# Synthetic ecology: A model system for cooperation

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Synthetic biology offers the promise of a better understanding of biological systems through constructing them. Unlike naturally occurring biological systems, which are generally complicated by multiple variables and difficult to isolate components, synthetic systems can be simplified to allow for experiments that would be too difficult to interpret if done in their full natural context. Up to now, synthetic biologists have primarily focused on gene circuits, such as oscillators and switches (reviewed in ref. 1). By building synthetic genetic circuits, researchers in the field have been able to learn more about the rules of gene expression and regulation, including fundamental issues regarding noise, timing, and signal fidelity, for example. In this issue of PNAS, Shou *et al.* (2) demonstrate an example of a new direction for synthetic biology, what might be called synthetic ecology. Rather than using gene modules as building blocks, they mix cell populations to construct a synthetic simple obligatory cooperative ecology and use it as the basis of a model for the robustness and boundary parameters of such systems.

Previous experimental and theoretical studies of cooperative or mutualistic ecologies have raised questions about the origin and stability of such systems (reviewed in ref. 3). Cooperation, by which one population or individual contributes to the survival of another at cost to itself, poses some problems. How could such a seemingly delicately balanced and evolutionarily problematic system (*i*) come about at all, (*ii*) persist through perturbations such as bottlenecks of one or both populations, and (*iii*) survive the threat posed by cheaters? Yet, cooperation is a widespread feature of biological systems. In natural environments, cooperation is found in the context of symbiosis (reviewed in ref. 4), coevolved pairs of plants and their pollinators (for example, figs and wasps; reviewed in ref. 5), social vertebrates (reviewed in ref. 6), and even cancer (7). In addition, cooperation is thought to be a precursor to multicellularity (8).

The questions posed have been approached theoretically mainly through the frameworks of game theory and experimentally in a variety of ways. Several model systems for cooperation or mutualism have been developed, with varying levels of genetic and experimental accessibility. Studies of natural systems have used organisms ranging from
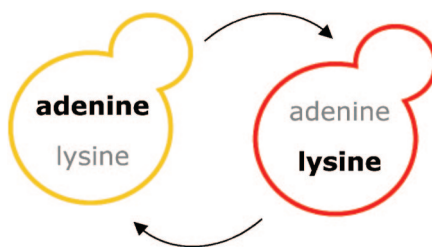


**Fig. 1.** The CoSMO universe: an obligate cooperative synthetic ecosystem composed of one strain of yeast, marked with YFP (yellow outline), that requires lysine and overproduces adenine, and another marked with DsRed (red outline) that overproduces lysine and requires adenine. Both strains are necessary for a viable system.

corals to wasps to meerkats. Such approaches have been particularly useful in documenting phenomena such as coevolution and the importance of relatedness to various modes of cooperation. In the laboratory, microbial experimental evolution cultures have repeatedly developed cooperative population structures in the forms of cross-feeding (9–11) and niches that facilitated cell interaction (12, 13). These experiments have hinted at an ease of creation of cooperative situations that the Shou *et al.* study bears out (2). As a means for studying cooperation, however, they leave something to be desired because other variables are also at play, including competition for resources and multiple selection pressures.

The current study circumvents some of these problems, at the necessary cost of verisimilitude, by engineering a simple mixture of two strains of yeast, each of which produces a nutrient required by the other. This was accomplished by a clever genetic trick involving feedback inhibition mutants that overproduced either lysine or adenine to supplement the corresponding auxotrophy of the partner strain (Fig. 1). In addition, each population was marked with a different fluorescent protein, allowing for simple and accurate characterization of population dynamics by flow cytometry. The resulting mixed cell population, termed CoSMO (for cooperation that is synthetic and mutually obligatory), demonstrated surprisingly complex behavior. Although the two populations did cooperate to support each other's growth, the overproduced nutrients were not actually available for consumption until the onset of cell death, leading to delays

in nutrient provision. The two populations were also differentially sensitive to starvation conditions, with one strain dying off faster than the other. This combination led to two distinct phases of growth for the culture, each of which was fueled by the death of one part of the population. Such behavioral quirks imply that even more complex aspects of cooperation might be incorporated into such synthetic ecologies.

In addition to the experimental work, Shou *et al.* (2) also mathematically modeled the behavior of the system. In general, modeling complex systems is fraught because assumptions are key and may be difficult to accurately estimate. The model for the simple ecology created by Shou *et al.* requires 16 parameters. More realistic systems can, of course, require many more. However, because of the simplified nature of CoSMO, the authors could actually accurately parameterize the model by measuring all of the relevant components. This close connection between the model and "real life" lent extra credibility, which was borne out when many of the predictions actually proved true. In particular, when considering the robustness of the system, the model predicted viable mixtures for starting strain ratios over 9 orders of magnitude. In experiments to test this prediction, strain ratios firmly on one side or the other of the boundary conditions always gave the expected result, whereas ratios near the boundary behaved stochastically. Along with the impressive robustness, initial conditions over 6 orders of magnitude converged on a narrow stable state, also predicted by the model.

The robustness of the system is a particularly interesting result, but even the modes of failure may be useful for considering natural conditions that would preclude the formation of such ecosystems or disrupt existing ones. Which parameters are important for the boundary conditions and various other behaviors could now be tested, to the degree to which the parameters are adjustable in the laboratory.

In fact, Shou *et al.* (2) went one better with this concept and did not limit themselves to improvements that they could envision. By evolving the system in the laboratory, the parameters of the new ecology tuned themselves. Over a series of dilutions mimicking bottlenecks, the cultures enhanced their ability to tolerate low starting densities. Selections that would act on other aspects of the system are easy to imagine. With the advent of whole genome mutation detection in yeast (14), it should be an easy task to find out how the cells accomplish parameter adjustments. If both cell populations are shown to adapt, CoSMO may even have something to say about cospeciation.

Finally, Shou *et al.* (2) suggest that their system could be useful for studying cheaters, i.e., cells that eat the nutrient without contributing any themselves. Cheaters pose a special challenge to the stability of cooperative systems and have arisen with interesting consequences in experimental evolutions of *Pseudomonas fluorescens* (12). Rich theoretical predictions also exist for how they should behave. CoSMO cheaters would be trivial to construct and could probably be easily incorporated into the model. The tolerance for cheaters is yet another example of the types of questions that can be addressed by the new synthetic biology (15). Over the next few years, we can look forward to seeing other complex questions framed as synthetic biology questions. Synthetic approaches can then be combined with analysis in the laboratory and in nature to triangulate the rules of natural systems.

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