COMMENTARY

The Origins of Cooperative Bacterial Communities

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ABSTRACT Bacteria live in complex multispecies communities. Intimately interacting bacterial cells are ubiquitous on biological and mineral surfaces in all habitats. Molecular and cellular biologists have unraveled some key mechanisms that modulate bacterial interactions, but the ecology and evolution of these associations remain poorly understood. One debate has focused on the relative importance of cooperation among cells in bacterial communities. Some researchers suggest that communication and cooperation, both within and among bacterial species, have produced emergent properties that give such groups a selective advantage. Evolutionary biologists have countered that the appearance of group-level traits should be viewed with caution, as natural selection almost invariably favors selfishness. A recent theory by Morris, Lenski, and Zinser, called the Black Queen Hypothesis, gives a new perspective on this debate (J. J. Morris, R. E. Lenski, and E. R. Zinser, mBio 3(2):e00036-12, 2012). These authors present a model that reshapes a decades-old idea: cooperation among species can be automatic and based upon purely selfish traits. Moreover, this hypothesis stands in contrast to the Red Queen Hypothesis, which states that species are in constant evolutionary conflict. Two assumptions serve as the core of the Black Queen model. First, bacterial functions are often leaky, such that cells unavoidably produce resources that benefit others. Second, the receivers of such by-products will tend to delete their own costly pathways for those products, thus building dependency into the interactions. Although not explicitly required in their model, an emergent prediction is that the initiation of such dependency can favor the spread of more obligate coevolved partnerships. This new paradigm suggests that bacteria might often form interdependent cooperative interactions in communities and moreover that bacterial cooperation should leave a clear genomic signature via complementary loss of shared diffusible functions.

> *Evolutionary adaptation is a special and onerous concept that should not be used unnecessarily, and an effect should not be called a function unless it is clearly produced by design and not by chance.*
> -George C. Williams (7)

Much of our understanding of bacteria is from studies of pure cultures. In some cases, single strains of single species have come to represent what scientists know about diverse bacterial lineages. Yet in nature, bacteria almost always exist in complex communities of other species that can be predators or prey, as well as competitors, commensals, mutualists, or pathogens. Despite our appreciation of the ubiquity and importance of bacterial communities, little is known about the cell-cell interactions that form these multispecies consortia. Molecular and cellular biologists have unraveled some of the key mechanisms that modulate bacterial interactions, but there is little understanding of the relative importance of cooperation and conflict in these associations. It is critical to understand the potential for cooperation among bacteria if we are to successfully manage multispecies infections (whether harmful or beneficial to the host) or if we are to engineer bacterial consortia to perform valuable biological tasks. It is also important to discern how and when natural selection can shape cooperation among bacteria. Toward these ends, the opinion piece by Morris, Lenski, and Zinser (1) offers a new perspective on the ecology and evolution of bacterial cooperation. They present a model that reshapes a decades-old idea: that cooperation among individuals can be automatic and based on little more than the production of by-products (e.g., see references 2 and 3). Beyond this core, the model has clear and testable predictions germane to bacterial genomic evolution, the design of novel *in vitro* methods, and the assembly and stability of bacterial communities. To appreciate the utility of this new model, it is important to introduce a recent controversy over bacterial cooperation.

One school of thought is that cooperation among bacteria has produced emergent properties in bacterial communities. Research on two systems, bacterial biofilms and quorum sensing, offers good examples. Biofilms are clonal or multispecies groups of cells defined by their secreted polymers, which help to glue cells together (4). Biofilm communities exhibit several properties that are hypothesized to require molecular communication and cooperation among participating bacteria, including differentiation among cells to produce highly resilient biofilm structures, species stratification to optimize productivity, and the formation of channels to maximize nutrient and oxygen flow (5). In a similar vein, emergent group properties have been highlighted in studies of bacterial quorum sensing, a process in which bacteria secrete and detect diffusible molecular signals to express traits in a context-dependent fashion, usually among members of the same species (4). Detection of released signals in cell groups is thought to allow bacteria to perform functions that would be beneficial only in large populations of cells. Classic examples include the group release of plant-cell-degrading compounds by bacterial phytopathogens and group secretion of virulence compounds in animal pathogens. The idea is that quorum sensing allows the pathogenic traits to be expressed only when the bacterial population numbers are high enough to overwhelm host defenses (4). Thus, both in

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biofilms and in quorum sensing, the paradigm has been that evolution has shaped cooperation to maximize the greater good of the group. Yet evolutionary biologists have called ideas of bacterial cooperation into question. The central evolutionary argument is that the relentless action of natural selection favors selfish behavior (6) and that group-level adaptations should be analyzed with care and invoked only when necessary (7). For instance, individual-based models of bacterial movement and attachment can lead to biofilm-like structures in computer simulations, inconsistent with the hypothesis that cell-cell communication is needed to create them (8). Moreover, quorum-sensing traits have also been modeled on the simple terms of individual cell action. Under the "diffusion-sensing" model, single cells selfishly monitor levels of costly diffused products as opposed to coordinating their activities with other cells (9). A difficulty for clarifying predictions for cooperation in bacterial communities has been the lack of predictive models specific to microbe-microbe interactions.

The Black Queen Hypothesis by Morris and colleagues (1) makes two basic assumptions about bacterial biology that build a foundation for cooperative community evolution. The first assumption is that some bacterial functions are often leaky. Specifically, bacteria unavoidably produce publicly usable resources, which become available to their local community. This assumption closely mirrors classic models of "by-product mutualism" (2, 3), in which one individual produces a by-product as an automatic effect of its biology and thus enhances the fitness of other individuals that can use that product. By-product mutualism might not seem like a typical form of cooperation, since the "cooperative" phenotype carries no cost and because the trait need not evolve in the context of the interaction. Yet by-products fit well into a broad definition of cooperation: a trait in one individual that increases another's fitness (10). The second assumption of the model is that a bacterium that uses another's by-products will benefit from deleting its own costly pathways for those products. Adaptive genome streamlining is known to be common in parasites and symbionts, both of which can benefit from deleting costly functions that are provided by their hosts. When gene loss of vital functions occurs, this builds dependency into the interactions (11). Importantly, these assumptions and the model as a whole can be applied to cooperation within or among bacterial species. Nonetheless, by-product mutualisms based upon resources most often occur among species, since they tend to exhibit divergent resource usage.

Morris and colleagues support their assumptions with a diversity of sources. For instance, many empirical studies have shown that bacteria produce by-products that are useful to other cells. Examples of leaky systems include catalase-peroxidase activity (enzymes that break down toxic hydrogen peroxide), production of iron chelators (which make iron soluble for metabolism), and the reduction of sulfur (which is often growth limiting). Such leakiness might be unavoidable because of the nature of diffusion gradients and the need for bacteria to maintain membrane permeability. Morris and colleagues also highlight potential links between by-product utilization and adaptive gene loss. Some very elegant evidence comes from recent studies of the marine bacterium *Prochlorococcus*. This species is fascinating because it cannot grow in its own natural habitat (sunlit seawater) as a pure culture (12). *Prochlorococcus* becomes poisoned under these conditions by hydrogen peroxide (which is generated by photooxidation). However, poisoning fails to occur in natural habitats because *Prochlorococcus* grows commensally with catalase-peroxidase-producing species, such as *Synechococcus*, which brings to the table enzyme activity that permeates its membranes and eliminates hydrogen peroxide in the local environment (13). Phylogenetic data suggest that the *Prochlorococcus* lineage ancestrally exhibited catalase-peroxidase function but that it has been subsequently lost, consistent with the hypothesis of adaptive streamlining (1).

Is this combination of by-product utilization and genome streamlining common in bacterial communities? If so, are most bacteria tied into obligate dependence upon partners in coevolved consortia? Morris and colleagues suggest that such interdependence is common and hypothesize that the failure of many bacteria to grow in pure culture is caused by obligate interspecies dependencies. They further suggest that whole bacterial communities could depend on rare keystone taxa that provide vital resources for many other species. The extinction of such a species would have dramatic effects on bacterial communities (8). This degree of bacterial cooperation might seem farfetched to some, but extensions of the classic by-product mutualism models suggest a clear route to the expanded evolution of such interdependence. One such extension, termed by-product reciprocity, predicts that natural selection will shape receivers of by-products to maximize these benefits by being cooperative to by-product producers (10, 14). Coupled with the two key assumptions of the Black Queen Hypothesis, this suggests a third step in which simple by-product interactions evolve into more-complex associations based upon costly resource exchange. Thus, the predicted evolutionary steps are as follows: (i) selfish usage of another species' by-products, (ii) genome streamlining to minimize production of resources that can be gotten from others, and finally (iii) the evolution of costly cooperative traits to maximize vital functions produced by others (14).

The Black Queen Hypothesis has implications for bacterial genome evolution, for the development of new *in vitro* techniques, and finally for the origins and stability of bacterial consortia. In terms of gene content, the model suggests that bacterial genomes should exhibit signatures of deletion mutations in gene pathways that produce transferable resources. Loss-of-function mutations are predicted to be most widespread among intimately interacting bacteria, such as in biofilm communities and rhizosphere consortia. The model suggests that within these communities deletions might often occur in a complementary fashion among interacting bacteria. Moreover, specific taxa might exhibit different loss-of-function deletions among populations that vary in community content. In terms of studying bacteria *in vitro*, taxa that cannot be grown in pure culture might be successfully cocultured with key interacting "helper" species (e.g., see reference 15). Finally, in terms of bacterial community assembly and stability, the model suggests that obligate interspecific interactions should be much more common in bacteria than previously suspected. Although these interactions are predicted to originate via simple selfish steps, such as by-product production and genome streamlining, these prerequisites can begin an evolutionary cascade towards more-intimate interactions. Once dependency has evolved—through the deletion of a vital pathway provided by another taxon—the evolution of costly cooperative phenotypes to maintain and optimize these interactions becomes much more likely (14). The Black Queen Hypothesis reminds us of Darwin's dictum that natural selection favors selfishness above all else. Cooperative

traits must result in fitness benefits to the individuals expressing those traits.

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