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Engineering microbial systems to explore ecological and evolutionary dynamics

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Abstract

A major goal of biological research is to provide a mechanistic understanding of diverse biological processes. To this end, synthetic biology offers a powerful approach, whereby biological questions can be addressed in a well-defined framework. By constructing simple gene circuits, such studies have generated new insights into the design principles of gene regulatory networks. Recently, this strategy has been applied to analyzing ecological and evolutionary questions, where population-level interactions are critical. Here, we highlight recent development of such systems and discuss how they were used to address problems in ecology and evolutionary biology. As illustrated by these examples, synthetic ecosystems provide a unique platform to study ecological and evolutionary phenomena that are challenging to study in their natural contexts.

Introduction

A major goal in biological research is to provide a mechanistic understanding of a system of interest. It is increasingly recognized that quantitative analysis is often critical to achieve this goal. Depending on the specific systems and the corresponding questions, different approaches can be adopted that use varying degrees of conceptual abstraction and simplification (Figure 1). For instance, a biological system can be described by a mathematical model, wherein a set of equations or rules describes the essential interactions constituting the system. Mathematical models offer the convenience of exploring the impact of system parameters in a well-controlled manner. However, a model is a closed system that is free of interference from cellular processes not accounted for in the model. As such, the applicability of the conclusions derived from modeling analysis ultimately requires experimental evaluation.

Testing model predictions is typically performed in natural biological systems. Effective integration of modeling and experiments has generated quantitative insights into the dynamic characteristics of cellular interactions. Nonetheless, such an approach faces the challenge of having to deal with many confounding factors in the natural context (i.e., “background” processes). For instance, one may be interested in the property of a particular regulatory module (Figure 1, shaded region in the right panel). In reality, however, the

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module cannot be completely insulated from other cellular processes that interface with it, which can be difficult to account for. For this reason, precise control of systems parameters is challenging. As such, experimental results that demonstrate certain model predictions are often subject to alternative interpretations.

Conceptually, engineered systems serve as an intermediate between these two extremes [1]. For example, to study the function of a regulatory module of interest, one may construct the module from scratch and test its function with minimal interference from background processes (Figure 1, middle panel). This property represents a major advantage of the engineered systems: due to the better insulation from background processes (albeit incomplete; see [2,3] for unintended interactions between engineered system and the background processes), it is more likely that one can examine the impact of specific system parameters in a more definitive manner. Also, when compared to mathematical models, synthetic systems retain biological reality and operate within the limits defined by physical laws. Such engineered systems can offer a form of proof for the biological phenomenon in question.

While the synthetic-biology approach has been successfully applied to elucidate design principles of gene regulatory networks in individual cells, such as oscillation [4,5] and genetic switch [6–8], there is now an increasing interest in applying this approach to studying microbial communities. In every imaginable habitat, microbes thrive in communities, where members engage in diverse interactions, such as cooperation, competition and predation. Historically, theoretical analysis and mathematical modeling have been extensively employed to study ecological and evolutionary interactions [9–11]. Since experimental tests of such predictions using natural systems are often challenging due to their inherent complexity [12–14], engineered microbial populations are advantageous in that they are equipped with well-defined interactions and components that can be easily modulated [13,14].

Cooperation in a single population

Cooperation is a ubiquitous interaction in microbial communities that facilitates growth and survival [15–17]. It is often mediated through the production and release of ‘public goods’ that are costly to make but beneficial for the population as a whole (Figure 2a). Public goods exist in many forms, such as virulence factors [18,19] and extracellular polymeric materials for biofilm formation [20,21]. For example, a human opportunistic pathogen *Pseudomonas aeruginosa* secretes siderophores that scavenge iron, a critical factor for growth, from the environment [16,22].

Public-good-mediated cooperation is inherently prone to exploitation by cheaters. Cheaters avoid the cost of public-good production but benefit from the public goods produced by cooperators in the population (Figure 2a) [16,17]. This conflict between individual- and population-level interests in public-good cooperation and its evolution are fundamental problems in ecology and evolutionary biology. To address this issue, Hamilton [23] formulated a simple rule to describe a condition under which a cooperative trait is evolutionarily favored:

In public-good-mediated cooperation b represents the fitness benefit to the recipient of public goods, c represents the fitness cost of public-good production, and r represents the genetic correlation between cooperators and recipients, called relatedness. Hamilton’s rule is appealingly simple. However, analyzing natural biological systems requires an understanding of how changes in both intrinsic and extrinsic parameters of the system translate into the parameters entering the rule. This task is challenging in natural systems due to many confounding factors.

To this end, Chuang et al [24,25] engineered a synthetic system in *Escherichia coli* to study how various experimental manipulations affect the parameters of Hamilton's rule. The system consists of a cooperator strain, which produces a quorum-sensing signal as a public good, and a cheater strain, which does not. Both strains express a receptor protein that recognizes the signal and drives expression of antibiotic resistance gene. Through a series of experiments, the authors demonstrated that the biological and environmental parameters associated with a particular system are not necessarily related in a simple way to the parameters entering Hamilton's rule. Specifically, they showed that increasing the receptor dosage in both strains disfavored cooperation although this manipulation was intended to increase b by making cells more sensitive to public goods. Further analysis revealed that the growth benefit was a nonlinear function of public goods, where b decreased with this manipulation. Their studies highlighted that even in a well-controlled synthetic system, bridging mechanistic parameters and factors in Hamilton's rule is non-trivial. Yet, use of a synthetic system has at least made the mapping tractable.

In a synthetic system created by Gore et al using the yeast *Saccharomyces cerevisiae* [26], cooperators express a sucrose invertase that hydrolyzes sucrose into glucose and fructose. These monosaccharides act as public goods by serving as a carbon source for growth. Cooperators can only directly capture 1% of the resulting monosaccharides; the remaining 99% diffuses into environment and is subject to exploitation by cheaters lacking the invertase [27]. The cooperators were also histidine auxotrophs. As such, the cost of cooperation could be tuned by the histidine concentration in the growth medium. When grown in co-culture, cooperators and cheaters were capable of mutual invasion; they increased their frequencies when rare, leading to stable coexistence. In game theory, this is a characteristic of the snowdrift game [28]. Through game-theoretic analysis and experimental validation, the authors showed that the determinants of snowdrift game dynamics in their system are non-zero glucose capturing efficiency of cooperators (proprietary benefit) and a sub-linear relationship between growth rate and glucose concentration. The authors further showed that by modulating histidine (cost) and glucose (degree to which cheaters rely on cooperators for growth) concentrations, the snowdrift dynamics could be transformed into a scenario where cooperators go extinct.

A similar system was recently used to explore the origin of multicellularity [29]. Transition from unicellular- to multicellular-organisms is a major evolutionary step in the history of life. Incomplete cell separation has long been suggested as the critical mechanism driving multicellularity [30]. While there have been several possible explanations as to what might select for incomplete cell separation [30], Koschwanez et al proposed intraspecific, public-good cooperation as a driving force (Figure 2b). Using an invertase system similar to that by Gore et al, they hypothesized that when cells are grown as a clump, sucrose hydrolysis becomes more localized, resulting in higher glucose concentration around cells than when cells are grown in a well-dispersed environment (Figure 2b). As such, there should be a sucrose concentration that allows growth of clumped cells but not dispersed cells. By engineering cells that grow in clumps due to incomplete cell separation, the authors indeed identified a sucrose concentration that is high enough for clumped cells but too low for dispersed cells to grow. This study illustrates how incomplete cell separation can become advantageous when coupled with public-good cooperation.

Cooperation between populations

In ecology, cooperation between populations, or mutualism, forms a foundation of biological communities. Mutualism can increase fitness of two populations when compared to scenarios where the two populations do not cooperate or exist in isolation [31]. While mutualistic relationships are ubiquitous in nature, the environmental and evolutionary

mechanisms by which they arise are poorly understood. Since there is growing interest in exploiting and creating novel microbial mutualistic relationships for industrial applications [32][33], studies using synthetic biology to examine the conditions under which mutualism can arise are increasing [34,35].

While mutualistic relationships have been achieved through either quorum sensing [36] or the exchange of essential metabolites [37], recent studies have focused on the latter mechanism (Figure 2c). Winternute and Silver examined the mutualistic relationships between pairs of *E. coli* lethal auxotrophs [38]. They observed that 17% of 1035 knockout pairs were able to complement each other's auxotrophic requirement. Using flux balance analysis of a metabolic network, they demonstrated that mutualistic relationships that involve the exchange metabolites that are required in small amounts and that are inexpensive to produce allow for the highest growth.

Members of a biological community can also adapt to form mutualistic relationship. A recent study by Hosoda et al demonstrated that two synthetic bacterial strains, which could not rescue each other's growth when grown as monocultures, could rapidly adapt their transcriptomes to form a mutualistic relationship during co-culture [39]. The authors constructed two auxotrophic *E. coli* strains, I^- and L^- . I^- synthesizes leucine but not isoleucine; L^- synthesizes isoleucine but not leucine. When grown separately, neither auxotrophic strain produced a sufficient quantity of amino acid to rescue the other. Interestingly, when co-cultured, they were able to grow to high density. A transcriptome analysis revealed that L^- increased expression of several genes involved in amino acid anabolism to become a high supplier of isoleucine. While the exact mechanism that resulted in this change remains elusive, this study demonstrated that bacteria could quickly alter their gene expression to develop a mutualistic relationship. These observations complement results achieved by Shou et al [37]. Using a similar system consisting of cross-feeding auxotrophic yeast strains, they observed that co-culturing both auxotrophs over a relatively short time frame (~70 generations) led to an enhanced mutualistic relationship. While the authors speculated about the underlying mechanism, they did not perform genomic or transcriptome analysis to elucidate this enhancement. The above simple synthetic systems can facilitate further investigation to understand molecular mechanisms underlying mutualistic adaptations. Furthermore, recent development of microfluidics technologies [40] [41] will catalyze the field by allowing well-controlled assembly and analysis of microbial communities.

Competitive synthetic ecosystems

Competitive interactions are as equally important as cooperative interactions in biological communities. For example, predator-prey relationships serve as important facets of the ecological food web. Recently, a synthetic predator-prey system consisting of two *E. coli* strains that communicate bidirectionally using quorum sensing was created (Figure 3a) [42]. This system has been used to examine the role of cell motility and spatial structure in maintaining biodiversity [43]. Song et al observed that motility had a negligible impact on biodiversity (i.e. coexistence of predator and prey) when the two strains were well mixed. In contrast, when the two strains were seeded at a distance from one another on an agar plate, motility exerted a significant effect on biodiversity; increasing motility decreased biodiversity (Figure 3b). Using a mathematical model, the study revealed that motility plays a critical role in biodiversity only when the length scale of predator-prey interaction matches the segregation distance.

While the predator-prey system represents a canonical example of competitive relationships, many competitive interactions in biology encompass more than two populations. Along this

line, a synthetic rock-paper-scissors system consisting of three *E. coli* populations was created to test computational predictions that spatial scales of dispersal and interaction are important factors in determining whether multiple competitive species can coexist (Figure 3c) [44]. Recently, a similar system was employed to address the evolution of restrained growth [45]. Prudent use of common resources (and hence restrained growth) is a form of altruism. It likely prolongs the persistence of the community, but such a trait has competitive disadvantage against less-restrained individuals who enjoy higher fitness than restrained ones [46,47]. Computational studies had previously identified that a nontransitive (rock-paper-scissors) interaction in a spatially structured community could lead to evolution of restrained growth [48,49]. If the rock increases its growth rate by mutation, it can drive the scissors away. The decrease in the scissors will benefit the paper, which in turn will be detrimental to the rock. In other words, the nontransitive interaction acts as a negative feedback. Although the mutated rock has competitive advantage over wild-type rock, when the community is spatially structured and the nontransitive interaction occurs locally, the negative feedback could favor restrained growth (Figure 3d).

The rock-paper-scissors system consists of three strains: a colicin-producing strain (P), a colicin-sensitive strain (S), and colicin-resistant strain (R) (Figure 3c). A nontransitive relationship is achieved as follows: P kills S, S outgrows R due to cost associated with colicin resistance, and R outgrows P due to cost associated with plasmid housing colicin gene and colicin immunity gene that protects P from colicin. S was also transformed with a plasmid that confers tetracycline resistance, and adding a low concentration of tetracycline in growth medium magnified the nontransitivity. Using a microtiter plate, the authors manipulated the degree of population structure by either having restricted or unrestricted migration, where migration occurs between adjacent wells or any wells, respectively. After 36 rounds of transfer, R propagated in the community with restricted migration evolved to show significantly lower growth rate than the case with unrestricted migration or when R was propagated alone (Figure 3d).

Concluding remarks

The past few years have seen an increase in the complexity of synthetic circuits that program novel behaviors. The increased complexity of synthetic circuits will allow biologists to address increasingly complex ecological and evolutionary interactions. While the above examples examine relatively simple ecological interactions, natural communities are usually composed of various types of interactions, which occur on different time and spatial scales. As such, we are only beginning to decipher the complexity of microbial ecosystems, which have tremendous implications for our health, environmental and industrial applications [35,50–52]. The current set of synthetic ecosystems has provided a strong foundation for this growing area of study. In the future, synthetic biology will continue to play an important role for deeper understanding of microbial ecology and evolution. In the long run, such understanding will be critical for devising effective antimicrobial strategies and for engineering microbial consortia for practical applications.

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Figure 1.

A set of complementary approaches toward quantitative, mechanistic understanding of cellular processes.

- a) A mathematical model consists of a set of equations and is the most abstract. It is also a closed system in the sense that the system is governed only by the equations that are of interest to the researchers (green).
- b) In synthetic biology, a system of interest (green) is programmed using well-defined components and interactions (orange). It may be engineered to be highly controllable. The resulting behavior is largely attributable to the synthetic system with reduced interference from organism's natural components and interactions (grey).
- c) A system of interest (green) in a natural context is often a part of larger system and likely affected by known (solid) and unknown (dotted) interactions.

Figure 2.

Engineering synthetic microbial systems to analyze mutualistic interactions.

a) General concept of public-good cooperation and cheating. Cooperators (red) produce public goods (yellow triangles) at a cost. Individuals who share the same environment gain a benefit from public goods. This includes cheaters (blue) who avoid the cost of public-good production.

b) Public-good cooperation as a driving force of evolution of multicellularity. In the spatial domain, the concentration of public good (yellow) forms a gradient around cooperators. This gradient becomes higher when cooperators aggregate (left) than when they are dispersed (right).

c) General logic of interspecific cooperation through metabolite exchange. Red and green cells release a metabolite (yellow and blue, respectively) that is required for the growth of the other cell.

Figure 3.

Engineering synthetic microbial systems to examine competitive interactions.

a) General logic of a chemically mediated predator-prey ecosystem. Growth of the predator population causes a decrease in growth of the prey population. As the density of prey population is decreased, the predator population also decreases, allowing the prey population to grow again. Both predator and prey secrete quorum-sensing molecules (purple circles and orange triangles, respectively) to modulate each other's growth.

b) When the length scale of the predator-prey interaction is comparable to the segregation distance of the two populations, increasing motility decreases biodiversity. Green arrows represent different motility rates qualitatively.

c) General logic of a rock-paper-scissors ecosystem. The colicin-producing population (P) outcompetes the colicin-sensitive population (S), which outcompetes the colicin-resistant population (R). The R population can outcompete the P population.

d) Evolution of growth rate is restrained when a community of rock-paper-scissor population is allowed to evolve with restricted cell movement (bottom). The evolved growth rate is higher when the community is evolved with unrestricted cell movement or a single cell type is evolved (middle and top, respectively). Green arrows represent different movement ability qualitatively.