervention needs to be earlier if we want to see a change in dominance. The same is the case for higher fitness of *ADE*↑*LYS*↑ although in this case intervention times would be extremely early on.

Discussion & Conclusion

Cooperation is instrumental at all levels of life and on all timescales. On the scale of ecosystems, the biosphere itself can be viewed as interacting networks of varying components. Although highly relevant, the behaviour of these complex interactions is difficult to study and nearly impossible to predict. Even simple interactions between a handful of cooperators form complex networks but when these networks are placed in fluctuating environments their complexity surges.

Synthetic biology systems involve applying engineering principals to an organism to promote a precise level of control and predictability over the natural behaviour. To this end, we leverage the simplicity of the engineered yeast system and the corresponding mathematical model. Our work examines both, the effects of how initial strain ratios and changes in the environment alter the dynamics of strain concentrations and influence mutualism stability. Under stable environmental conditions, as previously shown, the system is highly stable concerning the starting ratio of strains [15]. The stability under changing environmental conditions is an entirely different matter. The environmental changes have the potential to influence or outright disrupt mutualistic interactions [13]. Here we dissect these influences further with the aim of discerning how mutualistic interactions may be maintained. Going beyond the interactions between the biotic components we have integrated the abiotic factors such as the amount and timing of the environmental change into our system.

The final equilibrium of the mutualistic system depends more on the environmental supplementation shocks than on the starting ratio of the mutualist partners. As initial supplementation of adenine increased, favouring the $LYS\uparrow$ strain, the final ratio shifts in both the experimental system and mathematical model (Fig. 3). Confident in the exploratory power of our mathematical model, we determined the environmental conditions that promote mutualism and provide, what we refer to as, the Goldilocks zone. The modelled supplementation regimes are common in microbial culturing, but they also have clear ecological parallels. The non-supplemented culture is a niche baseline. Initial supplementation is akin to a single one-off event like the sinking of a massive carcass to the depths of the ocean resulting in extreme point-source enrichment [28]. Intermittent supplementation represents a season change like temperature fluctuations or the regular introduction of a nutrient to the gut of host animal. Understanding the effect of fluctuating resource availability is extremely important regarding invasibility [29]; a temporary reduction in competition due to nutrient excess can make the mutualism vulnerable to invasion [30]. Finally, continuous supplementation is a permanent change to a given environment as a permanent temperature change or evaporation of a large body of water. In general, we identified a gradually shrinking Goldilocks zone as one progresses through initial, intermittent and continuous supplementation regimes (Fig. 4). With environmental destabilisation becoming more and more concrete, the edges of the mutualistic system encroach upon even minimal supplementation levels. Stochastic effects may dominate these regimes where mutualism can then go extinct via chance events. Tracking the size of the zone is thus a good measure of the resilience of a mutualistic system under changing environments.

The mode of intermittent supplementation and in particular its timing offers some respite. The strains have asymmetrical starvation tolerance and do not release their overproduced

metabolite until near death [15]. Intermittent supplementation could thus act as a representation of death induced a periodic release of amino acids. It is important to note that the frequency of disturbance in complex communities can severely affect the assemblage of microbes [31]. We explored this by starting the intermittent supplementation at different time points and not just when the supplementation starts but also its frequency. SI. 1.4 highlights the importance of the supplementation parameters as well as the starting ratio of the two strains. We observe that when the environmental changes are minute then the initial ratios matter but if the change in the environment is drastic then the timing of the change is crucial.

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

The disruptive influence of this overproducer strain can be mitigated against. Intervention both early and with considerable supplementation permits continuation of the mutualistic interaction between $ADE\uparrow$ and $LYS\uparrow$Highlighting that targeted intervention in collapsing ecological niches, that depend on mutualism, and save these relationships or at least slow their collapse. Further experimental tests along these lines will provide insight into the role of the abiotic components on the resilience of the mutualistic system and inform intervention strategies.

Understanding environmental enrichment (or degradation) is imperative as we face climate change. The promise of bioengineering together with cooperation is enormous [32]. Besides providing an insight into the nature of interactions resulting in community stability [33], engineered systems have implications for designing complete ecosystems affecting the biosphere in general [34]. With continuing eutrophication of oceans, essential symbiosis may break down into parasitism [6]. Use of pesticides and synthetic nitrogenous fertilisers can disrupt the natural nitrogen fixation process – mutualism between leguminous plants and rhizobacteria [4]. Thus, enriched environments could pose a threat to long evolved mutualism which might lead to further catastrophic events [35]. Since mutualisms bind together organisms and enhance their survival, they come at the cost of binding the fates of all involved species together [36]. Changing the environment, inadvertently, or purposefully via anthropogenic activity and climate change can be costly not just to one particular set of species but the ecosystem at large. Besides being of interest in conservation biology, mutualistic interactions are also at the heart of translational biology affecting applied human health and biotechnology. For example, the normal functioning of the human microbiome is decisive for our well-being, and several pathologies emerge from deviation from the norm [37].

Microbes are often found in consortia rather than individual species. Communities of

bacteria are usually the pioneers in harsh environments from rocks formed from recent volcanic eruptions to hyperarid deserts [38, 39, 40]. A few members of the consortia survive by feeding off the inorganic matter, but most of the community survives by a cross-feeding network [41]. In such cases, it would be useful to know how the communities function, and, especially their fate when the environment has changed *due to their presence*. While the diversity of individuals in a consortia does now always guarantee robustness, engineered microbial consortia might prove to be more amenable to design robust pioneer communities in harsh environments [32]. Learning from natural well-established communities, we can better design the interactions between microbes making them robust against eutrophic environments and thus a useful tool in biotechnology. When developing techniques to tackle problems such as wastewater treatment, biofuel generation or oil spill cleanups, symbiotic communities of microorganisms potentially offer a more efficient pathway to the breakdown of complex substrates [42].

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

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Competing interests

The authors declare no competing interests.

SUPPORTING INFORMATION

1 Methods and Materials

1.1 Saccharomyces cerevisiae Strains

The synthetic system is made up of two amino acid overproducing strains previously developed in the w303 background provided by Professor W. Shou [15]. The first, WS950, is an adenine overproducing strain called *ADE*↑ through this manuscript. It has the genotype MATa ste3::kanMX4 lys2Δ0 ade4::ADE4(PUR6) ADHp-DsRed.T4. The second, WS954, is a lysine overproducing strain called *LYS*↑ here. This strain has the genotype MATa ste3::kanMX4 ade8Δ0 lys21::LYS21(fbr) ADHp-venus-YFP.

1.2 Culturing & Plate Counts

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

Experimental cultures were generated by selecting individual colonies from a streak plate and growing each colony in 5 ml SC medium for 48 hours. These cultures were pelleted, washed twice with SC-aa and resuspended in 5 ml of SC-aa. They were then grown for a further 24 hours. These cultures were pelleted, washed and resuspended as above and then were diluted 1 in 20 with SC-aa. Each data point was performed in triplicate starting from a single individual colony. The single strain growth tests were performed using 5 ml aliquots of the 1 in 20 dilution and supplementing to a final concentration with either 0, 0.1, 1, 10 or 100 µg/ml of adenine for the *LYS*↑ strain or lysine for the *ADE*↑ strain. The cultures were sampled at 0 and 24 hours. The experimental cultures were generated by mixing the individual strain dilutions at the volume ratios indicated in each experiment to a final volume of 5 ml. The $0, 0.1, 1, 10$ or 100 µg/ml adenine supplemented cultures were found to peak in the number of colony forming units, suggesting growth saturation, at 144, 120, 72 and 48 hours respectively. In each case, an equilibrium was reached before saturation. We determined colony forming units via plate counts on YPD solid media of culture dilutions at the time points indicated. Strain ratios were determined by replicate plating these YPD colonies onto solid media that permitted growth of only one strain, either SC without lysine or SC without adenine, and counting the respective colonies.

Estimating growth parameters. We now parametrize the Michaelis constants (k_{c_A} and k_{c_L}) and the growth parameters (r_1 and r_2) using experimental data. Single strain growth rates for *ADE*↑ and *LYS*↑ were determined on growth media supplemented with varying concentrations of the appropriate required amino acid. The two strains were grown in synthetic complete media either unsupplemented or supplemented with 0.1, 1, 10 or 100µg/ml of the corresponding amino acid. Growth after 24 hours was calculated as above. The growth curves were used to parametrise the model's growth components.

1.3 Data Analysis

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

1.4 Model extension for supplementation regimes

For initial supplementation we do not need to modify the model from what is presented in the Eqs. (2) and (4). For different values of initial supplementation we change the initial

conditions for the amount of amino acids. For *continuous supplementation* we modify the Eqs. (4) to,

$$\dot{c_A} = c_{Acont} + \beta_1 x_A - \frac{\gamma_1 c_A}{c_A + k_{c_A}} x_L \tag{5}$$

$$\dot{c_L} = c_{Lcont} + \beta_2 x_L - \frac{\gamma_2 c_L}{c_L + k_{c_L}} x_A \tag{6}$$

where the values of c_{Acont} and c_{Lcont} determine the amount of amino acid continuously added to the culture. For *intermittent supplementation* we make use of a hybrid dynamical system [23]. We start with the same initial conditions as for the other supplementation regimes, where the initial condition of the amino acid matches the amount used for supplementation. The dynamics proceed as per the equations in the main text. In total, they run for the same amount of time as the initial and the continuous supplementation before assuming equilibrium, but the time is split up into many cycles. Each cycle runs for a short period. Thus we have $cycle\ length \times number\ of\ cycles = total\ time\ until\ equilibrium$. At the end of each cycle, we add the predetermined amount of amino acids and then let the next cycle continue. In case of no external supplementation, the system takes a certain amount of time to equilibrate. If the supplementation keeps on disrupting this process by disturbing the transient dynamics, then the eventual outcome when evaluated at the same time point as the other supplementation regimes can be drastically affected (Fig. 5 and Appendix Fig. 7).

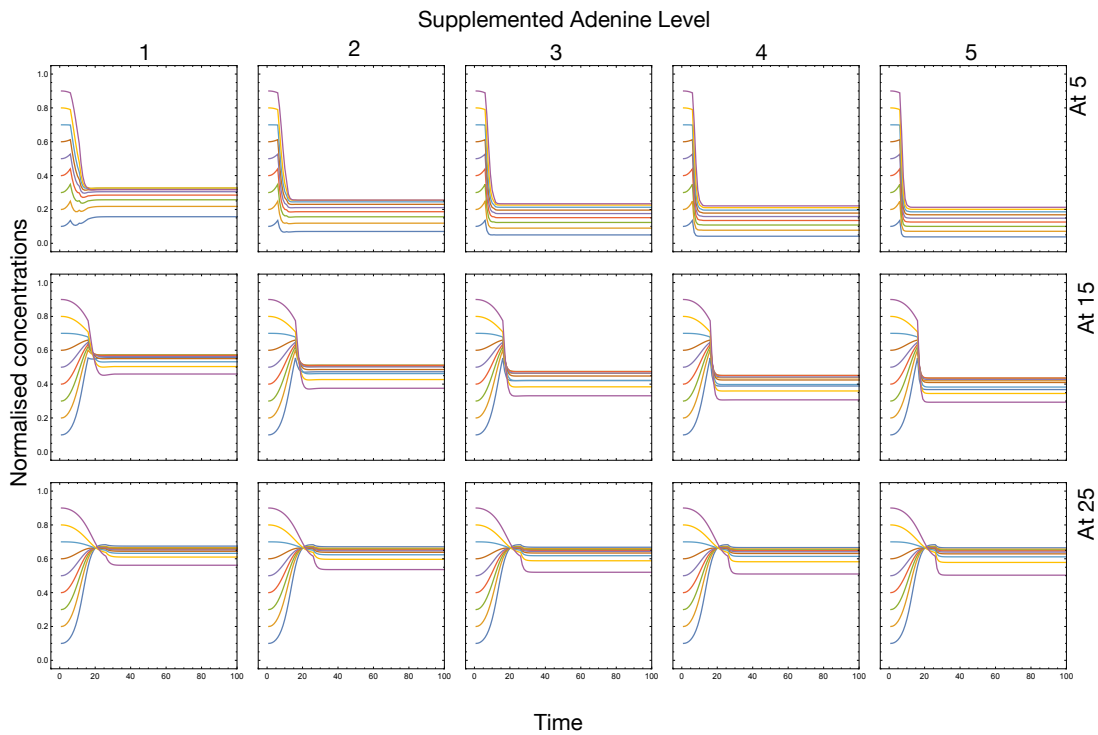

Figure 7: As shown in Fig. 5, the frequency and timing of the intermittent supplementation changes the equilibrium of the system but the initial conditions matter as well. In this figure we highlight this dependence. Starting the supplementation regime at time point 5 and then supplementing at every 5th 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 is 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 boosts the role of the initial conditions. In supplementations begins early (at 5) then the order of initial conditions is not affected as opposed to the case of starting the supplementation later (at 15 and at 25).