Edouard Jurkevitch
Robert J. Mitchell *Editors*

The Ecology of Predation at the Microscale

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This book is dedicated to the students, post-doctoral fellows, technicians and administrative staff that, along the years, have immensely contributed to the knowledge and understanding shared in it, and to enable our research.

Edouard Jurkevitch and Robert J. Mitchell

Preface

Fifteen years have elapsed since the publication of "*Predatory Prokaryotes – Biology, Ecology and Evolution*" the first and, as of today, only book describing the prokaryotic predators of prokaryotes. It also addressed some of the ecological issues pertaining to their distribution and a few factors affecting these remarkable organisms, as well as their diversity and how predator–prey dynamics could be explained.

Since then, the study of microbial communities has exploded. Microbial diversity is "larger than ever" and keeps on growing; spatio-temporal distributions of microbes can not only be described in great detail, but the underlying principles structuring their populations and communities are emerging. Genomes are being sequenced by the bucketload, and improved functional annotations, community structure-function analyses, genetic manipulations and other omics of single genomes, as well as of metagenomes, are uncovering novel functions to unknown gene sequences and the roles they play, from the cellular level all the way up to the ecosystem. We know much better the ecology of microbes, how communities are composed, how they fluctuate and what drives their changes; we also grasp nutrient flow between trophic levels and describe some specific interactions in great detail.

Symbiosis at large, as first proposed by Anton de Bary as "a phenomenon in which dissimilar organisms live together", has also greatly benefited from these advances. Yet, this book is on predation and one may ask about the connection between symbiosis, even in its broader sense, and predation. While predation between larger organisms is obvious, predatory interactions between microbes may be a lot more difficult to detect in the environment, or even to define. Is a ciliate phagocytosing a bacterium a predator in the same sense as myxobacteria lysing the colony of a nearby bacterium? Are these different than a *Bdellovibrio* and like organism (BALO) penetrating into the periplasm of another bacterium to feed on it, grow and replicate within it? Or are BALOs better described as parasitoids? To complicate this idea further, BALOs do not need their prey to be alive, as they can grow on dead or on reconstituted cells. So is it a scavenger? Continuing with this train of thought, in larger organisms, predatory features are well defined, i.e. anatomical and physiological adaptations for detecting, catching, killing and digesting prey. This leads

one to wonder about the "guts and claws" of predatory bacteria, be they myxobacteria or BALOs. Nevertheless, significant advances have been made to understand how bacterial predators detect, attach, kill, manipulate and exploit their bacterial prey. These understandings have made it possible to explore their potential as biocontrol agents of deleterious bacteria. One such application is therapy of antibiotic-resistant pathogens in humans and other animals, to provide part of a solution to the expending spread of antibiotic resistance.

The ability of predators to cull bacterial populations, such as specific pathogens or general biomass, renders them attractive for numerous applications. To rationally and wisely apply and exploit this potential, a good knowledge of their ecology is necessary. In this monograph, we tried this blend: bringing together applications and potential along with ecological knowledge of predatory bacteria. Towards this end, the chapter by Sester, Korp and Nett discusses secondary metabolites produced by predatory bacteria, focusing on myxobacteria and BALOs; Furness, Whitworth and Zwarycz detail predatory interactions and dynamics of myxobacteria with their prey at the population and biochemical level. Herencias, Salgado and Prieto explore industrial applications of BALOs, including their "domestication" for use as "cell crackers" and as *in-situ* modifiers of microbially produced biochemicals; Najnine, Cao and Cai describe the application of BALOs as biocontrol agents in aquaculture, and how they reduce pathogen loads, while Jurkevitch addresses BALO population dynamics and their role in wastewater treatment. By summarizing what environmental factors affect BALO predation, including prey effects, as well as physical and chemical variables, Im, Bäcker and Mitchell provide explanatory power to observed behaviours and "dos and don'ts" for applications. Finally, Kuppardt-Kirmse and Chatzinotas remind us that bacterial predators are not immune to themselves being eaten by other predators, and they present the principles of microbial intraguild predation and how this affects predatory networks.

It is the hope of all the authors included in this monograph that these chapters and the information provided within will stimulate young and older scientists alike to entangle the intricate dance between predator and prey at the microbe scale and enjoy the study of these remarkable interactions.

Enjoy!

R. J. Mitchell, A Personal Touch

It is my hope that for many young scientists, like it was for me when I read one of Edouard's articles many years ago, you will be fascinated by and captivated with predatory bacteria and that this passion will grow into a career.

Rehovot, Israel — Edouard Jurkevitch
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Contents

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About the Editors

Edouard Jurkevitch studied Soil and Water Sciences for his first and second degree and Microbiology for his third degree, all at the Hebrew University of Jerusalem (HUJI). He obtained his Ph.D. in Agricultural Microbiology in 1992 for a thesis on ecological and physiological roles of bacterial siderophores. He was a postdoctoral fellow for three years at CNRS in France, where he studied nodule formation in legumes. In 1995, he joined HUJI's Faculty of Agriculture in Rehovot. He has a deep interest in microbial ecology, reflected in his research topics: predatory interactions between bacteria from cellular processes to community interactions; the relationship between feeding strategies in flies and their gut symbionts; and soil microbial forensics, a cutting-edge application of microbial ecology.

Robert J. Mitchell followed his heart and traveled to South Korea, where in 2004 he received his Ph.D. in Environmental Sciences from the Gwangju Institute of Science and Technology (GIST) studying environmental toxicity sensing using bacterial bioreporters. After two postdoctoral fellowships studying oral pathogens and fermentations/bioenergy production, first at Harvard University and then at the Korea Institute of Science and Technology (KIST), respectively, he joined the Ulsan National Institute of Science and Technology where he continued to pursue his career as a professor. His lab has meshed all of his previous experiences to delve deeper into the fields of applied microbiology and pathobiotechnlogy, with a heavy emphasis given towards understanding predatory bacteria.

Predatory Interactions Between Myxobacteria and Their Prey

Eleanor Furness, David E. Whitworth, and Allison Zwarycz

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1 Introduction

In this chapter we explore what is currently known about myxobacterial predation. We provide a general introduction to myxobacterial biology, describing their diversity, distribution and social biology, before considering their predatory behaviour in more detail. Ecological factors affecting predation will be discussed, and rationalised with our current understanding of the predatory mechanisms employed by myxobacteria. We also highlight important gaps in our current knowledge of the ecology of myxobacterial predation.

The predatory strategy exemplified by myxobacteria is communal, involving secretion of predatory material into the shared environment; it has thus been described as group attack, or 'wolf-pack' predation.

2 Wolf-Pack Predation

2.1 The Many Strategies of Microbial Predation

Predatory microbes have evolved to exploit several distinct predatory strategies, which have been recently categorised depending on how predator cells encounter their prey, whether they attach to prey cells, and the molecular mechanisms by which prey cells are killed and consumed (Perez et al. 2016). In some cases the predatory

strategy is not easy to define: it can be difficult to discriminate between strategies (for instance whether predation requires contact between predator and prey cells, or whether cells just need to get very close), and some predators may employ multiple strategies simultaneously. The strategy employed by a predatory organism can even change depending on the prey organism being consumed or on prevailing predator/prey abundance (Perez et al. 2016).

Epibiotic predators attach to prey cells and deliver toxins and hydrolases into the prey cell through specialised secretion systems. The predators remain adhered to the outside of the prey cell while consuming its digested contents, and this predatory strategy is employed by genera including *Vampirovibrio* and *Micavibrio* (Soo et al. 2015; Wang et al. 2011). However, epibiotic predation can exhibit features that are usually associated with other predatory strategies. For instance, the epibiotic predator *Stenotrophomonas maltophilia* secretes diffusible antibiotics, while *Ensifer adhaerens* can attack prey as groups (Jurkevitch and Davidov 2007; Perez et al. 2016).

Endobiotic (or direct invasion) predators have a bi-phasic life-cycle. In attack phase, they hunt for susceptible prey and in growth phase they attach to the prey cell, force their way inside it and replicate within the host cell's cytoplasm or periplasm (Guerrero et al. 1986).

Prokaryotes that use a *group attack* strategy work cooperatively to lyse prey cells, either through the secretion of toxins and digestive enzymes into the extracellular space, or by direct contact with the prey (Perez et al. 2016; Velicer and Mendes-Soares 2009). Because the nutrients released by prey lysis are not privatised or ring-fenced by the predator, non-secreting predators and non-predatory bystanders can also benefit from the released nutrients (Mendes-Soares and Velicer 2013; Whitworth 2011).

2.2 *Myxobacteria Are Group Attackers that Employ a Wolf-Pack Mechanism*

A subset of group attack predators use a wolf-pack strategy, in which the predators use social gliding motility to move alongside prey, allowing subsequent contact-dependent lysis of prey cells (McBride and Zusman 1996; Pan et al. 2013). This is the strategy adopted by members of the myxobacteria, and it has also been described for species of the *Herpetosiphon* genus and *Lysobacter* strains (Livingstone et al. 2018b; Pan et al. 2017; Seccareccia et al. 2015).

As paradigms of the group attack wolf-pack strategy, myxobacteria are thought to require a minimum number of attacking cells (a quorum) in order to lyse prey, and require contact with prey for successful predation (McBride and Zusman 1996; Pan et al. 2013; Rosenberg et al. 1977). These are key defining features for distinguishing between the different strategies and sub-strategies of predation, and yet both features are contentious aspects of myxobacterial predation. For instance, myxobacterial

predators seem capable of killing at a distance through the secretion of diffusible secondary metabolites and outer membrane vesicles (Berleman and Kirby 2009; Evans et al. 2012; Findlay 2016; Xiao et al. 2011), while there is microscopic evidence of single myxobacterial cells being able to lyse prey (Berleman and Kirby 2009; McBride and Zusman 1996; Shilo 1970).

Myxobacteria cannot swim through liquid media, but can swarm slowly over surfaces through gliding motility (Mauriello et al. 2010; Munoz-Dorado et al. 2016; Nan and Zusman 2011). Thus their hunt for prey is considered social, as their motility is social. Without attachment of predator cells to prey cells, there must be transfer of toxins and enzymes from predator to prey through the environment. This predatory strategy therefore is likely to require life on a surface rather than in liquid, to avoid the dilution of secreted toxins/enzymes and ensuring that nutrients released from lysed prey do not get diluted below vital concentrations (Whitworth 2011). A related feature of group attack predation is that since predatory cells do not attach to or invade specific prey, instead secreting cocktails of antimicrobial substances into the extracellular space, they can consequently kill a very broad range of prey organisms (Livingstone et al. 2017; Morgan et al. 2010).

However, while wolf-pack predator prey range is broad, it is also patchy (Livingstone et al. 2017; Morgan et al. 2010), with patterns of prey susceptibility and predatory activity not congruous with phylogeny (of prey or predator). This suggests that both predatory activity and prey resistance are a consequence of multiple genes that are actively evolving – an archetypal microbial arms race that is highly specific to the particular strain of predator and the strain of prey being considered. Nevertheless, even when considering a single prey, the manifestation of predation can vary significantly depending on ecological variables. For instance, while wolf-pack behaviour is observed when prey cells are sparse, with small groups of myxobacteria surrounding prey cells, myxobacteria can also successfully prey upon dense colonies of prey cells (Berleman et al. 2008; Perez et al. 2011, 2014). The term ‘frontal attack’ is preferred for those situations rather than ‘wolf-pack’ (Perez et al. 2016), even though the predatory mechanism employed is likely the same.

Group attack predation by definition requires a group to attack, and it has been proposed that prey killing requires a group because communal secretion is required to reach extracellular concentrations of metabolites/enzymes high enough to trigger prey lysis (Rosenberg et al. 1977; Whitworth 2011). Such cooperativity is a hallmark of most aspects of myxobacterial biology (Whitworth 2008), so to contextualise myxobacterial predation we need to understand their socio-biology; the opportunities it creates, but also the vulnerabilities it exposes.

3 Myxobacterial Cooperativity

3.1 The Myxobacterial Life-Cycle Is Inherently Cooperative

Myxobacteria are facultatively multicellular – individual cells are viable entities in their own right, however, at higher densities cells increasingly interact with each other and new population-level behaviours emerge. Myxobacterial communities feed together through cooperative predation, but myxobacteria also respond to starvation as a community. When starved, a population of *M. xanthus* cells initially aggregates into raised mounds (Kuner and Kaiser 1982). Some cells within the nascent mounds are destined to autolyse, providing fuel for the surviving minority of cells to differentiate into dormant myxospores (Lee et al. 2012). Presumably, such a cooperative behaviour has evolved so that starvation causes myxobacteria to produce a population of myxospores. Therefore when food becomes available again, rather than an individual germinant, a population of germinants are released, – able to immediately start feeding efficiently as a population (Munoz-Dorado et al. 2016). Myxobacteria also cooperate when using motility machinery to move around their environment (Mauriello et al. 2010), when growing vegetatively they can share membrane damage (Vassallo and Wall 2016), charitably supporting less-able individuals between cells and sometimes culling them via outer membrane exchange of toxins (Vassallo et al. 2017). Thus every phase of the myxobacterial life-cycle is inherently cooperative (Fig. 1).

Motility *M. xanthus* can move across a surface by gliding motility, using one or both of two different motility engines (Li et al. 2005; Youderian et al. 2003; Youderian and Hartzell 2006). Each engine works better or worse under different environmental conditions, for instance on different percentage agar plates (Hillesland et al. 2007; Spormann 1999). The two engines are denoted A-motility (for adventurous motility, observed for single cells), and S-motility (for social-motility, requiring cell-cell contact). Myxobacterial cells are rod-shaped and their movement is characterised by gliding in the direction of their long-axis, with periodic reversals of direction (Kaiser and Warrick 2011; Wu et al. 2009). Movement leaves behind trails of slime, and other cells preferentially travel along pre-existing slime-trails or channels within the slime, giving rise to population-level patterns of motility (Berleman et al. 2016; Gloag et al. 2016; Stevens 2000). The engine for S-motility is type-IV pilus extension and retraction (Wu and Kaiser 1995). Pili are extended from the leading pole of a moving cell, and when a pilus tip adheres to EPS (exopolysaccharide) on the surface on another 'target' cell, pilus retraction is triggered, causing the moving cell to pull itself towards the target cell (Li et al. 2003). This engine thus requires cells to be close enough to touch each other with their pili, and S-motility is thought to help myxobacteria maintain population cohesion when migrating outwards during colony growth (Balagam and Igoshin 2015; Gloag et al. 2016; Kaiser and Warrick 2011).

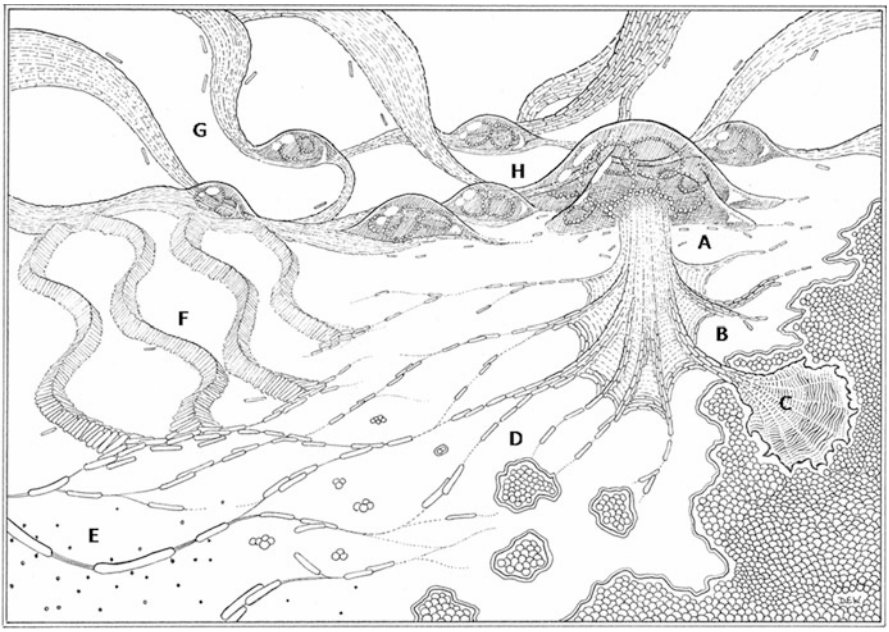

Fig. 1 The life-cycle of predatory myxobacteria. Spores within a fruiting body sense the availability of nutrients and germinate, swarming outwards in search of prey (**a**). As myxobacteria approach a prey colony, they can make a frontal attack (**b**), with predator cells penetrating the prey colony and rippling as they consume the prey (**c**). Alternatively, small groups of predatory cells move between patches of prey along slime-trails in a 'wolf-pack' mode of predation (**d**). Killing of prey is due to the secretion of toxins, enzymes and outer membrane vesicles (**e**). When all prey has been consumed and predatory cells are starving, they secrete A-signal and associate into rippling ridges of aligned cells (**f**). As starvation proceeds, ripples develop into streams of cells, which collide and form small, motile aggregates (**g**). Aggregates become progressively larger until the form a static fruiting body, within which cells differentiate into spores (**h**)

Cell-Cell Transport Adjacent *M. xanthus* cells are able to engage in a phenomenon called outer membrane exchange (OME). Cells belonging to the same TraA-mediated compatibility class are able to transiently fuse outer membranes, and exchange material between themselves (Pathak and Wall 2012; Pathak et al. 2012). This material can include membrane components, and this arguably allows distribution of membrane damage across all cells in a population and in doing so charitably enables individual cells to overcome otherwise fatal membrane damage (Vassallo et al. 2015; Vassallo and Wall 2016; Whitworth 2017). However, a similar, but surprisingly uncharitable phenomenon is also potentially used by *M. xanthus* to kill 'less-fit' siblings, with delivery of toxins via type VI secretion systems (Troselj et al. 2018). OME also enables exchange of toxins, killing non-kin that don't have the required anti-toxin, potentially promoting clonality of the population (Vassallo et al. 2017), although whether this mechanism operates in nature is unclear (Wielgoss et al. 2018).

Aggregation and Fruiting When a population of myxobacteria becomes starved, cells initially aggregate to form raised mounds. These mounds can migrate, split and merge, before stabilising into static fruiting bodies containing myxospores (Curtis et al. 2007b; Xie et al. 2011). Aggregation is preceded by a phenomenon called rippling, in which cells align side-by-side into ridges, which move backwards and forwards reflecting off one another (Igoshin et al. 2001; Welch and Kaiser 2001). Rippling is associated with a higher reversal frequency than during vegetative growth, but as starvation continues, reversal is suppressed and cells aggregate into motile streams of cells, whose 'collision' seems to nucleate aggregate formation (Cotter et al. 2017; Holmes et al. 2010). The aggregation phase of the life-cycle is regulated by a complicated gene-regulatory network and co-ordination of the population's behaviour is achieved through the exchange of two main inter-cellular signals (Kaiser 2004).

Intercellular Signalling The first signal (A-signal) is a mixture of peptides, proteases and the amino acids they generate (Kuspa et al. 1992a, b). It is believed to act as a quorum-signal, with the amount of A-signal indicative of the number of cells present and how starved they are (Kaplan and Plamann 1996). A-signalling leads in turn to the production of C-signal, a later signal of aggregation. C-signal is exchanged on cell-cell contact between cells in the population, with levels of C-signalling increasing with cell density as aggregation proceeds and cells find themselves in ever more intimate association with one another (Ellehauge et al. 1998; Sogaard-Andersen et al. 2003). C-signalling seems to be responsible for the increase and then decrease in reversal frequency exhibited during development, through regulation of the Frz chemosensory system (Igoshin et al. 2004; Jelsbak and Sogaard-Andersen 1999). It should be noted however, that there are conflicting models of how the C-signal (the CsgA protein) actually acts, whether it is a protein that docks with a receptor, or an enzymatic activity (Boynton and Shimkets 2015; Konovalova et al. 2012; Rolbetzki et al. 2008).

Differentiation At the high cell-densities found within fruiting bodies, C-signalling is high enough to trigger differentiation into myxospores. The peptidoglycan of cells is remodelled, changing the cells from rods into spherical spores, spore coats proteins are expressed and a polysaccharide coat is produced, encapsulating the myxospore (Bui et al. 2009; Dahl et al. 2007; Muller et al. 2010). However, spore formation is not the only example of differentiation during fruiting. Some cells are left outside fruiting bodies, and these 'peripheral rods' are thought to act as scouts for the availability of prey (O'Connor and Zusman 1991). In addition, more than 90% of cells entering the nascent fruiting body do not end up as myxospores, instead they are destined to lyse (Lee et al. 2012). Fruiting body sporulation is a process that takes days, yet is triggered by starvation, and autolysis of the majority of cells is likely required to provide surviving cells with the energy and nutrients needed to finish the developmental process (Wireman and Dworkin 1977).

Perils of Cooperation Cooperative societies are vulnerable to exploitation by non-cooperative individuals, and this is true of myxobacterial societies as much as

it is for human and other animal groups (Fiegna and Velicer 2003; Travisano and Velicer 2004). A myxobacterial mutant, whose genotype results in it not undergoing developmental lysis, will increase its proportion within a cooperative population. The fitness advantage of such 'cheating' is greatest when the cheat is a small minority of the population. When a population is predominantly composed of cheats, then it can face extinction, as not enough autolysis occurs to fuel sporulation (Fiegna and Velicer 2003; Velicer et al. 2000). Mechanisms have evolved to purge populations of cheats and to reduce the burden of cheaters on a population (Travisano and Velicer 2004; Velicer 2005). Various aspects of the myxobacterial cycle may have evolved as cheater-resistance mechanisms (Travisano and Velicer 2004; Velicer 2005). For instance, the amino acids of A-signal are particularly costly to synthesise, and their secretion as an early starvation signal may entice cheats/competitor strains to grow on the A-signal, alerting cooperative secretor cells to abort development (Whitworth 2015), while population bottlenecks enhance selection against sub-populations that contain cheats (Brockhurst 2007).

3.2 Cooperativity During Predation

Unlike epibiotic and endobiotic predatory strategies, group attack is reportedly a highly cooperative process. Members of the population secrete toxins and digestive enzymes into the surrounding milieu, and prey lysis releases nutrients into the same space. Thus predatory activity appears cooperative as it happens in a public space, which all members of the population can contribute to, and take from (Perez et al. 2016; Velicer and Mendes-Soares 2009).

In their now classic experiment, Rosenberg et al. (1977) studied growth of *M. xanthus* at different cell densities in shaken cultures. Cells were provided with either casein (protein), or hydrolysed casein as sole carbon and energy source. They found that the per cell growth rate was faster at higher cell densities when growing on casein, but was not density-dependent on hydrolysed casein. Their interpretation was that the rate-limiting step in *M. xanthus* growth was hydrolysis of casein into peptides and amino acids for cellular uptake. Providing pre-hydrolysed casein allowed every cell to grow at its maximum growth rate, but when provided with casein, the amount of liberated peptides and amino acids was dependent on secreted proteases. When more cells secreted protease, there was a disproportionate increase in the amount of available peptides/amino acids, allowing each cell to grow faster (Rosenberg et al. 1977). This lead to the proposal that myxobacterial predation is cooperative, although the unnatural system employed by the Rosenberg et al., experiment makes the extrapolation to predation on a surface debatable (Marshall and Whitworth 2019).

Nevertheless, myxobacterial predation also exhibits other features that rely on cooperation. As cells migrate through a prey colony they ripple (Fig. 2), as they do during starvation-induced aggregation (Berleman et al. 2006). The purpose of rippling during predation, if any, is unclear. It is possible that ripple formation is

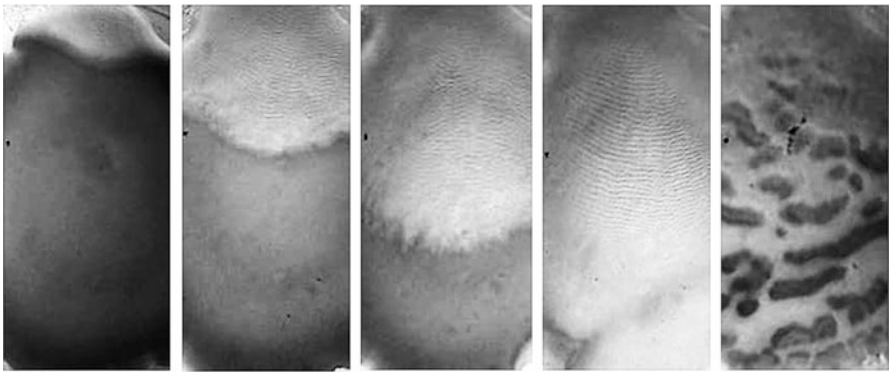

Fig. 2 Predation time-lapse. From left (day 1) to right (day 5), a myxobacterial isolate can be seen (top of the first image) progressively consuming a colony of *Escherichia coli* prey (darker mass comprising most of the initial image). With each day, the myxobacterial colony extends further into the prey, exhibiting rippling behaviour in the middle three images. Finally, upon complete prey depletion, fruiting bodies are left in the wake of predation (final image)

merely a behaviour that emerges when a population of cells has an increased reversal frequency. Increasing reversal frequency results in cells migrating less per unit time, and may be a beneficial adaptation during feeding (Zhang et al. 2012). Some authors argue that rippling is a predatory behaviour (predataxis), but whether rippling increases the efficiency of predation, or is merely coincident with predation remains to be seen (Berleman et al. 2006, 2008; Berleman and Kirby 2009; Zhang et al. 2012).

Another cooperative behaviour associated with predation is multicellular development (Berleman and Kirby 2007). Fruiting body formation is usually studied in monoculture by plating myxobacteria onto starvation medium. However, in experiments where spots of *M. xanthus* were plated alongside prey, fruiting was observed when the myxobacteria moved from an area of prey abundance to regions of relatively scarce prey. Conversely, encountering more prey impeded fruiting body formation. Thus fruiting body formation can be initiated independently of starvation, seemingly driven by interactions between the predator and prey (Berleman and Kirby 2007).

As with any other cooperative trait, myxobacterial predation is presumably open to exploitation by cheating genotypes. Cheats that did not secrete enzymes/toxins would presumably be fitter than secretor genotypes when at a minority, however would be incapable of predation when in pure culture. During wolf-pack mode predation, a small pack size would create a genetic bottleneck that could help purify the population of cheating genotypes (Brockhurst 2007). In small packs, the presence of a non-secretor would make a pack less competitive than a similar sized pack lacking cheats, whereas in larger packs the presence of a cheat would impose a negligible fitness penalty. If packs are generally small in the wild, then they will also amplify the effects of cheaters in subsequent stages of the life-cycle. For instance, if a hunting pack with five members contained a non-lysing developmental cheat, the

resulting population would become 20% cheat, and when undergoing development would be at a significant disadvantage compared to a population seeded from a pack of five cooperators.

While cheating during development has been investigated thoroughly (Velicer and Vos 2009), and predation has implications for cheater control throughout the life-cycle, predatory cheating *per se* remains to be demonstrated. It is also not clear whether there is a difference in behaviour towards cheating kin and non-kin competitors. Does clonal expansion foster cooperation with neighbouring cells as a general strategy, or is kin discrimination used to restrict cooperation to relatives? How closely related do kin have to be before they are considered kin to cooperate with, rather than competition? How often do myxobacteria encounter each other in the soil, and how different are they? Before even attempting to answer such questions, we first need to understand myxobacterial diversity and ecology.

4 Myxobacterial Diversity and Ecology

Myxobacteria (order Myxococcales) are diverse, abundant and widely distributed (Dawid 2000). Pure cultures of myxobacteria have typically been isolated by taking advantage of their ability to grow on either paper or prey organisms. They have consequently been generally classified into two (overlapping) functional groups: the cellulolytic and bacteriolytic myxobacteria, a grouping which broadly correlates with the formal taxonomy of the order.

4.1 Myxobacterial Taxonomy

The order Myxococcales, along with their close relatives the sulphate and sulphur reducing bacteria, belong to the class Deltaproteobacteria (Shimkets and Woese 1992). Presently, the myxobacteria are divided into three sub-orders, the basal Cystobacterineae (which includes the single most studied myxobacterial species, *M. xanthus*), and its two sister taxa Sorangiineae and Nannocystineae. Within the three sub-orders, eight families, around 30 genera, and nearly 60 species have been validly described to date (Garcia et al. 2010; Mohr 2018; Shimkets et al. 2006). This current taxonomic classification will inevitably expand – just 25 years ago only 2 sub-orders, 4 families, 12 genera and 38 species were known (Mohr 2018).

The majority of phylogenetic studies have examined myxobacteria from terrestrial environments (Dawid 2000; Garcia et al. 2010; Reichenbach 1999; Sproer et al. 1999), however, recent studies have included marine myxobacterial cultures and/or DNA sequences (Brinkhoff et al. 2012; Iizuka et al. 1998; Jiang et al. 2010). Marine myxobacteria are evolutionarily divergent from terrestrial species (Jiang et al. 2010), and an exclusively marine myxobacteria cluster (MMC) was recently found to be phylogenetically distinct from the three currently recognised suborders (Brinkhoff