Review

Black Queen evolution: the role of leakiness in structuring microbial communities

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Black Queen (BQ) functions are biological processes that yield neither purely private nor purely public products. This partitioning of benefits, also called 'leakiness', can produce negative frequency dependence of fitness in microbial communities, allowing coexistence between function-performing helpers and function-requiring beneficiaries. The ubiquity of leakiness favors a 'race to the bottom' as members of a community lose the ability to perform functions whose products are available from the environment. Rather than being social altruists, helpers are merely those populations that lost this race and got stuck in their role as function performers. Here I discuss many such BQ functions and the microbial communities that evolve around them. I also compile evidence from laboratory evolution experiments as well as phylogenetic reconstructions that show that organisms gain greater fitness increases from gene/function loss events than is commonly expected. Finally, I consider possible consequences of long-term BQ-stabilized coexistence, including sympatric speciation and the evolution of true mutualisms.

When less is more

Organisms that are obligately dependent on the activities of other organisms for survival are abundant in nature. Humans, for instance, require the provision of 13 vitamins and nine amino acids all of which are manufactured by other species that are consumed as part of our diet. Some of these resources, such as vitamins B1 and B12, are exclusively produced by bacteria, yet they are almost universally required by eukaryotes for core metabolic functions. Similar dependencies are commonly observed among microbes. In the case of symbiotic bacteria of insects and other animals, metabolic dependencies are often explained by the intimate contact between microbe and host [1]. However, dependencies are also widespread in free-living microorganisms. In both of these cases, the underlying cause of dependency is the evolutionary loss of particular genes in the dependent population. In the case of obligate endosymbionts, small population sizes and isolation from resupply of lost genes via horizontal gene transfer (HGT) can lead to gene loss from genetic drift [1]. In larger and more cosmopolitan populations, however, gene loss is probably driven by natural selection favoring resource conservation [2].

Here I discuss the BQ Hypothesis [3], which describes a selective force that allows some microbes to lose essential genes (Figure 1). When a community of organisms is dependent on a BQ function – that is, a function that is both essential and 'leaky', or partially available to the broader community – simple one-step inactivating mutations such as gene deletions can produce mutants that no longer perform the function and are able to invade and stably coexist with strains that retain the function. Communities structured in this manner are said to comprise function-performing 'helpers' and function-deficient 'beneficiaries'. In this review, I explore different types of leaky functions and consider evidence for the coexistence of helpers and beneficiaries for BQ functions in nature. I also discuss the generality of BQ evolution given the fitness effects of deletions and consider how long-term BQ-stabilized interactions might influence the development and evolution of microbial communities.

Helpers and beneficiaries: a microbial take on the tragedy of the commons

One of the first reports of intermicrobe dependency noted that *Haemophilus influenzae* grew on blood agar only as 'satellite colonies' at the periphery of colonies of other microbes [4]. Extracts from killed bacteria were even more effective facilitators of *H. influenzae* than live cells, suggesting that some diffusible enzyme was critical. Similar satellite colonies are a familiar nuisance to researchers using ampicillin resistance as a selectable marker [5] and have also been observed in experiments with iron-requiring [6] and hydrogen peroxide (HOOH)-sensitive bacteria [7,8]. Beneficiary microbes dependent on diffusible products produced by helpers appear to be very widespread in nature. For instance, most microcolonies from a soil community developed more quickly when other community members were growing nearby [9]. Many previously unculturable bacteria have been induced to grow in the laboratory using coculture either with mixed natural communities [10] or with specific helper microbes [6,8,11]. In many cases, including *H. influenzae* as well as the abundant marine microbes

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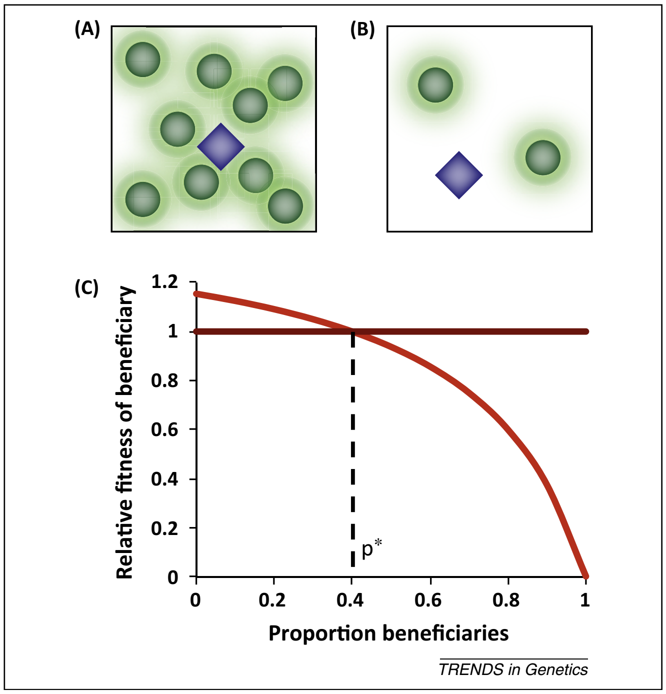

Figure 1. The Black Queen Hypothesis and negative frequency dependence of fitness. Helper cells leak a product (represented here by green halos) required by all community members. **(A)** When helpers (round cells) are abundant, beneficiaries (diamonds) have ready access to the leaked product. **(B)** When helpers are scarce, the leaked product is scarce and beneficiaries might not be able to access it. **(C)** As beneficiaries invade a population, their fitness relative to helpers (curved red line) decreases due to their lack of the leaked product. As long as beneficiaries have relative fitness >1 when rare and <1 when common, they will coexist with helpers. In this figure, the point where the curved line intersects the horizontal line indicates the equilibrium proportion of beneficiaries (p^*, shown by the broken black line).

Box 1. Cheaters versus beneficiaries

The literature on the evolution of public goods production often discusses the dynamics between cooperators and cheaters, roughly analogous to BQ helpers and beneficiaries. However, the two dichotomies are different in four important ways.

(i) In many cases leakiness is an inevitable characteristic of a function, due to either the chemical nature of the products or the required location of the reactions. In these cases production of public goods is not an act of cooperation but rather an unavoidable cost of doing business.

(ii) Although some classical examples of social cheating are also examples of BQ interactions (e.g., secretion of the enzyme invertase by yeast [24]), others are not. For instance, social functions that cause the individuals performing them to die or forgo reproduction (e.g., fruiting body formation in *Myxococcus xanthus* [96]) have no private benefit for the producer and therefore are not BQ functions.

(iii) The influence of many leaky functions extends well beyond any reasonable social context (e.g., see the discussion of N fixation in the text, where the cumulative activity of helpers over decades or centuries ultimately allows beneficiaries to thrive). In the absence of direct interactions between helpers and beneficiaries, the term 'cheater' is not particularly apt.

(iv) Whereas the verb 'cheat' is rarely used in common language except in a pejorative manner to describe active exploitation, 'help' is often used to describe passively beneficial activities or processes. For instance, one might say 'Yesterday's rain really helped my garden' without implying that the garden encouraged the rain to fall, that the rain benefitted from the garden's improved growth, or that any form of two-way interaction existed between the rain and the garden.

Thus, there is overlap between theories of social cheating and BQ evolution, but neither is a subset of the other.

Prochlorococcus and *Pelagibacter*, beneficiary status is associated with greatly reduced genome size relative to helpers [2,12,13], suggesting that gene loss may underlie dependence.

Superficially, interactions between helpers and beneficiaries appear altruistic and are therefore difficult to explain from an evolutionary perspective (Box 1). If beneficiaries gained a growth advantage by losing a function, what prevents the helpers from doing the same thing? Traditional evolutionary theory predicts that unless helpers are able to restrict access to the products of the leaky function to other helpers, the function will be lost from the community even if all community members suffer – a phenomenon known as the tragedy of the commons [14]. Microbes can avoid the tragedy of the commons by growing in structured environments surrounded by their close relatives (i.e., through kin selection) [15], but many of the best-characterized helper/beneficiary interactions involve pelagic marine bacteria that grow in environments with no spatial structure. How, then, can helpers and beneficiaries stably coexist in such an environment in the light of evolution?

One way that the tragedy of the commons can be avoided in well-mixed environments is through negative frequency dependence of fitness (Figure 1). When two competing organisms interact in such a way that their relative fitness is a function of their relative abundance, it becomes possible that the two can coexist even in the absence of relatedness. When essential leaky products are abundant in the environment (i.e., when beneficiaries are rare relative to helpers), beneficiaries gain a growth advantage equivalent to the cost of producing those products. However, as the beneficiaries become more common relative to the helpers, the leaky product becomes rarer and the fitness of the beneficiaries decreases. As long as each has a higher growth rate than the other when rare, the fitnesses of the two types will be equal at some ratio and a stable equilibrium ratio will be reached (Figure 1C). Because the loss-of-function mutations leading to beneficiary status are so easily obtained, leaky functions can be seen as reliable generators of negative frequency dependence and consequently act to create and preserve genetic diversity within microbial communities.

What is leakiness and where does it occur?

Theoretical discussions about the evolution of public goods production often consider functions to produce either entirely public or entirely private goods (Box 1). In reality, all functions occupy a spectrum between these two extremes; that is, they exhibit some degree of leakiness (Figure 2). One can envision four broad classes of function whose natures tend toward greater leakiness: (i) functions with membrane-permeable products; (ii) functions that occur outside the cell; (iii) functions whose products outlive the cells that make them; and (iv) functions that physically modify the organism's habitat. I consider examples of each below.

Functions with membrane-permeable products

Biological membranes are intrinsically permeable to small, uncharged molecules and some leakage is inevitable when