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Is "Wolf-Pack" Predation by Antimicrobial Bacteria Cooperative? Cell Behaviour and Predatory Mechanisms Indicate Profound Selfishness, Even when Working Alongside Kin

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For decades, myxobacteria have been spotlighted as exemplars of social "wolf-pack" predation, communally secreting antimicrobial substances into the shared public milieu. This behavior has been described as cooperative, becoming more efficient if performed by more cells. However, laboratory evidence for cooperativity is limited and of little relevance to predation in a natural setting. In contrast, there is accumulating evidence for predatory mechanisms promoting "selfish" behavior during predation, which together with conflicting definitions of cooperativity, casts doubt on whether microbial "wolf-pack" predation really is cooperative. Here, it is hypothesized that public-goods-mediated predation is not cooperative, and it is argued that a holistic model of microbial predation is needed, accounting for predator and prey relatedness, social phenotypes, spatial organization, activity/specificity/transport of secreted toxins, and prey resistance mechanisms. Filling such gaps in our knowledge is vital if the evolutionary benefits of potentially costly microbial behaviors mediated by public goods are to be properly understood.

1. Introduction

Predatory bacteria are becoming increasingly recognized as the apex predators of microbial communities, honed by evolution into proficient killers of other microbes.[1–3] Some micropredators, such as *Herpetosiphon* spp. and myxobacteria, are able to kill a broad range of prey, including Gram-negative bacteria, Gram-positive bacteria, and fungi.[4–6] Such broad range predation involves the secretion of toxic materials (hydrolytic enzymes and secondary metabolites) into the extracellular milieu, which can, therefore, be considered public goods—a shared resource produced communally.[7–9] This mechanism of predation is therefore often referred to as "wolf-pack" predation, as it apparently requires high densities of predatory cells, and is social—i.e., all predators contribute to the same public goods.[10]

Nevertheless, the molecular mechanisms of social predation are relatively poorly understood in myxobacteria and other microbial wolf-pack hunters. It is known that myxobacteria secrete enzymes with antibacterial activity,[11] and that the outer membrane vesicles (OMVs) they secrete are also intrinsically antimicrobial.[12] However, even though they have low-complexity proteomes,[13,14] we largely do not know which OMV cargo proteins are responsible for killing a particular prey, nor (except in a few specific cases) can we explain why a particular prey is more or less susceptible to communal attack by myxobacteria.[15–18]

In contrast, we know considerably more about the molecular mechanisms and evolutionary forces at play during fruiting body formation, a cooperative process that myxobacteria engage in when prey is scarce.[19]

1.1. Myxobacteria Respond Socially to Starvation

Nutrient limitation triggers populations of the model myxobacterium *Myxococcus xanthus* (*M. xanthus*) to engage in multicellular development, which requires cellular differentiation as well as a minimum population size. Upon starvation, around 100 000 cells aggregate into a raised mound (fruiting bodies), within which about 10% of the cells sporulate.[19] It is thought that fruiting body sporulation ensures that when prey becomes available again, a predation-efficient population of germinants will emerge, rather than a predation-inefficient single germinant.[20]

During development, cells differentiate into discrete cell types. Peripheral rods around the base of the fruit seem to have

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a role in "scouting" for nutrient availability, whereas most cells entering the fruit are destined to autolyze, providing energy for surviving cells to complete sporulation and differentiate into spores.[19] Such complex multicellular behavior requires sophisticated regulation, with coordinated intracellular and intercellular signaling. Myxobacteria consequently have a huge regulatory potential, through a multitude of two-component system signaling pathways and Ser/Thr kinases.[21–23]

During nutrient limitation, each cell senses its own starvation and communicates it to other cells in the population.[24] Intercellular signaling is mediated by the "A-signal," which is a secreted mixture of peptides, proteases, and amino acids. The A-signal is a "quorum signal," as the amount secreted reflects the number of starving cells present,[24] and development only proceeds if sufficient cells (a quorum) are available to form a fruiting body. Later stages of development require the coordination of motility (by regulating the frequency of reversals in the gliding direction) and sporulation, and this is orchestrated by the "C-signal." The C-signal is a cell surface-associated signal that stimulates signaling in other cells upon cell–cell contact.[25,26] Thus, the C-signal responds to cell density, which progressively increases during development as cells aggregate. Only at the high cell densities found within a fruiting body is enough C-signaling possible for sporulation to commence.[26]

Development is thus cooperative, and although 90% of cells forming a fruiting body are destined to undergo programmed cell death (autolysis), they thereby provide the energy and nutrients required by the remaining 10% to sporulate.[27] However, this makes fruiting body formation vulnerable to "cheating" genotypes. For example, a genotype that undergoes autolysis at a frequency <90% will be disproportionately represented among the spores within a fruit and in the resulting population of germinants. The fitness advantage of the cheat depends on its relative frequency in the population.[28] When a cheat constitutes a substantial proportion of the population, the number of cells undergoing autolysis can be reduced to a point where none of the surviving cells in the population are able to sporulate. Cheating genotypes emerge spontaneously, are abundant in natural populations, and can drive entire populations to extinction.[29] Thus, cheaters represent a burden on populations that social organisms need to mitigate.[30]

1.2. Myxobacterial Predation Is also Communal

Myxobacterial predation is performed by groups of neighboring cells and employs a wolf-pack mechanism; the population of predators secretes public goods (antimicrobial material) into the commons (a public space where public goods can accumulate). That cooperativity is intrinsic to feeding was first proposed by Rosenberg et al.,[20] who showed that the *M. xanthus* growth rate increased with cell density in medium containing protein (casein) but not medium containing amino acids (hydrolyzed casein). The amount of secreted protease and hydrolyzed casein

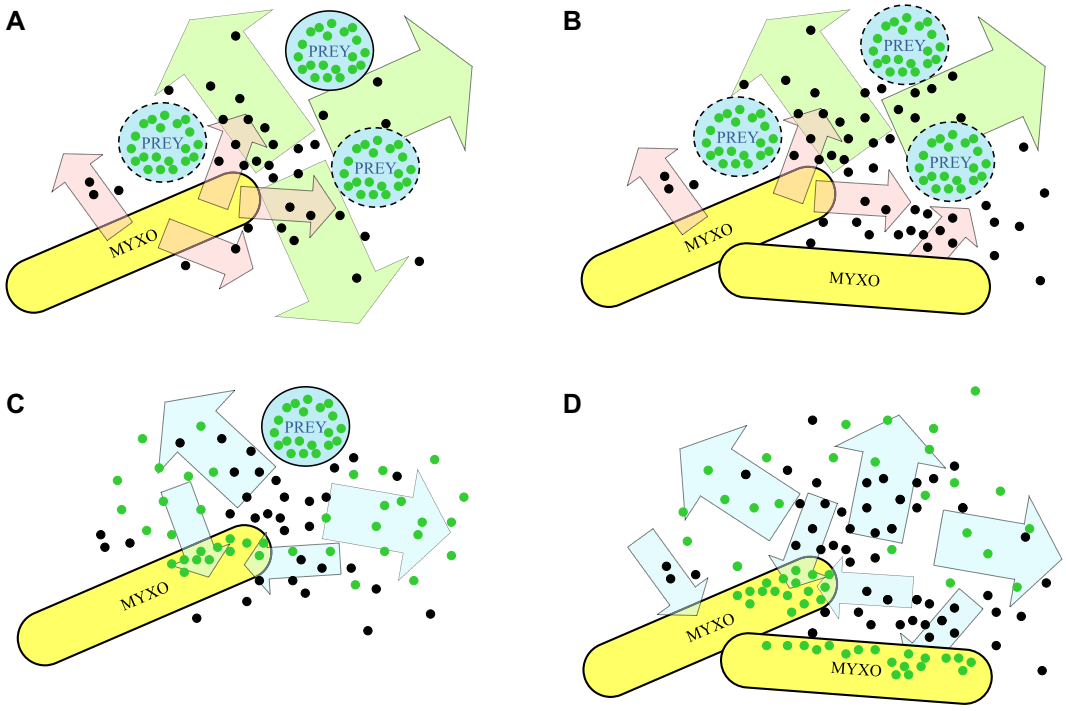

Figure 1. A) Secretion (red arrows) of antimicrobial substances (black circles) that diffuse away from (green arrows) the producing myxobacterial cell (Myxo). Neighboring prey cells (blue) are killed (dashed boundary). B) Dead prey release nutrients (green circles) into the medium and some are taken up by the predator (blue arrows). C) Increasing the number of predator cells increases the secretion of antimicrobials, which kill prey at a greater distance (including the topmost prey cell). D) More nutrients are released, although on average further from the producing cells, and these are shared by the predators.

was proportional to the cell number, leading to the proposal that communal secretion of protease increased the conversion of protein into amino acids that fueled cell growth. The relationship between growth and casein hydrolysate concentration was hyperbolic, giving rise to density-dependent stimulation of growth, interpreted as evidence of cooperativity.[20]

Myxobacteria are surface-dwelling organisms, incapable of swimming through liquid media. Although the Rosenberg et al. study was undertaken in a shaken liquid culture, it seems straightforward to apply its findings to predation on a surface (**Figure 1**). A *M. xanthus* cell secretes antimicrobial substances (OMVs, secondary metabolites, proteases, etc.) into the commons, which then migrate away from the producing cell by diffusion and other transport phenomena. Neighboring prey cells lyse and also release nutrients into the commons, some of which would be taken up by the predator cell. Doubling the number of predatory cells doubles the amount of predatory secretions, killing more prey and allowing the uptake of more nutrients (Figure 1).

However, if the number of predators was doubled, more than twice as many preys would need to be killed for each predator to benefit by acquiring more nutrients than if it was feeding alone (Figure 1). This apparent difficulty can be resolved conceptually in several ways. If prey lysis requires a threshold concentration of predatory secretions, doubling the number of predators in an area might be required to exceed that threshold concentration, or as secretions diffuse away, it might be more than double the area over which the threshold is exceeded (**Figure 2**), potentially killing more than twice as many preys, thereby benefitting the secretor and its neighboring cells.[31] However, doubling the concentration of predatory secretions at a distance could also be achieved by solitary predators secreting for twice as long or by doubling the rate of secretion. In either case, the benefits of communal action could potentially be achieved by cells acting individually.

So, is myxobacterial predation really cooperative? Forty years later, the only evidence for cooperativity during predation is the Rosenberg et al. study, which was performed in liquid culture. Rationalization of predation on a surface as being cooperative requires invoking many arbitrary assumptions or situations that may be rare in nature. Predator and prey density and proximity, the mobility of predator and prey, regulated secretion of toxins/defense molecules, relative transport rates of predatory secretions and prey nutrients, and the presence of nonsecretor or competitive genotypes could all affect the efficiency and apparent cooperativity of predation. The available evidence for and against cooperative predation will be discussed later, but first we need to define "cooperativity" as there are several overlapping definitions and uses of the term.

2. A Variety of Mechanisms Allow the Evolution of Cooperativity

Cooperativity has been investigated experimentally in diverse animal systems, resulting in a theoretical framework of social evolution.[32] Cooperative behaviors are usually evaluated in terms of a resultant pay-off, manifesting as increased reproductive fitness. Sometimes pay-offs are direct, e.g., shoaling by fish makes them less vulnerable to predation than swimming singly.[33] In other cases, pay-offs are indirect (and interspecific),

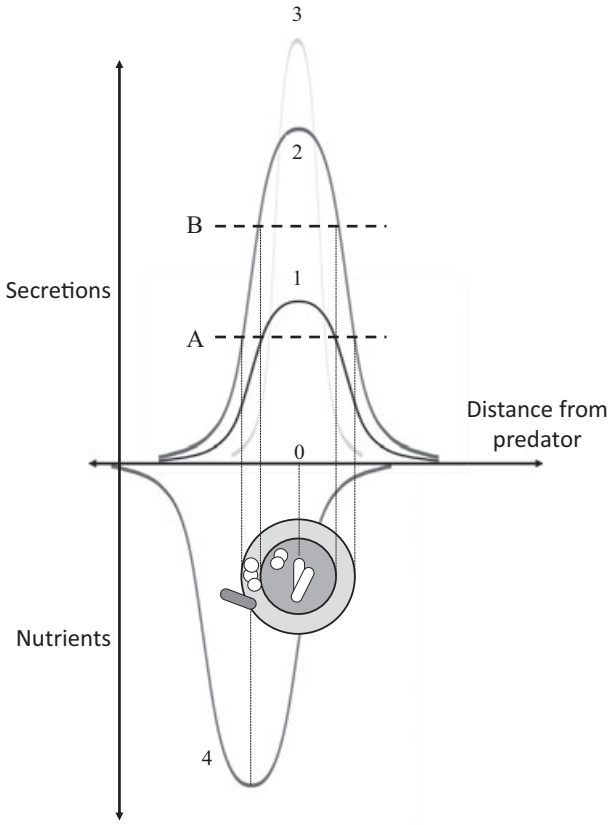

Figure 2. Myxobacteria (white rods) secreting diffusible enzymes/toxins (curve 1) lyse prey (white circles) in the dark gray area, releasing nutrients. Doubling the number of myxobacteria doubles the concentration of enzymes/toxins at any distance (curve 2), extending the zone of killing to the light gray circle and reaching a higher threshold (B) potentially required for killing the recalcitrant prey. Threshold B can also be exceeded by reducing the transport of secretions from the producer (curve 3), perhaps by packaging them within OMVs, which also has the advantage of restricting killing and prey nutrient release to the dark gray circle, thereby reducing competition with other genotypes/competitors (dark gray rod). The concentration of nutrients available (curve 4) is dependent on the distance at which prey are lysed. In this example, lysing cells in the light gray circle disproportionately benefit the competitor rather than the predator.

e.g., some ants farm fungi which benefit by growing faster but later the ants benefit by eating some of the fungi.[34] Discrete mechanisms of cooperation have been conceptualized in attempts to define categories of cooperative behavior.[35,36] A selection of more commonly described mechanisms is presented below:

- Positive reciprocity comprises beneficial behavior reciprocated by recipients, e.g., mutual grooming[37] and food sharing by vampire bats.[38] This behavior involves actors "investing" in the recipients.
- Negative reciprocity occurs when noninvestment results in an individual being penalized, e.g., retaliatory aggression toward individuals that reduce the actor's fitness.[39]
- Positive indirect reciprocity occurs when overt investment by an individual stimulates observers to also invest (this

mechanism is reliant on "reputation" or "image-scoring" by observers).[40,41]

- Negative indirect reciprocity occurs when the failure to invest is overtly penalized. There is little evidence of indirect reciprocity outside humans, although cleaner wrasse alter their cooperative behavior depending on the abundance and size of observing client fish.[42]
- By-product mutualism does not require investment in a recipient. Two actors behave independently, to their own benefit, but in doing so benefit each other anyway. An example is provided by the crèching behavior of baby penguins, who aggregate to reduce aggression from non-parental adults.[43]
- Positive pseudoreciprocity occurs when the investment appears to be selfless, but there is a delayed pay-off (exploitation by the investor). Ants farming fungi is a good example of this mechanism.[44]
- Negative pseudoreciprocity occurs when the investors avoid investing in the recipients that would have a delayed negative effect on the investor. For instance, leafcutter ants preferentially select against invading "suboptimal" strains of fungi.[45]

Owing to uncertainties regarding the ecological significance of observed behavior, it is often difficult to categorize observed behavior into one of the above mechanisms, and sometimes multiple mechanisms can occur within the same observed cooperative behavior.[46,47]

Nevertheless, most of these mechanisms of cooperativity (except by-product mutualism) require conditional or regulated investment directed at particular recipient partners, sometimes dependent on whether the recipient had reciprocated/invested previously. Therefore, such mechanisms require repeated interactions between partners, the ability to discriminate between potential interaction partners, the ability to assess other partners' behaviors, and a memory of how those partners have behaved previously.

2.1. Can the Social Evolution Framework Be Applied to Microbes?

It is certainly not straightforward to apply the lessons learned from animal cooperativity to microbial systems. Although a thorough treatment of this subject is beyond our word limit, some excellent reviews and discussions are available.[48–52]

For cooperativity to evolve, there must be a resulting increase in fitness that more than compensates for the cost of cooperative behavior. For microbes, this fitness benefit tends to be equated with increases in growth (or survival) rates when compared with competing noncooperating organisms.[49] As bacterial populations are largely clonal, neighboring cells in a colony will tend to be phenotypically identical/similar, hence precluding cooperation based on the division of labor or discrimination between individuals. Differentiation into discrete cell types can occur (e.g., during myxobacterial fruiting), and apparent kin discrimination between strains has also been observed.[53,54] However, these are relatively rare phenomena in bacteria. The absence of long-term memory and mixing of cells within bacterial populations also means that most of the well-explored cooperative (reciprocity-based) mechanisms in animals are not applicable to microbes. Prior interactions with each other cannot be assessed by bacteria for conditional reciprocation. As they cannot discriminate between other individuals, there is no guarantee of repeated interactions between particular individuals, and other individuals' behavior cannot be observed from afar, judged, or remembered.

A different vocabulary and set of preoccupations have, therefore, been adopted in the studies of microbial cooperativity:

"Quorum signaling" involves the secretion of a signal, usually an acyl homoserine lactone (AHL) in Gram-negative bacteria. The concentration of the secreted signal indicates the size of the population. At high signal concentrations, quorum-dependent behavior is switched on.[55] The communal activity of this sort thus also manifests as "density-dependence," in which larger numbers of cells exhibit greater per cell activity.

"Social behavior" is also commonly referred to in the literature, along with its subtypes "altruism" and "synergism." However, social behavior also includes negative interactions, such as "spite," "antagonism," "cheating," and "exploitation." "Multicellularity" is a term used to describe cells working together to achieve something that individual cells could not, e.g., rippling pattern formation in myxobacteria.[56]

Care should be taken with all these terms though, as their meanings can be vague and poorly defined, resulting in them being used almost interchangeably. Even in theoretical or animal studies of behavior, there can be confusion with nomenclature and definitions.[49,57] The use of particular terms can also imply certain features, e.g., "multicellularity" suggests a beneficial regulated biological phenomenon, which may not always be the case. Rippling in myxobacteria may merely be an emergent behavior manifested at particular reversal frequencies, rather than an adaptively evolved characteristic.[58]

2.2. Microbes Exhibit Distinct Modes of Joint Action

Problematically, there are varying scientific definitions of "cooperative" behavior, and cooperativity can manifest in profoundly different ways.[49,59] Dictionary.com defines[60] "cooperative" as "an act or instance of working or acting together for a common purpose or benefit; joint action." It is easy to imagine scenarios exemplifying distinct types of joint action that could be employed by microbes.

Figure 3A shows the relationship between population fitness and a number of actors for three fundamental modes of joint action, which we label as "proportionate," "synergistic," and "quorum-dependent."

In proportionate joint action, individuals work together to achieve a goal more efficiently. For instance, if two fruit pickers work together to harvest the fruit in an orchard, they will take half the time to harvest the fruit than a single picker would take. If they were joined by another two pickers, the time taken would be halved again. The efficiency of fruit harvesting increases proportionately with the number of pickers.

In quorum-dependent joint action, a beneficial outcome requires a minimal number of workers. For instance, moving a large rock might require four people to work together. If five helped, the rock would be lifted more efficiently (requiring less effort per contributor), but three people would be unable to lift the rock at all.

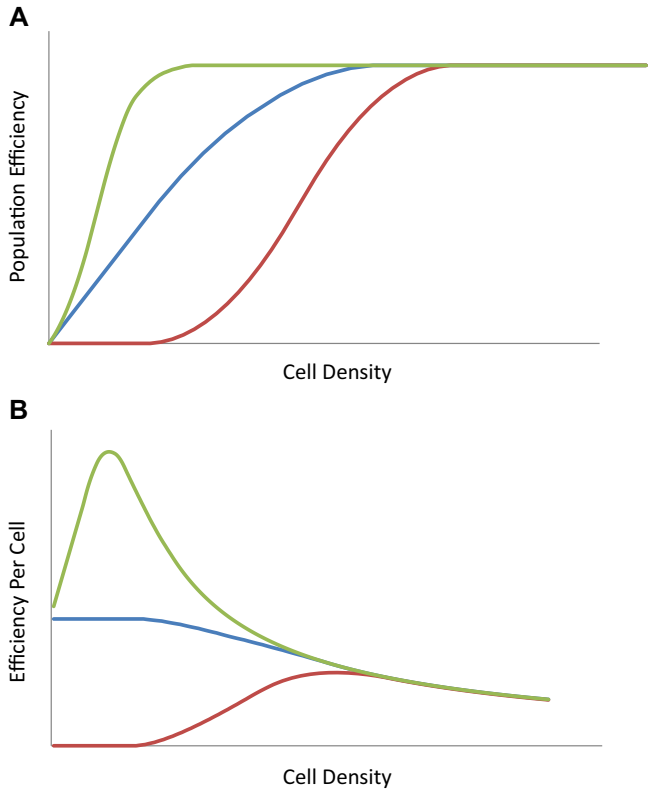

Figure 3. Benefits to the A) population and B) cell of different modes of communal behavior (green—synergistic action, blue—proportional action, and red—quorum-dependent action).

Finally, in synergistic joint action, individuals benefit disproportionately from the assistance of other actors, producing a combined effect greater than the sum of their individual contributions. For instance, bees finding food sources communicate the location to other members of the hive, increasing the per capita acquisition of food nonlinearly.[61]

Note that in all three modes of joint action, there is a maximum population fitness that can be achieved (akin to saturation), e.g., when each fruit tree is being picked, when every bee is harvesting the most abundant food source, and when the rock has been raised. Also note that in the examples above, there is no division of labor: every actor is performing the same task.

2.3. The Evolution of Cooperation Requires a Selective Advantage

A separate issue to the mode of joint action is the evolution and maintenance of cooperation. In evolutionary terms, cooperation is an adaptive trait, which is selected for when a costly behavior provides a selective advantage to an actor, by enhancing the fitness of recipients of that behavior. For myxobacterial predation to be considered evolutionarily cooperative, the (presumably costly) secretion of lytic factors must confer a selective benefit to all recipient cells, which should outweigh the cost of secretion. Whether the three modes of joint action would be "cooperative" in its strictest sense will be discussed below in the context of predation.

3. Are Any Modes of Joint Action Manifested During Predation?

Quorum-dependent predation: There is no evidence of minimal population size for myxobacterial predation. Although there are anecdotal observations suggesting *M. xanthus* colonies cannot grow from individual cells,[20] there is plenty of evidence for single-celled predation.[62] Quorum signaling is a regulated reduction in a defined behavior at low cell densities, where it would be counterproductive to attempt the behavior. When the regulated behavior being considered is feeding, it is hard to imagine a fitness advantage associated with deliberately reducing the efficiency of feeding under conditions when it is already inefficient, although reducing the costs of unproductive secretion could potentially help a cell persist until food became more abundant.[63]

Quorum signaling does happen in *M. xanthus*, where the A-signal regulates the initiation of fruiting body formation. However, although *M. xanthus* can respond to AHLs produced by prey organisms during predation,[64] it cannot produce its own, nor is there any evidence of other quorum signals being involved in predation. Although A-signal components affect predation efficiency, not all do, indicating that A-signal production is not required for predation.[65]

Synergistic predation: The only evidence for synergistic joint action during feeding comes from Rosenberg et al.,[20] who assessed the growth rate in homogenously dispersed cultures with casein as the nutrient source. The natural situation is very different from the one studied by Rosenberg et al. making extrapolation of its conclusions to predation difficult.

The foraging of bees is synergistic because signaling between individuals enhances each individual's ability to locate food. Myxobacteria have an extensive repertoire of signaling proteins.[14,21] They respond to molecules secreted by some prey, and motility-based behavioral changes have been observed during predation.[66,67] Nevertheless, virtually no transcriptional changes were observed in the predator when exposed to prey *Escherichia coli* (*E. coli*),[68] precluding cell–cell signaling dependent stimulation of predatory gene expression.

Proportionate predation: Rosenberg et al.,[20] demonstrated that, in liquid culture, the amount of secreted protease was proportional to the number of producing cells, and Livingstone et al.,[68] found no evidence for regulation of secretion in response to prey availability. Secretion is, therefore, apparently constitutive and proportionate to the number of secreting cells, unaffected by cell density or prey availability. This would be expected to give rise to a growth rate that is independent of cell density (proportionate joint action), especially when secretions are rate-limiting for nutrient liberation.

3.1. Cell Density and Time Are Important Determinants of Cooperation

Nevertheless, the synergistic increase in growth rate observed by Rosenberg et al. is an example of how a proportionate

behavior (secretion) can become synergistic due to nonlinearity in response to the concentration of public goods. For instance, nonlinearity in growth rate can arise if the secreted public goods are enzymes, such as proteases. At low ratios of secreted protease concentration when compared with the substrate (casein), the amount of nutrient source (casein hydrolysate) would increase proportionately with the amount of secreted protease. However, at high protease/casein ratios, each protease would hydrolyze less casein per unit time, as proteases compete for substrate.

Nevertheless, all casein would be hydrolyzed eventually, regardless of how much protease was secreted. If casein hydrolysis takes place orders of magnitude faster than cell division, a more efficient predator would be one that secreted less protease, incurring a lower fitness cost by contributing less to the public goods (i.e., nonsecretors would be selected for). Additionally, involving more predators would reduce each predator's feeding efficiency as they would compete with one another for casein hydrolysate (i.e., there would be a density-dependent fitness reduction).

Time must, therefore, be considered, as fitness costs and benefits are both time-dependent. How does the cost of producing public goods faster compare to the benefit of being able to feed faster? An increased growth rate is usually equated with increased fitness but speedy predation can also be beneficial because it denies prey the opportunity to out-reproduce the predator or become predator-resistant by building biofilms or counterattacking with antimicrobial compounds of their own.

Cooperativity would also be dependent on the cell density of predator. Figure 3B shows the fitness per cell as a function of cell density for the quorum-dependent, proportionate, and synergistic joint action curves presented in Figure 3A. Per cell benefits depend on the mode of action and cell density. Quorum-dependent and synergistic joint action each exhibit a discrete efficiency, maximum at particular densities; and of the three modes, synergistic action can give the greatest per cell efficiency—cooperation occurring when efficiency per cell is greater than the efficiency of cells working independently. Proportionate action gives maximum benefits to cells at low cell densities (i.e., working independently) and as more cells are involved per cell efficiency decreases. To paraphrase, cells jointly acting proportionately are equally or less efficient than cells working separately. Therefore, proportionate action is certainly not cooperative, instead, it is competitive.

3.2. Spatial Structure Dictates the Outcome of Public Goods Secretion

In Rosenberg et al.'s study,[20] secreted public goods were immediately accessible to the whole population due to mixing within the culture. In such a situation, nonsecretor genotypes would thrive as they would not incur any fitness costs from producing public goods, yet would feed on the public goods generated by others, increasing their efficiency of feeding at the expense of secreting cells. However, on a surface, the benefits of nonsecreting are reduced, as public goods are generated in the vicinity of secreting cells and, therefore, nonsecretors would find themselves in a nutrient-impoverished microenvironment compared with that of the secretor cells.

For secretor cells, it would be beneficial to concentrate secretions immediately around the cell so that prey killed were as close as possible, maximizing nutrient uptake and minimizing nutrient lost from the vicinity by diffusion/transport. This "selfish cooperation" would also reduce competition with other secretors when at high density and protect against piracy by nonsecretors or other competitors. Reducing the transport of secretions away from the secretor can be achieved by increasing the size of secreted material, tethering secretions to the cell surface, reducing the half-life of secretions, reducing the amount of material secreted, and/or reducing the distance traveled by the secretor.

There is evidence that all the above-mentioned mechanisms are involved during *M. xanthus* predation. Diffusion resistance around cells is suggested to be increased compared to normal medium,[20] while the packaging of metabolites and hydrolases into OMVs increases the effective size of the secreted molecules, decreasing their diffusion rate.[31] OMVs seem to be tethered to the cell surface and their component hydrolases have reduced half-lives compared with the same enzymes from other bacteria,[12] while starvation stimulates OMV production around twofold.[31] Finally, cells actively preying on *E. coli* ripple (a phenomenon that emerges from an increased reversal frequency), reducing cell migration.[69] There is also evidence that myxobacterial predation is effectively contact-dependent: prey killing is observed only in the immediate vicinity of (potentially touching) myxobacterial cells.[70]

Mechanisms that restrict the benefits of secretion to producing cells and their neighbors have traditionally been proposed to support cooperation because within an unmixed environment closely positioned cells are more likely to be kin, while a spatially unstructured mixed environment would be considered to destabilize cooperativity.[49] However, whether proximal killing supports cooperative behavior or is suggestive of noncooperation, depends on ecology, whether secreting cells are surrounded by kin or competitors.

4. Is *M. xanthus* Predation Cooperative?

The model of selfish cooperation described in the previous section could be viewed as a manifesto for noncooperation, reducing the impact of other cells (whether nonsecreting competitors or other secretors). Because the mechanism of predation requires the secretion of lytic factors into the commons, it is hard to imagine a less cooperative way to do so than that manifested by the myxobacteria. This leads us to question whether the synergism observed by Rosenberg et al. was merely an artefact of the artificial experimental setup employed. If so, is myxobacterial predation actually cooperative?

On the basis of the three contentions below, we propose the testable hypothesis that myxobacterial cells do not cooperate per se during predation but instead merely work alongside each other.

- There is no evidence of cooperativity during feeding except for a single study using an artificial situation in a shaken liquid culture.

- Predator cells do not affect each other's secretion of predatory factors—secretion is proportionate, scaling linearly with cell density.
- Predation is associated with several mechanisms that promote selfishness during feeding.

We argue that working side-by-side is not cooperation in any meaningful biological sense. Considering proportionately joint-acting fruit pickers, doubling the number of pickers will halve the time it takes to harvest an orchard. However, whether two pickers work together in the same orchard, or each work separately in different orchards, does not affect the amount of fruit harvested in a certain period of time. In a proportionate joint action, there is no cooperation, merely (at best) an absence of hindrance. This also distinguishes proportionate joint action from by-product mutualism, which is superficially similar. In by-product mutualism, working alongside another picker would cause an increase in the speed of picking by each picker.

It also seems likely that historically associating the term "wolf-pack" with descriptions of myxobacterial predation has led many authors to implicitly assume that predation was synergistic, as predation by wolf-packs is one of the seminal examples of synergism in biology.[71]

4.1. Toward a Model of Myxobacterial Predation

To test the hypothesis of noncooperativity in "wolf-pack" predation, a mechanistic and holistic model for myxobacterial predation is needed that takes into account ecology, social genotypes, transport dynamics of secretions and nutrients, predator dand prey proximity as well as density, physiology, and life history. Mathematical models of related phenomena have proven powerful tools for investigating the importance of particular parameters and the emergence of different behaviors, in particular regions of parameter space.[72,73] Such models applied to public goods predation would need to be easily parameterized but not too simplistic so that the significance of mechanistic knowledge can be extrapolated to understanding a realistic ecology. As well as requiring computational biologists to develop a formal framework for parameterizing and simulating predation, testing the model will require integrating expertise from diverse experimental disciplines.

Microbial ecologists have at their disposal well-established methods for manipulating microbial growth conditions, measuring microbial growth/fitness, recovering cells from cultures and enumerating viable and nonviable cells, allowing them to quantify growth rate and efficiency of predator and prey under different predatory contexts. This is required as testing the hypothesis requires direct assessment of whether efficiency increases at higher cell densities. Doubling the amount of secreted toxins might be achieved by doubling the number of secreting cells or by waiting for twice as long, but the feasibility of that approach depends on the cellular reserves available to each secretor. The current cooperative dogma can be most easily explained when a small number of predators are surrounded by large numbers of prey, but this is likely to be a fairly unusual situation as myxobacteria tend to move together even at low cell densities,[74] and are unlikely to find themselves suddenly immersed among prey.

Should we even be considering predation from a cellular perspective, where individuals are surrounded by other cells of varying degrees of phenotypic and genotypic relatedness, or should we take a population-centric perspective? Maybe we need to reconcile both perspectives, as cells can give rise to populations and vice-versa. Although the effects of cheaters, defectors, and other social genotypes have been studied in great detail during fruiting body formation, there have been no descriptions of cheating during predation. Defectors are genotypes that choose not to engage in cooperative behavior. Therefore, it would be interesting to see whether it is possible to isolate and distinguish between "cheaters" and "defectors" during predation. If predation is cooperative, we might expect uncooperative defectors that withheld public goods secretion completely to be unviable in isolation, as we would for nonsecretor "cheats." And if predation is not cooperative, we might predict that it would be impossible to grow nonsecreting "cheaters/defectors" (which are fitter when mixed with cooperators) even in the presence of cooperative genotypes. Although this approach risks being self-confirmatory, this is a challenge for evolutionary biologists—isolating asocial genotypes from predatory populations, which will likely require developing predatory regimes (of ecological relevance) that confer a selective advantage to asocial variants.

Biochemists also have a fundamental role to play, as the molecular details of predation are important to consider. What are the active molecules of predation and how fast do they migrate? Is delivery via OMVs required for prey cell entry? Do the active secretion components act stoichiometrically (as antibiotics typically do) or catalytically (e.g., digestive enzymes)? Do we need to consider prey cell resistance mechanisms (and are they stoichiometric or catalytic)? Can myxobacteria fall prey to their own secretions at high concentrations, and how does relatedness affect susceptibility? Which of the nutrients released from prey is rate-limiting for growth, and how fast does it migrate? Do myxobacterial secretions change the diffusive properties of their immediate surroundings to promote proximal killing?

To answer such questions requires the fractionation of active components from predatory secretions, coupled with assays of antimicrobial activity. Characterizing the dose–response relationships of active components will answer several of the questions above, allowing comparison of predatory efficiency and susceptibility. Characterization of molecular size and diffusion/transport rates will also be extremely important and tools, such as scanning electrochemical microscopy,[75] are available to allow assessment of migration rates in naturalistic contexts.

5. Conclusions and Outlook

Is myxobacterial predation cooperative? There seems little reason to think so. At the moment, predation has been described as cooperative because it involves multiple cells secreting into the same environmental compartment. However, a more precise way of defining cooperativity is needed, and we suggest that "synergistic" be used in place of "cooperative"

when describing a process that is more advantageous per individual when undertaken by a larger group. We would argue that proportionate joint action (just working alongside others) is not a form of cooperation, and we would also encourage abandoning the term "wolf-pack" when describing myxobacterial predation.

With a clearly defined model of extracellular predation, experiments can be designed to test additional aspects of predation.[76,77] By assessing how cell density affects growth rate on a surface, and also how prey cell density affects predator growth rate, it should be possible to test experimentally whether predation is/can be synergistic. Is there a combination of predator/prey densities where predation does become synergistic and are such situations likely to arise in the wild?

Our argument of noncooperativity in a public goods-mediated microbial behavior is also relevant beyond predation. For instance, bacterial iron acquisition is mediated by secretion of public siderophores but whether it is actually a cooperative behavior has been debated recently.[51,52] Computational models of public goods-mediated behaviors also suggest that such behaviors can be noncooperative in different ecological contexts.[78,79]

If we can start to address the fundamental questions regarding the cooperativity and evolutionary ecology of public goods-mediated behaviors, it will enhance our ability to rationally exploit them, to develop truly novel antimicrobial therapies as well as increase our understanding of what cooperation may mean (if anything) to a bacterium.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

cooperativity, multicellularity, *Myxococcus xanthus*, predation, public goods, secretion

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