Review

Microbial Models for the Evolution of Predator-Prey Interactions

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Abstract: Predation is a driving force of organismal ecology and evolution. Predator-prey interactions, constrained by other environmental factors and historical contingency, shape physical and behavioral phenotypes of organisms on both sides of the interaction arrow. Yet despite the universality of trophic interactions in biology, study of the ecology and evolution of anti-predator defense is challenging because these interactions tend to be brief and unpredictable, and thus gathering direct evidence requires patience and serendipity. The evolution of prey defenses can be studied by DNA sequencing followed by phylogenetic analysis, and the ecology can be studied by field observation and bringing tractable systems into the lab. However, animal models generally do not allow genetic manipulation, strict control of environmental variables, or detailed observation of evolution in real time, all of which are important for demonstrating evolutionary causes and consequences. Developing complementary microbial predator-prey systems can help overcome this hurdle. There is an extensive body of work on microbial experimental evolution, and many microbes come with well-characterized genomes and established methods for genetic manipulation. As understanding of microbial ecology and the mechanisms of their trophic interactions grows apace, these systems are poised to make valuable contributions to our understanding of how predator behaviors evolve, why certain anti-predator defenses evolve and not others, and how multiple defenses function together in a single defense portfolio. These systems will enable us to both test hypotheses formulated from the study of larger organisms and to propose new ones that can be tested in larger organisms with existing methods.

Keywords: predator-prey interactions; evolution; prey defense; microbial communities; food webs; multiple defenses

1. Introduction

Prey capture and anti-predator defense are energetically costly endeavors, and selection can be assumed to carefully regulate investment into them. This is a fundamental organismal interaction, yet large knowledge gaps remain as we try to understand the balance between innovation and constraint (Caro, 2005; Rutxon *et al.*, 2018). Under what circumstances do organisms invest in single versus multiple defenses? When do prey evolve early-acting versus late-acting defenses, or weak versus strong defenses (Kikuchi *et al.*, 2021)? How quickly do predator-prey relationships shift in ecological and evolutionary time, for example in response to climate change (Burgess *et al.*, 2018; Thakur, 2020), and which factors drive these shifts? Understanding how these interactions evolve gives insight not only into the past and evolutionary contingency but also into the future, as environments change and ecological succession occurs. Classic ecological and evolutionary approaches bring insight into these questions; new techniques make sequencing studies more informative than ever and more complex laboratory experiments possible.

Triangulating the mysteries of biology requires researchers to continually develop new techniques and apply existing tools in innovative ways. For studying predator-prey interactions and the evolution of anti-predator defenses, researchers primarily use animal model systems and rely on phylogenetic comparative methods, field observation, and laboratory behavioral tests (Caro, 2005; Rutxon *et al.*, 2018). These methods provide a wealth of information on ecological realities and historical patterns of change, but they often depend on chance to understand evolutionary processes, selective pressures, and mechanisms of change. Deeper understanding requires more control and fewer confounding variables.

Microbial systems can offer this. For nearly 150 years, microbial experimental evolution has provided broad insight into the processes of evolution (Cable, 2021). Although laboratory studies of microbial evolution are limited in the ecological context they can replicate, they are powerful tools for studying specific sets of circumstances (Collins, 2011; Kawecki *et al.*, 2012; Adams & Rosenzweig, 2014; LaCroix *et al.*, 2017; Van den Bergh *et al.*, 2018). Microbial systems allow close control of community and environmental variables over evolutionary time. Researchers can control intra-population variability, resource abundance, rates of change of abiotic factors such as temperature and pH, and food web complexity. This fine-scale control allows us to clarify the roles of phenomena of interest, such as mutation rates, and to answer questions about competition among predators, generalism versus specialism, and overall effects on diversity. In addition, many microbes now boast fully sequenced genomes and well-established molecular methods of genetic manipulation and mutant construction. Microbial systems would allow study of genetic mechanisms behind predator and prey behaviors, as well as exploration of evolutionary dynamics in real time in response to different ecological scenarios.

Microbes offer a rich metaphor for animal predator-prey biology. Microbes and animals fill equivalent trophic roles, and it may be possible to integrate microbes into animal-dominated food chains (Steffan *et al.*, 2015). As with animal communities, microbial communities are complex and structured by trophic interactions (Goldford *et al.*, 2018; Granato *et al.*, 2019; Rodrigues *et al.*, 2021). Predation among microbes has been studied for decades (Jurkevitch & Mitchell, 2020), since the discovery of single-celled predators, *e.g.* the myxobacteria and dictyostelid amoebae, and the development of small predatory animals such as the nematode *Caenorhabditis elegans* into model systems. Studying these organisms has provided us with a growing body of knowledge about modes of microbial predation (Jurkevitch & Mitchell, 2020) and the roles of microbial predators in nutrient cycling (Hungate *et al.*, 2021) and structuring microbial communities (Trap *et al.*, 2016; Johnke *et al.*, 2017a; b; Petters *et al.*, 2021). Recent research has begun to explicitly investigate the ways in which microbes employ defensive strategies not only against competitors but also against predators (Laaberki & Dworkin, 2008; Nair *et al.*, 2019; Nair & Velicer, 2021), and these results provide an ideal foundation for developing them into model systems for studying the evolution of predator-prey interactions.

Microbial predators come in three categories: prokaryotes, unicellular eukaryotes, and small multicellular eukaryotes. Prokaryotic predators include organisms such as *Bdellovibrio* sp. (often known as *Bdellovibrio* and like organisms, or BALOs) (Jurkevitch *et al.*, 2000; Gallet *et al.*, 2009) and the myxobacteria (Muñoz-Dorado *et al.*, 2016). They encompass a range of predation strategies – BALOs invade the periplasm of a prey cell and extract nutrients through the inner cell membrane (Summers & Kreft, 2022), while the myxobacteria form a “wolf pack” which surrounds a prey colony and digests cells via secreted enzymes (Thiery & Kaimer, 2020). Unicellular eukaryotes, such as the ciliates *Tetrahymena* sp. (Ruehle *et al.*, 2016) and the dictyostelid amoebae (Dunn *et al.*, 2018), tend to ingest bacterial prey by phagocytosis or otherwise consuming cells whole. Small multicellular eukaryotes, including *Daphnia* (Stollewerk, 2010; Miner *et al.*, 2012), copepods (Broglio *et al.*, 2001; Turner, 2004), and bacterivorous nematodes such as *C. elegans* (Gray & Cutter, 2014; Teotónio *et al.*, 2017) and *Pristionchus pacificus* (Hong & Sommer, 2006), also consume prey whole.

In this review, I discuss how microbial model systems can be an ideal complement to existing methods for studying predator-prey evolution. I describe the qualities that are desirable in new model systems. I explain how microbial predator-prey interactions can be understood in terms of the predation sequence. I provide details of selected microbial systems and suggestions for future experiments. Finally, I argue that developing microbial models explicitly to complement animal studies of the evolution of predator-prey interactions is likely to make important contributions, to predator-prey biology as well as to microbial ecology itself, both because of the similarities between microbes and larger animals and because of their differences.

Start of box 1 --

Box 1: What is a microbe?

The term "microbe" is usually used only to refer to microscopic, single-celled organisms. Here, I describe several common microbial predator-prey systems, focusing on those with bacterial prey. I include small animals such as nematodes and *Daphnia* in the term "microbes" for the sake of simplicity. These animals are often microscopic, and they bridge the world between what humans can see with the naked eye and what they cannot. This review focuses on organisms of this size and smaller, and so I prefer not to make a distinction between small multicellular predators and single-celled predators and prey.

End of box 1 --

2. Microbes as model systems for the evolution of predation and defense

Model systems for studying the evolution of predator-prey interactions and of anti-predator defense portfolios should have certain traits. They should be easily culturable in the lab on a variety of media, to simulate different environmental conditions. They should reproduce rapidly, with a generation time on the order of hours or days, so that adaptive responses can be readily observed. Ideally, the genome should be fully sequenced and annotated, so that predatory or defensive phenotypes can be readily linked to genes or regulatory elements and evolutionary mechanisms, *e.g.* of emergence of multiple defenses in a single prey species, can be better understood. These organisms should be genetically manipulable, with standard tools available for constructing mutations in genes of interest. Finally, they should be preservable, for example by freezing at -80 ^{o}C, so that different genetic or phenotypic variants can be stored and revived for later experiments.

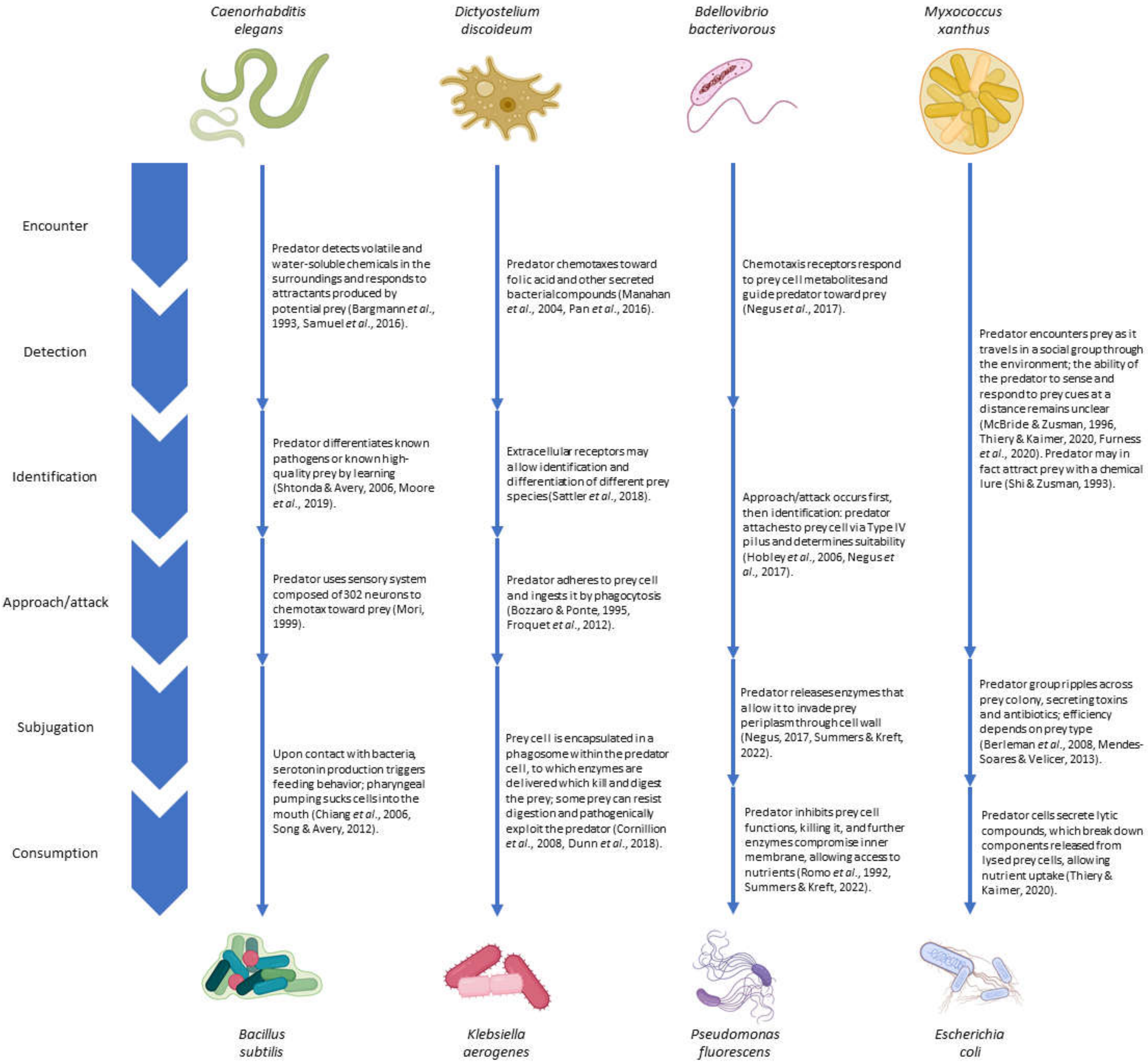
Many microbial predators are studied in the lab which fulfil these criteria. Suitable predators may be found among the predatory bacteria (*e.g.* the social soil bacterium *Myxococcus xanthus*), bacterivorous protists (*e.g.* the ciliates *Tetrahymena* species), bacterivorous nematodes (*e.g. C. elegans*), and aquatic invertebrates (*e.g. Daphnia* species). Bacteriophage viruses are often considered by microbiologists to be predators, and there is much research on their mechanisms of action and ecological effects (Abedon, 2009; Ofir & Sorek, 2018); however, their reproductive and population dynamics may not be sufficiently analogous to those of larger organisms. Researchers must decide for themselves if this system offers the potential for relevant insight. Additional predator types may provide unique benefits and reveal novel biology, but they may not yet be sufficiently developed to constitute useful models. Some tardigrades, for example are known to prey on bacteria (Bryndová *et al.*, 2020) and nematodes (Sánchez-Moreno *et al.*, 2008), although there is yet no type culture collection of tardigrades, and only two species are regularly cultured and available, which feed on algae (Gabriel *et al.*, 2007; Goldstein, 2018).

When selecting a microbial model, researchers must take into account certain considerations regarding differences between microbes and common animal models. The primary consideration is how to compare senses. Many of the most-studied anti-predator defensive traits of larger organisms involve the visual sense – camouflage, startling, warning signals – but microbes lack this sense. However, senses and the role they play in predation can still be studied with microbial systems. There is an extensive literature on chemosensing and chemotaxis in bacteria (Manson, 2018; Keegstra *et al.*, 2022), including review of bacterial chemoreceptors (Hazelbauer *et al.*, 2008) and discussion of information processing in bacteria (Lan & Tu, 2016). An experiment by Clausznitzer and colleagues endeavored to use what is known about chemosensing in *Escherichia coli* to predict what chemical gradients it is likely to encounter in nature (Clausznitzer *et al.*, 2014), providing a foundation for understanding why certain sensory apparatuses evolved. Chemosensory pathways and chemotactic behavior are relatively well understood in two of the species I highlight here, both of which can function as predator or prey: *C. elegans* (Mori, 1999; Ferkey *et al.*, 2021; Suzuki *et al.*, 2022) and *M. xanthus* (Zusman *et al.*, 2007; Berleman *et al.*, 2008). In particular, *C. elegans* has different receptors to detect volatile compounds versus water-soluble compounds, suggesting that it has distinct systems for smell and taste (Bargmann *et al.*, 1993; Taniguchi *et al.*, 2014).

Another consideration for the choice of microbial model is the effect of scale. While some microbial predators are somewhat similar in size, movement rate, and reproductive rate to their prey (such as *M. xanthus* or *Tetrahymena* and their bacterial prey), others are much larger, faster, and reproduce more slowly (such as nematodes and *Daphnia*). The latter may be more similar to a whale consuming plankton than to a bird hunting an insect or a lynx hunting a rabbit. To understand the ecological implications and to usefully generalize across systems, we must therefore consider what constitutes an individual: a single prey cell or a colony of prey cells. In the case of single-celled predators, it makes more sense to consider individual prey cells. In the case of a much larger predator, such as a nematode, we might choose between considering individual prey cells, in which case prey individuals are removed from the colony population, or individual prey colonies or biofilms, in which case cells are parts of the individual and the individual is damaged during predation but not necessarily killed. When considering how microbes respond to selective pressures, it is important to determine at what level selection is acting – at the level of the single cell or at the group level – and therefore what constitutes an “individual” in the evolutionary process of interest. Multi-level selection (Gardner, 2015; Goodnight, 2015; Kramer & Meunier, 2016) and evolutionary transitions in individuality (Godfrey-Smith, 2009; West *et al.*, 2015; Lidgard & Nyhart, 2017; Ratcliff *et al.*, 2017; Black *et al.*, 2020), as well as controversies surrounding their conceptualization, have been extensively reviewed elsewhere.

3. The microbial predation sequence and anti-predator defenses

The predation sequence (Caro, 2005) is a conceptual model describing the steps a predator generally goes through in order to capture and process a prey item (Jeschke *et al.*, 2002). It usually includes as steps: encounter, detection, identification, approach/attack, subjugation, and consumption (Endler, 1986, 1991). It is highly useful for characterizing predator and prey behaviors and for understanding how prey defenses evolve in response to these interactions. For example, by discussing the process of prey capture as individual steps, researchers can more easily define when prey defenses are used and explore how multiple defenses might be deployed during a single attack. The predation sequence was formulated to describe predator-prey interactions of animals, but it can be applied to microbes as well (Figure 1). Considering microbial predator-prey relationships in terms of the predation sequence offers researchers a way to choose ideal organisms to answer certain questions, and it provides a structure for understanding this aspect of microbial ecology, which can motivate future research directions.



Predator detects volatile and water-soluble chemicals in the surroundings and responds to attractants produced by potential prey (Bargmann *et al.*, 1993, Samuel *et al.*, 2016).

Predator differentiates known pathogens or known high-quality prey by learning (Shtonda & Avery, 2006, Moore *et al.*, 2019).

Predator uses sensory system composed of 302 neurons to chemotax toward prey (Mori, 1999).

Upon contact with bacteria, serotonin production triggers feeding behavior; pharyngeal pumping sucks cells into the mouth (Chiang *et al.*, 2006, Song & Avery, 2012).

Predator chemotaxes toward folic acid and other secreted bacterial compounds (Manahan *et al.*, 2004, Pan *et al.*, 2016).

Extracellular receptors may allow identification and differentiation of different prey species (Sattler *et al.*, 2018).

Predator adheres to prey cell and ingests it by phagocytosis (Bozzaro & Ponte, 1995, Froquet *et al.*, 2012).

Prey cell is encapsulated in a phagosome within the predator cell, to which enzymes are delivered which kill and digest the prey; some prey can resist digestion and pathogenically exploit the predator (Cornillion *et al.*, 2008, Dunn *et al.*, 2018).

Chemotaxis receptors respond to prey cell metabolites and guide predator toward prey (Negus *et al.*, 2017).

Approach/attack occurs first, then identification: predator attaches to prey cell via Type IV pilus and determines suitability (Hobley *et al.*, 2006, Negus *et al.*, 2017).

Predator releases enzymes that allow it to invade prey periplasm through cell wall (Negus, 2017, Summers & Kreft, 2022).

Predator inhibits prey cell functions, killing it, and further enzymes compromise inner membrane, allowing access to nutrients (Romo *et al.*, 1992, Summers & Kreft, 2022).

Predator encounters prey as it travels in a social group through the environment; the ability of the predator to sense and respond to prey cues at a distance remains unclear (McBride & Zusman, 1996, Thiery & Kaimer, 2020, Furness *et al.*, 2020). Predator may in fact attract prey with a chemical lure (Shi & Zusman, 1993).

Predator group ripples across prey colony, secreting toxins and antibiotics; efficiency depends on prey type (Berleman *et al.*, 2008, Mendes-Soares & Velicer, 2013).

Predator cells secrete lytic compounds, which break down components released from lysed prey cells, allowing nutrient uptake (Thiery & Kaimer, 2020).

Figure 1. The predation sequence of selected microbial predators. The steps of the standard predation sequence (Endler, 1986, 1991) are shown on the left as wide blue arrows going from top to bottom. Thin blue arrows show how each the prey capture process of predator matches up to these steps, according to our best understanding. Due to their particular mechanisms, some predators don't distinguish between some steps or otherwise perform them at the same time. At the bottom are example bacterial prey species for each predator, but the predatory process is expected to be generally similar across each predator's prey range. Organism images were created with BioRender.com.

A model of predator functional responses (Jeschke *et al.*, 2002) has previously been applied to microbial predator-prey interactions in order to understand the evolution of bacterial defenses against protist and nematode predators (Jousset, 2012). This model includes a search phase, encounter/recognition, attack/ingestion, and digestion, and it explores prey traits which may repel attack at each phase as well as predator traits which may aid in each phase of the attack. The known range of anti-predator defenses of animals has been covered extensively (Caro, 2005; Rutxon *et al.*, 2018), and there is a large and growing body of literature on microbial predation and defense (Pérez *et al.*, 2016; Jurkevitch & Mitchell, 2020). Bacterial anti-predator defenses have been reviewed in detail elsewhere (Jousset, 2012; Granato *et al.*, 2019), so here I will simply summarize them.

Microbes have evolved a range of predatory actions and anti-predator defenses, which may be considered comparable to some animal defensive strategies (Table 1). Here I group defenses into three categories – physical, chemical, and perception-based. The most common physical form of microbial anti-predator defense is biofilm formation (O'Toole *et al.*, 2000; Donlan, 2002). Growing evidence suggests that most microbes exist in biofilms in nature (Ansari *et al.*, 2012), and biofilm-associated traits can evolve *de novo* in the lab in response to predation (Nair *et al.*, 2019). Spore formation in response to environmental stress is another physical defense which can be found across taxa (Lennon & Jones, 2011), and which has been shown to protect prey from predators (Laaberki & Dworkin, 2008; Müller *et al.*, 2014) and to be induced by the presence of predators (Pérez *et al.*, 2011; Müller *et al.*, 2015). Type VI secretion systems (T6SS) are also found in many different bacterial species (Coulthurst, 2019) and can deliver toxins as well as puncturing an aggressor's cell membrane. Finally, some bacteria (in particular, strains of *Pseudomonas aeruginosa*), can make structures known either as pyocins or tailocins, which resemble phage tails and are used to punch holes in an aggressor's cell membrane (Michel-Briand & Baysse, 2002; Scholl, 2017).

Table 1. Types of defenses in animals and microbes, with examples (Caro, 2005; Jousset, 2012; Rutxon *et al.*, 2018; Granato *et al.*, 2019).

Physical		Chemical		Perception	
Animals	Microbes	Animals	Microbes	Animals	Microbes
grouping	biofilms	internal toxins	secreted toxins	mimicry	???
armor	spore formation	injected toxins	injected toxins	camouflage	
spines	type VI secretion system stabbing		vesicles	dazzle	
weapons	tailocins		nanotubes	aposematic coloring	
countermeasures			outer membrane exchange		

In terms of chemical defenses, bacteria produce a wide variety of toxins, which can be induced or constitutively produced, secreted into the environment or packaged within vesicles or T6SS, or delivered directly into an aggressor cell via nanotubes or outer membrane exchange (Cornforth & Foster, 2015; Granato *et al.*, 2019; Niehus *et al.*, 2021). I include perception-based defense here because it forms a large part of defensive repertoires among animals, although to date no bacterial analogue to camouflage or mimicry has been described as such. It may be argued that the ability of *C. elegans* to detect and avoid pathogenic bacteria by their secreted compounds (Meisel & Kim, 2014) may constitute a form of aposematic signaling by the bacteria, although it remains to be demonstrated whether any bacterial compounds are used explicitly to warn off predators.

4. Applications for microbial models

Microbial model systems can evolve fast enough and in a controlled and manipulable enough way to answer a range of questions about predator-prey evolutionary biology which are challenging or impossible to address using animals. The most well-developed microbial systems include the bacteria *M. xanthus* and *E. coli*, the nematodes *C. elegans* and *Pristionchus pacificus*, and the protists *Tetrahymena* and *Dictyostelium*. Here I describe their application to questions of trait evolution in predators and prey, predator senses and cognition, how predators choose among prey, natural variation in predator behavior and prey defensive phenotypes, and how prey react to invasion of new predators and to multiple predators. Additionally, further work on these systems can continue the exploration of whether predator-prey interactions function as a co-evolution (Gallet *et al.*, 2009; Kaitala *et al.*, 2019; Nair *et al.*, 2019) or as an escalation, where the predator exerts selective pressure on the prey but there is little selective pressure in the reverse direction (Brodie & Brodie, 1999).

4.1. Trait evolution

Some prey defensive traits may be multifunctional or may have originally been used for another purpose and been co-opted for defense (Caro, 2005; Stankowich, 2012), but in many systems this is difficult to disambiguate. *M. xanthus* may be an ideal model for studying the process of co-option of existing life history traits for defense and vice versa. This social soil bacterium has a complex life cycle and is therefore a model system for studying cooperation and cheating (Velicer & Vos, 2009). It is capable of preying on a wide range of Gram-negative and Gram-positive bacteria (Morgan *et al.*, 2010; Livingstone *et al.*, 2017), as well as some fungi (Bull *et al.*, 2002). Its predation abilities can evolve in the lab (Hillesland *et al.*, 2009), and we now know that it uses different mechanisms to process Gram-positive and Gram-negative prey items (Arend *et al.*, 2021). Its cooperative behaviors, such as swarming and fruiting body development, have been hypothesized to play a role in predation (Velicer & Vos, 2009); more recently, it has been suggested that these behaviors are also involved in anti-predator defense (Mayrhofer *et al.*, 2021). *M. xanthus*' variety of social behaviors, large predation range, and complex mechanism of predation offer many possibilities for traits to change function over time. This suggests exciting opportunities for future evolution experiments using this organism as either a predator or a prey.

As the *M. xanthus* secondary metabolome becomes better understood, it may also become an interesting model for studying the role of historical contingency in the evolution of predator behavior and anti-predator defenses. *M. xanthus* has been shown to be capable of re-evolving traits that have previously been lost during laboratory evolution (Fiegna *et al.*, 2006; Yu *et al.*, 2016) or due to engineered mutations (Velicer & Yu, 2003). It may therefore be possible to engineer mutations that limit the adaptive trajectories available to *M. xanthus* as it evolves in the presence of different predators, such as nematodes or protists, or of different prey. Addressing such questions can help us understand how adaptation may or may not be constrained as trophic interactions shift over time. Such experiments could examine the effects of adapting as a prey organism on *M. xanthus*'s own predatory ability (and vice versa), as a measure of how viable such an adaptation might be in nature.

4.2. Predator senses and cognition

Nematodes may be a useful laboratory model for studies of predator cognition and the evolution of senses as they relate to predation (Gray & Cutter, 2014). *C. elegans* is a soil nematode which is often studied as a model for aging, immune response, and neural signaling (Corsi *et al.*, 2015). It can be used for experimental evolution (Gray & Cutter, 2014; Teotónio *et al.*, 2017), and it is readily genetically manipulable. *C. elegans* is capable of both positive (Kauffman *et al.*, 2011) and aversive olfactory learning (Zhang *et al.*, 2005), by associating certain compounds with the presence of either palatable bacteria (Kauffman *et al.*, 2011) or pathogenic strains (Pradel *et al.*, 2007; Meisel & Kim, 2014). This learning can be heritable (Palominos *et al.*, 2017; Moore *et al.*, 2019; Pereira *et al.*, 2019). *C. elegans* has separate neural systems for sensing volatile and water-soluble compounds, which are analogous to smell and taste (Bargmann *et al.*, 1993; L'Etoile & Bargmann, 2000), and these senses play a role in predatory behaviors (Zhang *et al.*, 2005; Shtonda & Avery, 2006). This nematode is therefore ideal for studying the evolutionary relationship between a predator's sensing of prey and prey defenses, especially since the genetics and mechanisms of

C. elegans' chemosensory systems are well characterized (Mori, 1999; Schulenburg & Ewbank, 2007; Ferkey *et al.*, 2021). Disabling one or both of the systems could show how these senses are used to interact with different prey species: perhaps by characterizing prey as strongly or weakly defended (Kikuchi *et al.*, 2021) and thus allowing the worm to choose appropriate prey depending on its capability of handling the prey's defenses, *e.g.* toxin load (Palominos *et al.*, 2017).

Pristionchus pacificus is in many ways similar to *C. elegans*, but it can prey on other nematodes as well as bacteria (Hong & Sommer, 2006). It has two different mouth morphologies, one suitable for consuming nematodes and the other optimized for bacteria, which appear at different rates within a population depending on various environmental factors (Susoy & Sommer, 2016; Namdeo *et al.*, 2018). This invites studies of competition and intraguild predation in food webs consisting of prey bacteria and multiple nematode species. Indeed, given evidence that some copepods can feed on bacterivorous nematodes (Muschiol *et al.*, 2008), there are many possibilities for constructing complex food webs, featuring nematodes, which consist of a range of different interaction types.

4.3. Choosing prey

Ciliates of the genus *Tetrahymena* have been increasingly used in evolution experiments to study adaptation, mutation accumulation, and eco-evolutionary dynamics (Plum *et al.*, 2022). They can reproduce sexually and asexually, and sexual reproduction can be induced in the lab (Ruehle *et al.*, 2016). They can be grown with or without prey, offering flexibility for researchers assembling model food webs, who can use it as a prey organism, a mesopredator, or an apex predator. *Tetrahymena* has been shown to change its morphology and behavior in response to prey species (Cairns *et al.*, 2020). This may be a good organism to use in studies of how various environmental factors influence the evolution of prey defense portfolios, as well as the role of sexual reproduction in adaptation of either predators or prey, depending on the role of *Tetrahymena* in a particular community.

Dictyostelium discoideum, the best-known of the dictyostelid amoebae, is a model system for studying cooperation and social conflict (Strassmann *et al.*, 2000; Queller & Strassmann, 2018) as well as the mechanisms of particle adhesion and phagocytosis (Dunn *et al.*, 2018). The latter focus is primarily used to understand mammalian immune defenses and the activity of macrophages (Cosson & Soldati, 2008), but it is also relevant for studying selective pressures on physical defenses of prey. *D. discoideum* is a particularly interesting model system for mechanisms of prey recognition, as it has been shown to respond differently to different prey species and to make metabolic distinctions when processing them (Cosson & Lima, 2014). The molecular mechanisms of prey sensing and chemotaxis are well-described (Manahan *et al.*, 2004).

4.4. Natural variation in predator behavior and prey defensive phenotypes

Laboratory studies of animals, such as birds (Exnerová *et al.*, 2010), can show how different individuals respond to similar circumstances. This allows researchers to clarify how much natural variation is present in a given predator behavior or prey defensive strategy, with implications for the probability of successful prey capture during a predation event and therefore the overall impact of the predator-prey relationship on the evolution of both organisms. However, these studies are often limited in the number of individuals who can be tested, and researchers usually do not know the genotypes of the tested individuals. This limits the extent to which natural variation can be explored and used to explain observed evolutionary trends.

M. xanthus and *C. elegans* are both excellent organisms for investigating these questions. Natural populations of *M. xanthus* have high levels of genetic diversity, even at small spatial scales (Vos & Velicer, 2006; Kraemer & Velicer, 2011). Many strains have been isolated from nature and used to investigate diversity in cooperative and antagonistic behaviors (Vos & Velicer, 2009; Kraemer *et al.*, 2010; Rendueles *et al.*, 2015). *C. elegans*' natural ecology has recently come into focus (Petersen *et al.*, 2015; Schulenburg & Félix, 2017; Zhang *et al.*, 2017), providing new insights into the bacterial prey it may interact with in nature. Studies of natural phenotypic variation have resulted in the creation of standard sets of natural types, which are available from the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR, https://elegansvariation.org/) and can be used to measure variation in behavior and responses. These natural isolates of *M. xanthus* and *C. elegans* are a valuable resource for investigating questions about natural variation, especially because both organisms can act as either predator or prey depending on community context.

4.5. Predator invasion and defense against multiple predators

E. coli is a palatable prey item for many known microbial predators and has been shown to evolve defenses against predation (Nair *et al.*, 2019) and to form biofilms for defense against a range of predators (DePas *et al.*, 2014). It could therefore be used to test how prey defense portfolios, evolved to protect against one predator, respond to invasion of the environment by a new predator. Such experiments might examine how prey defenses evolve when the original predator remains versus when the original predator is extirpated and replaced by the new one. It would be of particular interest to see whether the prey adopts new defensive strategies or adapts the existing ones to address the new threat by tracking phenotypic frequencies and monitoring for *de novo* mutations over time. This could be tested with a range of different predators to look for correlations between predator traits and the evolutionary outcome of prey defenses. For example, defense strategy may depend on predator size relative to prey; when predators are much larger, aggregation may be a useful defensive strategy, as shown by Kapsetaki *et al*. (Kapsetaki *et al.*, 2016), where clumps of algal prey may avoid ingestion by *Daphnia*, or even survive in the predator’s gut and be transported to a new location. *E. coli* may defend itself differently against nematodes and tardigrades than against bacterial and amoeboid predators.

In addition to showing plastic responses to prey (described above), *Tetrahymena* can drive prey adaptation. Experiments with food webs topped by *Tetrahymena* have shown that resource limitation can constrain defense adaptation in prey (Friman *et al.*, 2008) and investigated effects of trophic complexity on prey outcomes (Friman *et al.*, 2016). One study, which used *Tetrahymena* as the predator to investigate the role of predation in diversification and adaptive radiation, demonstrated experimentally that predator-induced diversification occurs slowly because prey population sizes are reduced (Meyer & Kassen, 2007). As an organism which has been used in studies of range expansion (Fronhofer & Altermatt, 2015), *Tetrahymena* may be a useful secondary predator in experiments such as the one mentioned above, in which prey (*e.g. E. coli*) must adapt to a new predator which is introduced to the community. Some of the studies mentioned above exploit different dietary ranges of different *Tetrahymena* species, some of which are intraguild predators (Friman *et al.*, 2016), and I encourage interested researchers to explore how the variations available with this system lend themselves to addressing questions about fluctuating interactions between predators and prey.

5. Conclusion

Microbial model systems offer many advantages for studying the evolution of predator-prey interactions beyond simple population dynamics and offer a timely, propitious, and necessary complement to existing methods employed with larger organisms. They are suitable for addressing mechanistic evolutionary questions about predator-prey interactions, the steps of the predation sequence, and regulation and deployment of defenses, as well as questions about ecological dynamics, evolution of diversity, the effect of community trophic complexity, and adaptation in the presence of multiple predator or prey species.

Microbial systems offer advantages at the scale of an interaction between two individuals and at the scale of dynamics of entire communities. Studies using microbes can benefit from improving RNA sequencing technologies (Kukurba & Montgomery, 2016), which can track changes in gene expression levels in response to predators or prey and at different stages of the predation sequence. At a community scale, microbial systems allow communities to be constructed which contain more than two interacting partners (Johnke *et al.*, 2017b; a; Ratzke *et al.*, 2020; Mayrhofer *et al.*, 2021), which is important in understanding realistic dynamics, as pairwise interactions do not necessarily predict ecologically realistic outcomes (Friman *et al.*, 2016; McClean *et al.*, 2019).

Some model microbes come with well-curated and extensive informational databases, such as WormBase (https://wormbase.org/) for nematodes and dictyBase (https://dictycr.org/) for the dictyostelid amoebae. Such resources enable these organisms to be readily adopted by research groups whose primary focus is another system. I encourage the development of similar databases for other bacterial and protist predator types. Efforts may be underway to establish a tardigrade culture collection, in addition to the primary model *Hypsibius exemplaris* strain Z151 (Goldstein, 2018), which is distributed by the educational supply company Carolina Biological (Carolina Biological Supply Company, 2022). These organisms are significantly less well understood in terms of their basic biology and trophic ecology than the other organisms I have highlighted in this review, but their outstanding overall hardiness suggests that research into the prey-handling abilities of bacterivorous species, particularly regarding toxin load and handling of noxious prey, might be fruitful. Other systems, like BALOs, copepods, and *Daphnia*, are likely to offer unique insights of their own, but these systems may be further from animal predator-prey relationships. The unusual intracellular predation strategy of BALOs (Summers & Kreft, 2022) is a striking difference to animal predation strategies. The aquatic invertebrates, on the other hand, are many times larger than their prey, and these systems lack the genetic methods and robust knowledge of sensory perception which make nematodes particularly useful despite similar size differences.

Every biological metaphor has limitations as well as advantages. While using microbes as analogies for animals can open the way for asking useful and relevant questions, this approach may inherently neglect the unique aspects of these microbial systems. However, collaborations between microbial ecologists and animal evolutionary biologists can allow us to exploit these systems to test hypotheses from phylogenetic or observational studies, or to form hypotheses that can be tested with those methods. Such collaborations, in addition to providing new systems and methods for studying the evolution of predator-prey interactions, can provide valuable insights into the natural ecology of microbial species (Rivera-Yoshida *et al.*, 2020), who are so often studied in isolation.

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