Eco-evolutionary dynamics of complex social strategies in microbial communities

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Microbial communities abound with examples of complex social interactions that shape microbial ecosystems. One particularly striking example is microbial cooperation via the secretion of public goods. It has been suggested by theory, and recently demonstrated experimentally, that microbial population dynamics and the evolutionary dynamics of cooperative social genes take place with similar timescales, and are linked to each other via an eco-evolutionary feedback loop. We overview this recent evidence, and discuss the possibility that a third process may be also part of this loop: phenotypic dynamics. Complex social strategies may be implemented at the single-cell level by means of gene regulatory networks. Thus gene expression plasticity or stochastic gene expression, both of which may occur with a timescale of one to a few generations, can potentially lead to a three-way coupling between behavioral dynamics, population dynamics, and evolutionary dynamics.

Introduction

Microbes are not only social organisms,1 but they may also be strategic gamers. Many instances have been found in recent years of evolutionary games, situations where the fitness of a particular microbe depends on the complex interplay between its actions (its strategy) and those of the other microbes in the population. To name a few examples, the budding yeast S. cerevisiae plays a snowdrift game when collectively degrading complex extracellular sugars;2–5 Pseudomonas fluorescens play a stag-hunt, or coordination game when they form biofilms on the air-water interface;6 signaling games allow Vibrio fischeri to coordinate via low cost quorum sensing molecules before making energetically expensive investments, such as synthesizing bioluminescent proteins within jellyfish.7

Most research on microbial games has indeed focused on their evolutionary importance. However, it is becoming increasingly clear that these social interactions may also have important ecological consequences. Due to the rapid evolutionary dynamics that are typical in microbes, and to the fact that the payoffs of the different strategies in a social game often depend on the size of the population,8–11 the ecological and evolutionary dynamics may be strongly coupled via feedback loops. Therefore, the outcomes of the social games played by microbes may have ecological, as well as evolutionary consequences, and the two need to be studied together in a unified approach: that of eco-evolutionary dynamics.

A second important aspect of microbial social dynamics is the fact that microbes can implement complex social strategies. While they lack a central nervous system to help them make decisions, microbes have gene regulatory networks that allow them to adopt different phenotypic states in response to their environment and the actions of other microbes.3,12 This genetically coded ability to adopt different phenotypes in response to the actions taken by the other cells in the population, allows these relatively simple unicellular organisms to go beyond simple (i.e., always cooperate with others, always defect) evolutionary strategies. Interestingly the behavioral process of selection of a phenotype among the many alternatives allowed by the genotype, (a process that is often referred to as “cellular decision making”13), also occurs with timescales that are comparable to those of population and evolutionary dynamics; for instance, switching between alternative phenotypic states can occur with timescales in the order of one to a few generations.14 In consequence, there is the potential for microbial behavioral dynamics to interface with population and evolutionary dynamics,15 in a complex three-way feedback loop whose consequences are yet unknown.

The goal of this mini-review is to put the spotlight onto these two aspects of microbial sociobiology: first, we discuss recent findings on the interplay between ecology and evolution of social microbial traits. In the second part, we discuss how complex strategies may be implemented by gene regulatory networks, and hypothesize what their effect may be on the evolutionary dynamics of social behaviors in microbial communities, and how they may affect ecological interactions and rewire ecological networks in a short timescale.

Eco-Evolutionary Dynamics of Social Microbial Traits

Eco-evolutionary dynamics, the study of the coupling between population dynamics and evolutionary dynamics, has been discussed within the scientific community for many years of evolutionary games, situations where the fitness of a particular microbe depends on the complex interplay between its actions (its strategy) and those of the other microbes in the population. To name a few examples, the budding yeast S. cerevisiae plays a snowdrift game when collectively degrading complex extracellular sugars;2–5 Pseudomonas fluorescens play a stag-hunt, or coordination game when they form biofilms on the air-water interface;6 signaling games allow Vibrio fischeri to coordinate via low cost quorum sensing molecules before making energetically expensive investments, such as synthesizing bioluminescent proteins within jellyfish.7

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The transformation of the environment from sucrose dominated to a mainly glucose and fructose dominated environment is achieved cooperatively by a population of invertase expressing cells. As it is often the case with cooperative behaviors, cooperators that break down sucrose can be exploited by non-cooperative “cheaters,” or “free-loaders,” which consume fructose and glucose but do not express invertase. In spite of the competition from cheaters, cooperators have a higher fitness than cheaters when the population density is low, and the medium consists mainly of non-hydrolyzed sucrose. Under these conditions, cooperators replicate faster than cheaters, thanks to the small fraction of glucose and fructose that they are able to capture after sucrose hydrolysis. This leads to the initial evolutionary advantage of cooperators when their density is low, which results in a slow but steady growth in numbers of cooperators in the population. However, as the density of cooperators increases so does the public supply of glucose and fructose. Environments with high concentrations of glucose and fructose favor cheater phenotypes, which can grow rapidly on the glucose and fructose without having to pay the metabolic cost of producing invertase. This leads to an increase in cheaters at the expense of cooperators that eventually reduces the number of cooperators in the population; and with them the public goods (glucose and fructose) to the point where cheating is no longer favorable, and the eco-evolutionary feedback loop can begin again. The consequences of this eco-evolutionary feedback extend beyond iterative dynamics. Under some conditions the equilibrium between cheaters and cooperators can be near the separatrix that divides the eco-evolutionary phase space between stable equilibria and population collapse, making the whole population more prone to extinction in response to environmental perturbations (Fig. 1). It is worth noting here that the possibility that the evolution of cooperation may lead to population collapse has also been experimentally found as well in other single-species microbial ecosystems.

The observed eco-evolutionary trajectories behave just as expected by an ecological public goods game. This may perhaps be surprising, given that the biochemical details that describe the interaction between cooperators and cheaters in yeast, as well as the experimental design (unlike in the model, yeast cells do not form local interaction groups and the system is well mixed) and the physical constraints in these experiments do not adjust to the assumptions by the model. This speaks highly of the ability of this model to capture the essential features of public goods interactions, which are characterized by an Allee-effect-like, non monotonic dependence of the fitness of cooperators on their density, and by a higher fitness of cooperators at low density and a higher fitness of the cheaters at high density. Notably, a simple ad hoc phenomenological model that just captured these two features was also able to reproduce the experimentally observed dynamics. However, the same features emerge naturally and without imposing any ad hoc assumptions from the ecological public goods game studied by Hauert and Doebeli.

In addition to affecting the resilience of a population, evolution of cooperation in sucrose breakdown may affect other ecological parameters. In a recent study, MacLean and Güdeli found that the evolutionary competition between cooperators...
and cheaters had important effects on another ecological parameter: ecosystem productivity (in this case, measured as an increase in total biomass). When considered as a single population, mixed cultures of cheaters and cooperators growing in plates were found to lead to larger net populations than would be achieved by pure cooperators. A similar, though in this case transient effect was also observed in well-mixed populations. In equilibrium, this second study found that both mixed and pure cooperator cultures had very similar population sizes, with the slight advantage being for the pure cooperator populations. The sucrose system has also been used to investigate the interplay between the evolution of cooperation and ecological interactions between different species. In another experiment that explores the effect of ecology on the evolution of cooperation, Celiker and Gore investigated a two-species ecosystem of P. aeruginosa and S. cerevisiae and E. coli growing together in sucrose. The authors found that the presence of the bacteria limit the density of yeast. This ecological effect leads to lower densities of yeast, thus promoting the evolutionary success of cooperation.

**Complex Social Strategies in Microbial Populations**

In the previous section, we discuss the competition between “cooperator” strains that produce a public good, and “free loader” strains that take advantage of it without contributing to its production. However, the picture is more nuanced than this, and microbial social strategies can move more complex than simply “always defect” or “always cooperate.” In spite of their relatively simple nature, even unicellular microbes are able to make sophisticated behavioral decisions and implement complex social strategies, with the aid of gene regulatory networks. These complex strategies are characterized by phenotypic plasticity: the social behavior or phenotype adopted by a microbe depends on the behaviors of the other microbes in the population. In some instances, the social strategies adopted by a microbe may be characterized by a continuum of expression levels of the cooperative genes as a function of the actions of other cells in the population. In other instances, microbes may choose between a set of alternative phenotypes (which can vary in the degree of cooperation), stabilized by (genetic) positive and negative feedback loops. Here we present some examples that have been recently discussed in the literature and which we believe are particularly promising in order to help us understand the evolutionary dynamics of social microbial behavior.

The first example concerns ferric uptake in *P. aeruginosa*. In a set of recent studies, Kümmerli et al. have demonstrated that extracellular iron scavenging proteins are released in greater or smaller levels depending on the amount of iron in the local environment. This finding indicates that cooperative release of iron scavenging proteins is not unconditional, but depends on the environment; an environment that is in turn transformed by the collective actions of all the cells in the population. Phenotypic plasticity also allows microbes to tune their level of investment in a public good in response to the presence or absence of other species in the population and the phenotypes adopted by those other species. For instance, *P. aeruginosa* has been found to upregulate the secretion of iron scavenging molecules when in presence of *S. aerus*, which acts as an interspecific cheater. Finally, the ability to switch phenotypes between the expression of two alternative iron scavenging molecules has also been reported to be superior evolutionarily to conditionally expressing each phenotype in fluctuating environments. Thus, phenotypic plasticity allows *P. aeruginosa* to maximize its fitness by allowing it to differentially adopt a different social strategy depending on the environmental conditions, which in turn may be affected by the combined actions of all cells, leading to the potential for feedback between behavior and ecology.

A second example of complex social strategizing takes us back to the sucrose degradation by *S. cerevisiae*. While the models used to understand cooperation in this system assume cooperation to be constitutive for the sake of simplicity (also note that within the experimental constraints imposed by previous researchers, expression of invertase is approximately constant), it is known that the expression of invertase (and thus the degree of cooperation) can be modulated by the amount of glucose in the environment: the higher the glucose concentration is, the lower the expression of SUC2. This represents a particularly appealing example of a complex social strategy implemented by gene regulatory circuits: As the cells transform their environment by increasing the extracellular concentrations of sucrose and fructose, they can also modify their behavior by reducing the expression levels (and the cost associated to it) of the enzyme invertase. This regulation mimics the winning strategy in a snowdrift game: cooperate when the majority of cells in...
the population are defecting, and defect when they are cooperating. It is possible that this complex strategy (as opposed to a “simple” strategy consisting on unconditional cooperation) may help the cooperators to fare better when competing against the cheaters. Whether or not this is the case, and what the “optimal” gene regulatory function would be in the presence of cheaters, remains to be investigated and represents a promising area of future research.

Although most studies of sucrose breakdown cooperation in \textit{S. cerevisiae} focus on the invertase gene \textit{SUC2}, other members of the SUC family provide similar functionality and may present a unique opportunity for evolutionary studies. With the exception of \textit{SUC2}, members of the SUC family are located in the sub-telomeric region. It has been found that SUC genes in these subtelomeric regions can be epigenetically silenced. This epigenetic silencing represents an important form of phenotypic plasticity from the perspective of evolutionary theory: it allows lineages to temporarily deviate from their genetically-encoded plasticity from the perspective of evolutionary theory: it allows epigenetic and genetic means. This possibility remains to be investigated and represents a promising area of future research.

Discussion: Ecological Implications of Cellular-Decision Making in Social Behaviors

Gene regulatory networks allow microbes to implement complex social strategies; these include both continuous tuning of the investment in a public good by the regulation of cooperative genes, as well as the choosing among alternative, discrete phenotypes driven by stochastic switching. In both of these instances, the dynamics of phenotype switching can occur within a timescale of one to a few microbial generations, which is comparable to the timescale of evolutionary and population dynamics. Therefore, it is conceivable that phenotypic dynamics (changes in the adopted phenotype at the level of a single cell) may also be coupled with ecological and evolutionary dynamics.
in a three-way feedback loop. Furthermore, we believe that phenotypic dynamics of social traits may have profound ecological implications, allowing for rapid re-wiring of microbial ecological networks over a timescale of just one or a few generations.

Two possible scenarios where phenotypic dynamics may affect eco-evolutionary dynamics are illustrated in Figures 2 and 3. In the first such scenario we consider a synergistic “public goods” interaction between two bacteria, which cross feed each other via the secretion of extracellular glycosidases that break down two different polysaccharides (Fig. 2). The sudden arrival of a better carbon source (perhaps secreted by a third species) may make it unnecessary to pay the cost of secreting the enzymes. Instead of cooperating, the two species would now compete for the higher quality sugar. This hypothetical situation is quite plausible; for instance, catabolite repression is a widespread regulatory process by which bacteria shut down operons responsible for metabolizing lower-quality sugars when a better quality sugar is available. It is thus likely that synergistic interactions can be suddenly transformed into competitive interactions by the repression of single operons on the mutualistic species. A second attractive possibility is suggested by recent experiments that indicate that very simple mutational processes can transform a transcriptional activator into a transcriptional repressor, or turn a constitutive gene into a regulated one. As before, it is plausible that evolutionary changes in cellular decision making circuitry could rapidly re-wire ecological networks within a very short time. For instance, consider the case of a bacterium that contains more than one “public good” gene; to keep with the previous example, consider these code the expression of two different exoenzymes that break down two different polysaccharides releasing nutrients to the environment. Rather than secreting the two exoenzymes constitutively at the same time, a bacterium can “learn” to secrete them sequentially, thanks to mutations that allow the cells to downregulate the expression of one of the exoenzymes when the other exoenzyme is being expressed (See Figure 3). This would in turn affect any commensal bacteria that depend on the monosaccharides released by the producer strain. Thus, without the need to lose a social gene, and just by acquiring the ability to regulate its expression, a bacterium can change its ecological interactions with the various partners with which it coexists.

These two examples are just illustrations of what the consequences of coupling between population, evolutionary, and phenotypic dynamics may be in microbial communities. While we have no direct evidence of this three-way feedback yet, many results, particularly those reviewed above concerning plasticity in the expression of iron scavenging proteins, suggest that phenotypic dynamics occurring over a one to a few generations timescale may be a major force in determining social interactions between microbes.

The specific examples of complex social interactions that we have discussed above come mainly from laboratory experiments with exceedingly simple, single-species ecosystems. The advantage of these reductionist experiments is that they allow us to put theoretical predictions to the test, and thus allow us to advance in firmer ground by benchmarking our theoretical understanding of eco-evolutionary dynamics in social microbial communities. There is every reason to believe that these interactions are very important in natural communities as well. Processes such as the decomposition of complex organic matter involve the secretion of extracellular enzymes that break down this complex matter and release smaller nutrients, which become public goods. For instance, bacteria such as Akkermansia muciniphila (an important commensal of the human gut and a biomarker for a healthy intestinal track) grow in the human colon by breaking down the complex human glycoproteins that form the matrix of the intestinal mucosa. Since the small sugars released by the action of these enzymes may benefit other species (as well as potentially “cheater” A. muciniphila mutants), public goods interactions similar in nature to those in the sucrose system in yeast may likely ensue. Similar public goods mediated ecological interactions also take place in oral cavity: several Streptococcus species secrete extracellular glycosidases that break down salivary mucin glycans and allow the oral microbiome to survive even periods of prolonged starvation. The fact that bacteria may adopt complex (in this case probabilistic) social strategies in the wild is supported by the observation of stochastic expression of a lethal strategy (a fraction of cells commit suicide to allow other members of a clonal population of Salmonella typhimurium to infect the host). In this case, stochastic phenotypic switching is critical for the “self-destruction cooperation” strategy to be successful.
Microbes represent the majority of biomass on Earth, and they are most commonly found forming complex communities consisting of many different species interacting with each other. A small number of microbial ecologies have been discussed in this review, exemplifying the complex strategies often followed by seemingly simple unicellular organisms, and the eco-evolutionary dynamics resulting from these social interactions. While the game dynamics of some of these systems have been confirmed, most still require quantitative evidence of game dynamics. By understanding microbial games quantitatively, not only will we further understand the role of microbes within us from teeth to gut, but our own role in interacting with these strategic organisms. After all, pound-for-pound microbes occupy more of this planet than humans.

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