UNIVERSITY OF CALIFORNIA SAN DIEGO

Characterization of *Pristionchus pacificus* as a nematode model of interspecific aggression

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by

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2019

The Dissertation of Kathleen T Quach is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-chair

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Chair

University of California San Diego

2019

DEDICATION

To the many worms whose lives I sacrificed in order to answer the fundamental questions of this dissertation. I wish I could have done more with less.

EPIGRAPH

. . .Non entia enim licet quodammodo levibusque hominibus facilius atque incuriosius verbis reddere quam entia, veruntamen pio diligentique rerum scriptori plane aliter res se habet: nihil tanturn repugnat ne verbis illustretur, at nihil adeo necesse est ante hominum oculos proponere ut certas quasdam res, quas esse neque demonstrari neque probari potest, quae contra eo ipso, quod pii diligentesque viri illas quasi ut entia tractant, enti nascendique facultati paid ul um appropinquant.

ALBERTUS SECUNDUS
tract, de cristall. spirit.
ed. Clangor et Collof. lib. I, cap. 28.

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ACKNOWLEDGMENTS

I am very proud to affirm that the story that frames the work in this dissertation is very much my own. However, I could not have carried out this work to its current state of cohesion and sophistication without the support, inspiration, and unfiltered conversation from many people. Adam Calhoun directed my attention to the Chalasani Lab and the unexpected appeal of worm research. Jagan "Zooman" Srinivasan and Ray Hong for oriented me to the idiosyncrasies of working with *P. pacificus*. Lyle Muller, my comrade-in-arms, instilled an uncompromising dedication to rigor and an unapologetic compulsion to poke holes. As the lone ethologist in the lab, I was always glad to have Gerald Pao come by to talk about animal diversity. Both Lyle and Gerald have been the rare type of friends that seemingly know a little, if not a lot, about everything. Our discussions are lively cross-pollination of many topics. My lab community, past and present formulations thereof, has always been a welcoming and sociable bunch - sometimes overwhelmingly so, but in such cases I'd rather extricate my introverted self rather than dim the vitality of the lab. Shrek fostered an environment of self-determination and freedom that made for an empowering work environment. It has been refreshing to work in a space that is so dense with diverse minds working on diverse projects. Few would do what Shrek did to promote individualism and creativity at the risk of complacency and inefficiency. The most important lesson I've learned from Shrek is the ubiquity of failure in science and the promise of persistence. In science and in life, Shrek has been an unquestioning cheerleader who reflexively launches into support mode when I experienced experimental troughs and personal crises. His support has always been a relief for the aching joints of the scientific process. Still, I find the academic atmosphere sterilizing at times - I have difficulty truly relating to the problems of most people I meet. In these

times of isolation, my family gave me solace in the way that only survivors of a shared trauma can. For levity in everyday life, Sissi consistently made me laugh so vigorously that the laughter would reverberate into the following days and erupt as aftershocks of chuckles and giggles during an idle moment. Finally, in the words of Aldous Huxley, "Even if I could be Shakespeare, I think I should still choose to be Faraday".

Chapter 2 includes material being prepared for publication and is included with permission from all authors: Kathleen Quach and Sreekanth Chalasani. The dissertation author will be the primary author of the prospective paper.

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ABSTRACT OF THE DISSERTATION

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Aggression is a complex behavior in which harm is intentionally inflicted onto another individual. Most definitions of aggression require competition as the motivation for harm. In contrast, most aggression taxonomies exclude predation because it is proximally motivated by nutrition and not competition. Many neuroscience studies of aggression have focused on aggression between males for

mate access, in which the motivation is unquestionably competitive and behavior is typically stereotyped. There have also been a considerable number of studies that have argued for the aggressive nature of predatory attack based on separability of killing and feeding on behavioral and neuroanatomical levels. However, very few studies have attempted to disentangle competitive and predatory motivations in intraguild predation, which is the predation of a potential competitor. The intraguild predator and intraguild prey exploit the same resource. When the intraguild predator kills the intraguild prey, it simultaneously achieves nutritional and competitive benefits, whether or not interference competition was intended. Unlike in male-male aggression, there are no obvious behavioral clues about the degree to which competition motivates intraguild predation. The aim of this dissertation is to devise a strategy for dissecting the nebulous motivations of intraguild predation and identify neural signaling principles for flexibly switching between competitive and predatory motivations. To achieve this aim, I used the facultative predatory nematode *Pristionchus pacificus* as the intraguild predator, *Caenorhabditis elegans* as the intraguild prey, and bacteria as the shared resource.

In Chapter 1, I review key literature that establish the foundational principles that inspire this work and introduce the tripartite members of the intraguild predation model studied in Chapter 2.

In Chapter 2, I articulate behavioral models of predatory and competitive motivations of *P. pacificus* biting that I used to determine that intraguild predation is primarily motivated by competition with *C. elegans* for a limited bacterial resource when *P. pacificus* is well-fed. I then describe a model of how D2-like and octopamine receptor signaling communicate bacterial information to influence biting across various food and hunger contexts.

In Chapter 3, I discuss further conclusions and future directions.

Chapter 1

Introduction

Aggression and its relation to intraguild predation

'Aggression' is an unbound term used to refer to a subset of complex social interactions. Although numerous definitions of aggression have been proposed, none concisely encapsulate the behavioral diversity of aggression, and many versions are fraught with stipulations about motivations that are not readily observable. Despite lack of consensus, it is generally accepted that a hallmark feature of aggression is intentional harm or injury to others [1]. From this, a basic operational definition of aggression can be framed as **any behavior that is intended to inflict aversive or noxious stimuli or harm to another individual** [2][3][4][5].

This minimal definition inherently possesses little value for discriminating between aggressive behaviors and does not capture the multifaceted complexity of aggression. Several taxonomies have been developed to meaningfully characterize differences between aggressive behaviors and sort them into discrete subtypes. These classification systems vary in which dimensions of aggression they use to compare

aggressive behaviors. These dimensions include behavioral expression, eliciting stimulus, motivation, functional value, and underlying neurophysiological mechanisms [5]. Of these classification dimensions, motivation is the most difficult to evaluate because it must be inferred from the others. In all aggression taxonomies, competition is the most representative and often defining function of aggression. Some of the most distinctive aggressive behaviors are subtypes of competitive aggression. For example, aggression associated with male-male competition for mates is marked by conspicuous behavioral expression (ritual combat) that is specifically elicited (by male targets) for a singular observable function (access to mates). In this idiosyncratic case, one-to-one mapping between behavioral expression, eliciting stimulus, and function provide unambiguous support that mate competition is the driving motivation of aggression.

However, aggressive motivation eludes simple inference when overlaps occur between sets of behavioral expression, eliciting stimulus, and function. This problem is especially pronounced when a behavioral expression confers multiple benefits. In intraguild predation, a predator kills and sometimes feeds on an interspecific potential competitor, thereby achieving the dual function of nutrition and competition [6]. Behavioral expression of killing by the intraguild predator appears identical regardless of whether killing is motivated by hunger, competition for a shared resource, or a combination of both. We are confronted with the problem of determining whether competitive benefit is a side effect of predation, or if killing can be at least partially attributed to competitive aggression. While many studies have explored the ecological ramifications of intraguild predation on a community level, many unknowns remain about the nature of the motivation that drives attack behavior on the individual predator level. While field studies of intraguild predation have ecological validity and access to the full complexity of an open system, they have limited ability to control and manipulate elements of the environment that instigate the

predator to kill.

A key goal of ecology is to link phenomema across multiple scales of interaction between organisms and their environment. Here, the dissertation focuses on connections between behavioral ecology and community ecology. Specifically, the question that drives this dissertation is **how does competitive aggression at the individual predator level contribute to the ecological consequences of intraguild predation?** In intraguild predation, the presence of some non-predatory component is detected when the intraguild predator chooses to not feed on a proportion of killed potential competitors [7]. While this post-hoc indicator is a positive measure of hunger and satiety, it leaves unanswered questions about the causal effect of resource availability on mediating the competitive aggression aspect of the motivation underlying intraguild predation. Fully answering these questions requires meticulous manipulation of environmental variables that can disparately elicit predation and competition, which is not feasible in the field setting. Mammalian and insect studies have been prolific in tracing aggression from sensory input to behavioral output in a laboratory setting. However, most of these studies use intermale competition for mates as their behavioral substrate for aggression, and few are interested in studying a nebulous form of competition that is inextricably alloyed with predation. Intraguild predation is a widespread predator-prey interaction pattern that pervades many food webs [8]. It is possible that intraguild predation may allow for study of aggression in animals that otherwise do not exhibit any form of aggression. Predatory nematodes such as *Pristionchus pacificus* may be prime candidates for understanding aggression in a simple nervous system. In this way, the doctoral research presented here will provide a more complete understanding of aggression in its more nuanced and nonspecific manifestations.

To begin to disentangle competitive aggression from intraguild predation and

determine whether competition is even intentional, this introduction chapter reviews relevant key findings of literature from the fields of aggression and ecology and identifies guiding principles as well as limits of knowledge. First, predation is reviewed to explore which predatory behaviors allow for the possibility for predatory attack to be separable from feeding and potentially intentional. Second, field observations and theoretical predictions of intraguild predation are outlined as a conceptual framework for the doctoral research presented here. Finally, *Pristionchus pacificus*, *C. elegans*, and bacteria and their trophic relationships are characterized as the focal intraguild predation model of this dissertation.

Predation

In order to accurately relate predation to aggression, predation must first be explicitly defined. Unlike aggression, predation has a generally accepted definition: an organism killing another organism for nutritional purposes [9]. This definition differs from the previously described definition of aggression (any behavior that is intended to inflict aversive or noxious stimuli or harm to another individual) in three important ways : (a) killing, rather than harm of any intensity, is required, (b) harm does not need to be intentional, and (c) a function for the behavior is specified, nutrition. This last point is the main cause of contention regarding whether predatory killing should be included as a subtype of aggression. In 1968, Moyer was first to outline a stimulus-based taxonomy of aggression, in which predatory aggression was defined as behavior that is elicited by and targeted at prey [10]. However, a subsequent classification scheme, based on function rather than the eliciting stimulus, rejected predation as valid form of aggression because it did not fulfill any competitive, protective, or parental purpose [11]. In this section of the introduction,

we will not yet impose these exclusionary criteria based on function, though they should be acknowledged for their classification value. Instead, this section will explore in which ways predation may overlap with aggression, based on the broad definitions of predation and aggression expressly articulated thus far. Specifically, this section will focus on the **intentionality of the harm** inflicted during predation, which is the only requirement of aggression that predation does not automatically fulfill.

Predation: nonaggressive forms

Predation likely first evolved when the first unicellular life forms appeared, and has since evolved independently many times across all domains and many kingdoms of life [9]. However, aggression is typically considered only in interactions between animals. A key factor for disqualifying simpler predators from aggression is whether predatory attack and feeding are simultaneous or separate. Unicellular organisms can predate on each other by using phagocytosis to engulf a whole prey. For example, predatory phagocytosis is strongly implicated in the origin of mitochondria and chloroplasts as resident prokaryotes that survived engulfment [12] [13]. Engulfment is a simple and compressed form of predation in which killing and feeding are achieved simultaneously - there is no separate attack phase. In this case, killing of the engulfed prey is incidental to feeding on the prey and is generally not considered intentional harm, a requirement for aggression. A similar logic can be applied to exclude multicellular suspension/filter feeders from being considered aggressive.

Other instances in which predator-prey interactions are not deemed aggressive concern the prey's response. For example, herbivores that kill plant or algae in the process of grazing are considered predators. Unlike engulfers and suspension/filter feeders, predatory grazer-type herbivores can kill and feed in separate steps. For

example, sea urchins can use its rasping teeth to incrementally carve away and feed on portions of kelp without necessarily killing it first - that is, sea urchins do not have to subjugate the kelp first to reap nutritional rewards [14]. The kelp only dies when it receives a critical amount of damage, and once again, killing is a side effect of feeding. Feeding without killing is possible when the prey is too large for engulfment and does not physically evade harm. Plants certainly can suffer from harm inflicted by herbivores and have accordingly evolved anti-herbivore defenses, such as chemical defenses and tolerance to herbivory [15]. However, these plant defenses are largely passive or invisible to the herbivore, and therefore the predatory grazer lacks discernable cues for associating its own harmful actions with a correlated harm response from the prey. From an epistemological perspective, the predatory grazer cannot intend harm if it does not know that its grazing is is harmful or noxious to the prey.

Predation: potentially aggressive forms

The potential for predation to be aggressive arises as prey become more difficult to kill and predation transforms from a simple process into a complex sequence of steps. Predation exerts a stronger selective pressure on prey than on predators. Referred to as the 'life–dinner principle', failure costs the prey its life, whereas it only costs the predator a meal [16]. Mutations that are disadvantageous for predation survive longer in the predator gene pool than in the prey gene pool. This suggests that prey can quickly evolve antipredatory adaptations and accelerate co-evolution between predator and prey. Such antipredatory adaptations, such as increased size and speed, make prey more resistant to harm and ingestion and more able to escape. As prey become too big to swallow and motile instead of sessile, engulfment and

grazing cease to be adequate predatory strategies. Instead of achieving harm and feeding in the same step, predation now requires considerably more effort to capture the prey before feeding can even begin. The predatory process leading to capture can be subdivided into a sequence of escalating steps: encounter, detection, pursuit, attack, and capture [17]. The prey has the opportunity to escape at any of these points of escalation. In this elongated predation process, harm is temporally separated from feeding.

The particular temporal order of harm and feeding affects the degree to which intentionality of harm can be inferred. As previously described, it is difficult to disprove that harm is incidental when killing coincides with or follows feeding. In contrast, when killing precedes feeding, a causal relationship between the two becomes available as a possibility. More specifically, predatory attack may be vitally instrumental in capturing prey and contribute directly to the predator's ability to feed on prey. In order to argue a case for predatory aggression, it must be demonstrated that harm inflicted by predatory attack is intentionally perpetrated. However, the close sequential proximity between killing and feeding insinuates that killing may be directly associated with feeding as part of a cohesive feeding behavioral sequence, which would rule out aggression. Predatory attack can fulfill the intentionality requirement of aggression only if it can operate separately from feeding. Therefore, studies that argue for an aggressive quality to predation have outlined ways in which predatory attack is a deliberate and separate behavior that can operate in an uncoordinated way from feeding.

Behavioral evidence for an incongruous relationship between tendency to kill, tendency to feed, and hunger have existed for some time. The most prominent indication comes from widespread observations that predators often kill prey in excess of what they need to fulfill their nutritional requirements, with numerous instances in

which killed prey is abandoned without being consumed. Surplus killing behavior has been readily observed in the field for a variety of predators, especially in wolves [18], but also in wild dogs [19], wild cats [20], mongooses [21], weasels [22], rats [23], mice [24], birds [25][26], and insects [27]. Experimental efforts to differentially influence killing and feeding behavior largely come from studies of muricide by rats. Rats are known to predate on mice in the wild and in the laboratory [28][29]. When presented with mice, a small proportion of laboratory rats kill mice [28]. Notably, rats that kill will only eat a portion of killed prey and with variable latency after killing. These 'killers' attack regardless of whether they are hungry are fully satiated [28]. Further exploration into water deprivation, severity of food deprivation, and time of testing relative to an animal's regular scheduled feeding time failed to show any significant effect on the tendency of killers to attack mice [30][31]. Conversely, 'nonkillers' cannot be coerced into killing mice with extreme food deprivation - some rats were reported to have even starved to death in the presence of prey [28]. Studies have also shown that the respective tendencies to kill and eat are not mutually reinforcing and do not follow each other as one is selectively repressed or promoted. For example, killing does not decrease when the rat is prevented from feeding on its prey [32][33][34], and killing experience is sufficient to promote killing tendency [35]. However, killing does not potentiate subsequent feeding. Rats presented with pre-killed prey were just as likely to feed as rats who were allowed to kill their own prey [36]. Therefore, promoting killing does not always enhance feeding, nor is the inverse true. Altogether, this body of evidence suggests that predation does not always proceed as a unitary behavioral chain of killing and feeding. Rather, predatory attack can be influenced by factors other than those that influence feeding. Predatory attack may be more aptly described as an aggressive behavioral module that is intentionally, though not necessarily, deployed as a means to acquire prey.

Mouse killing is peculiarly situated in between two other rat behaviors that involve harming others: predation of other less-related species and aggression against conspecific intruders. Unlike mouse prey, predation of other species is characterized by much higher and more consistent rates of attack and subsequent feeding of prey such as frogs, turtles, chicks [37][23], and insects [38]. In one study, nearly all tested rats attacked frogs or turtles placed in their cages, while only 17% of rats attacked mice placed in their cages [37]. Killing of frog and turtle prey was almost always accompanied by eating of the killed prey [39]. Mouse killing therefore differs starkly from predation by rats of their most common prey food, and brings into question whether mouse killing possesses some nonpredatory component [40]. Since mice are phylogenetically close to rats, it is tempting to surmise that mouse killing shares aspects of conspecific aggression between rats. Rat colonies are known to attack strange intruder rats, and experience with intruders increases attack behavior [40]. If mouse killing resembles offensive aggression against conspecific intruders, then the increase in aggression induced by exposure to conspecific intruders should also lead to an increase in aggression against intruder mice. However, previous aggressive experience with conspecifics failed to induce any change in readiness to attack either mouse or roach targets [38]. If predatory attack is indeed aggressive, it is not influenced by the same factors that govern intraspecific aggression. Thus, the behavioral and neurophysiological signatures of intraspecific aggression cannot be referenced for identification and validation of predatory attack as a form of aggression. Predatory aggression must be judged on its own merits with reference to the definition of aggression.

In addition to behavioral evidence, hypothalamic stimulation studies in cats have shown that that feeding and killing are separable on the neuroanatomical level. While some hypothalamic sites can elicit both predatory attack and eating [41],

stimulation of a particular site in the lateral hypothalamus in cats has been shown to selectively elicit predatory attack [42][43][44]. In order to ascertain that this lateral hypothalamic site is indeed specifically dedicated to the attack aspect of predation, Flynn and associates conducted an exhaustive set of behavioral experiments in which they attempted to coax eating behavior out of the cats while they were stimulated [45][46][47][48]. First, the researchers attempted to increase the stimulation to the stimulation site that reliably induces a cat to attack a rat. Since, there exists a hypothalamic site that elicits eating at lower stimulation intensities and attack at higher intensities [41], perhaps the inverse is true for this putative predatory attack site. Indeed, this is true, but only for a small number of cats tested; most of the cats did not eat their captured rat prey with increased stimulation intensity. In addition, persistent stimulation duration past the point of attack did not lead to consummatory feeding after predatory attack of a rat had already been evoked. Second, the researchers presented easily attainable non-prey food to reduce the effort needed to eat. When a dish of non-prey food was presented, most cats attacked the dish of food when stimulated but never consumed the food. When horsemeat is placed closer than an anesthetized rat prey in relation to a cat, stimulation induces most cats to pass over the horsemeat and attack the rat. Finally, the researchers increased motivation eat by starving cats for three days. The starved cats were then fed non-prey food and stimulated while eating. Amazingly, most of the cats halted eating of the non-prey food and proceeded to attack a nearby rat. Altogether, these cat studies indicate that a predatory attack site of the lateral hypothalamus exists that is functionally selective in influencing the attack component of predation, and is neuroanatomically distinct from other neighboring sites that influence eating or the predatory process as a whole. Combined with previously described behavioral experiments of muricide by rats, a strong body of evidence suggests that predatory attack is dissociable from feeding,

thus opening up the possibility for predatory attack to be applied for other functions.

Intraguild predation, a combination of predation and competition

While predatory attack as described above has been labeled as predatory aggression by a cohort of aggression researchers, consensus remains far out of reach. One explanation for this hesitancy is that it is not sufficient to show that predatory attack can be dissociated from feeding - something else must replace feeding as the motivation for and function of the attack. For many, the most convincing motivation and function is competition [11][49]. I will therefore introduce a more stringent ethological definition of aggression that I will refer to as competitive aggression: **any behavior that is intended to 1) inflict aversive or noxious stimuli or harm to another individual and 2) deal with competition**. It is important to note that this definition requires that both harm and competition be intentional.

One class of interspecific interaction that can potentially satisfy both competitive motivation and function of predatory attack, and thus aggression in a more widely accepted sense, is intraguild predation. In intraguild predation, a predator kills and sometimes eats a potential interspecific competitor [6]. A guild consists of a group of species that exploit the same resource in a similar way [50]. From a food chain perspective, intraguild predation is the set of relationships between three trophic levels: the intraguild predator, the intraguild prey, and the shared resource. **A basic model of intraguild predation has the following trophic structure : 1) Both the intraguild predator and intraguild prey consume, and thus compete for, the same shared resource; and 2) the intraguild predator is facultative and can also eat the intraguild prey** (Fig. 1.1.A) [6][51][52]. This type of intraguild predation is

assymmetric, because only one of the guild species consistently predates on the other.

Two general forms of competition are involved in this basic form of intraguild predation. First is exploitative competition, in which two species indirectly negatively affect each other by consuming the same resource and thereby reducing resource abundance [53][54][55]. If two species have the exact same resource needs and only engage in exploitative competition, the species that is more efficient at consuming the shared resource should theoretically emerge as the winner, while the less efficient consumer is driven to extinction or a different niche [55]. In order for intraguild predation to be robust and its participating species to coexist, it must include a second form of competition, interference competition [56][55][57]. In interference competition, one species reduces the ability of the other to exploit the shared resource [53][56][55]. Intraguild predation involves a severe form of interference competition in which the competitor is killed. With these two forms of competition in mind, there are three key predictions of a simple model of stable intraguild predation [51][52]:

1. The intraguild prey is superior in exploitating the shared resource.
2. The intraguild predator should have greater fitness from predating on the intraguild prey than from competing on a purely exploitative level.
3. The intraguild predator, by reducing the population of the more efficient consumer species, indirectly increases the abundance of the shared resource at equilibrium.

Interference competition is the component of intraguild predation that is most relevant to demonstrating that predatory attack can be aggressive. By definition, predation of the intraguild prey eliminates competitors for a shared resource and thus fulfills a competitive function for the intraguild predator. Competitive motivation, on the

other hand, is difficult to prove in intraguild predation. The set of interactions that comprise intraguild predation are notoriously difficult to disentangle. Predation and interference competition are especially difficult to delineate because they usually occur simultaneously, which add another dimension to intentionality: in addition to harm being intentionally inflicted, is competition also intentional? Or is it an accidental benefit that emerges from facultative generalists that consume multiple trophic levels? Unfortunately, most intraguild predation research focuses on the ecological effects on intraguild predation on community structure, rather than on the individual scale. Specifically, much of the interest in intraguild predation lies in understanding how intraguild predation promotes species coexistence and biodiversity, as a well as building theoretical models of more complex variations of intraguild predations with more species, more intricate interactions, and intraspecific contributions.

Intraguild predation: uneaten intraguild prey

Meanwhile, very little research has been done to dissect the motivations of an intraguild predator, even when field examples seem to conform to the simplest form of intraguild predation. When the intraguild predator sucessfully kills and eats the intraguild prey, nutrition and competition benefits are simultaneously achieved and thus the corresponding motivations are difficult to distinguish. However, when the intraguild predator does not consume a proportion of intraguild prey that it kills, an opportunity arises to use the percentage of uneaten intraguild prey as a proxy indicator of non-predatory motivation.

This idea is reminiscent of the aforementioned studies of mouse-killing by rats (page 8), in which some mouse prey are left uneaten after being killed [28]. Since both rats and mice and are taxonimically similar, it was previously surmised and then

rejected that perhaps the killing of mice mimicked intraspecific competition against invader rats [38]. Instead of intraspecific competition, taxonomic similarity may more strongly suggest that rats and mice may eat similar food. Indeed, field studies indicate that rats and mice compete intensely for the same food resources and reciprocally affect each other's population number [58][59]. Rats have also been previously described as intraguild predators of competing mice [60]. Field studies of poisoned or trapped rats have shown that mice dramatically increase in abundance when rats are removed, even if mice were also being eradicated at the same rate [61][62][63]. In what is sometimes referred to as 'competitor release', the increase in mouse population from rat removal is much higher than expected from exploitation competition alone and strongly implicates interference competition through predation [64][61][65]. In order to validate whether this interference competition against mice is intentional, or just simple predatory behavior with incidental competitive benefits, the approach taken in the field has been to look for 1) threat and display features associated with intraspecific aggression, and 2) uneaten prey. Results taken from wild rats indicate a lack of threat and display features towards mice, and all well-fed and starved rats ate at least a portion of euthanized mice [66]. These findings led researchers to conclude that interference competition in this case was predatory behavior and not intentionally competitive.

There are two important caveats to these field results. First, it is important to note that here, just as in aforementioned mouse-killing studies, the researchers used similarity to intraspecific competition as an indirect metric for whether interference competition is intentional. Similarity to intraspecific competition does not address competition in a definitional sense that directly accounts for resource motivations. The similarity metric also does not allow for non-binary interactions between predation and interference competition - it does not capture situations in which a combination of

predation and intentional competition contributes to mouse-killing. Additionally, intraspecific competition, especially for mates, evolved display postures and ritualized fighting as a way to establish dominance without killing of conspecifics [67] [68][69] [70][71][72][73]. These social methods of communicating aggression and determining the winner may serve as species-preserving restrictions on the severity of harm, and as such may not be applicable to interspecific competition. Second, it is known that wild rats consume most killed mice, while laboratory rats consume only a small portion of killed mice [28]. Laboratory rats were used to demonstrate that feeding and killing were behaviorally dissociable components of predation. While wild rats are more pertinent for ecologically valid representation of an actual ecosystem, laboratory rats may have been more valuable for disentangling competitive and predatory motivations for eating or not eating prey.

A field study of wild lynxes and foxes differs from intraguild predation studies of wild rats in two ways: 1) intraguild predation is compared to conventional predation, rather than to intraspecific competition, and 2) the intraguild predator abandoned a proportion of intraguild prey without eating [74]. In this study, lynxes are the intraguild predator and foxes are the intraguild prey. Lynxes and foxes both predate on smaller animals such as roe deer and mountain hares. Since they do not compete with lynxes, roe deer and mountain hares are referred to as 'true' prey species. Predation of true prey species is considered 'true' foraging, because it only serves nutritional purposes and does not confer competitive benefits. If nutritional need is the only factor motivating killing of foxes, then the proportion of uneaten fox corpses should closely match the proportions of uneaten roe deer and hares. On the other hand, if something other than nutrition also motivates killing of foxes, then killed foxes should be left uneaten more often than roe deer and hare. The latter prediction is vindicated; 37% of foxes killed by lynxes are uneaten, while 2% of roe deer and 0% of hares were

uneaten. This finding is similar to the previously mentioned behavior of lab rats that attack and eat almost all frog, turtle, or insect prey but only a small percentage of mice [37][23], and insects [38]. The notable difference between these rat/mouse/true prey studies and the lynx/fox/true prey study is that intraguild predation relationships were only articulated in the latter. This opens a line of questioning about interference competition, rather than intraspecific competition, as a potential "other" factor for driving killing of the intraguild prey.

While it may be tempting to conclude that competition is the putative other factor that motivates lynxes to kill but only sometimes eat foxes, the field study was unable to account for relative abundance of foxes, roe deer, and hares. Specifically, they could not account for how often lynxes encountered foxes or true prey by coincidence. Even if the absolute population counts of true prey were large, they may be effectively scarce to lynxes if true prey are good at evading lynx detection. On the other hand, foxes may be effectively abundant if they were poor at evading lynx detection and lynxes encountered them more often by chance. In the latter case, lynxes may find that the extra immediate energy required to subdue a fox prey may be worthwhile if they do not require as much time and energy for prey search. In short, scarcity of true prey species should increase uneaten fox corpses, while abundance of foxes should increase eating of foxes. Without full control and understanding of the relative abundances of intraguild prey and shared resources, it is difficult to concretely attribute uneaten intraguild prey to competition. Firm evidence of competition must be acquired before competitive aggression can be argued for.

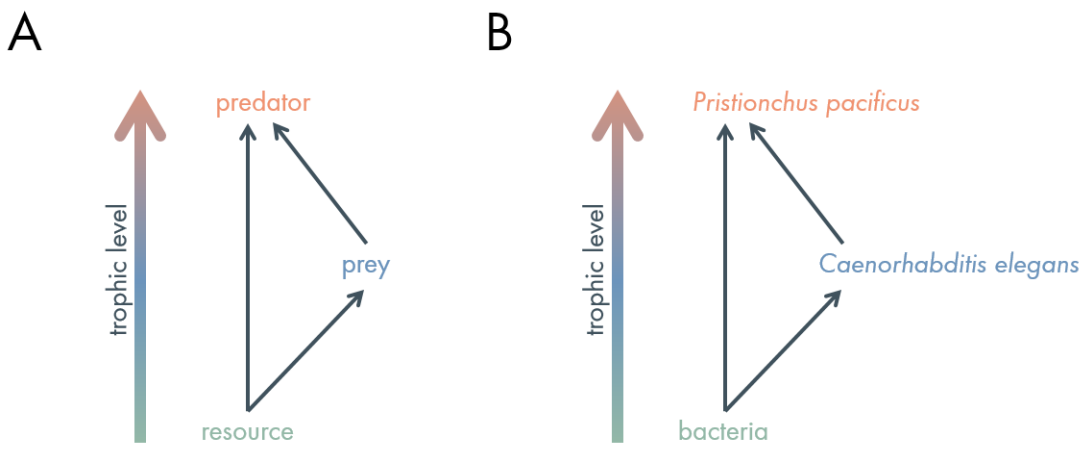

Figure 1.1: Basic model of asymmetric intraguild predation. Arrows indicate the flow of energy towards higher trophic levels. (A) The intraguild predator is facultative and consumes both the intraguild prey and the shared resource. The intraguild prey only consumes the shared resource. (B) Intraguild predation involving a guild of nematodes (*Pristionchus pacificus* as intraguild predator and *Caenorhabditis elegans* as intraguild prey) and bacteria as the shared resource.

Nematode intraguild predation

In order address the practical and ethical limitations of previously described experiments, one solution towards pinpointing competitive motivation in intraguild predation is to study how perturbations in resource abundance influence nematode interactions in a lab setting. Specifically, the nematode guild of interest features *Pristionchus pacificus* as the intraguild predator, *Caenorhabditis elegans* as the intraguild prey, and bacteria as the shared resource (Fig. 1.1.B). *Pristionchus* and *Caenorhabditis* species have been found to co-occur in nature on rotting plant material [75]. Relocation from the field to a lab setting allows for meticulous experimental manipulation of shared resources and hunger state. Use of nematode intraguild prey circumvents ethical qualms of purposefully subjecting vertebrates to being painfully killed and eaten as prey. Additionally, both nematode species have large brood sizes and short life cycles of only 3-4 days in optimal conditions [76][77], allowing for fast quantification of fitness consequences. In this section, I will review literature about *P. pacificus* and *C. elegans* as it pertains to their relation to each other and to bacteria. The goal of this section is delineate what is known and what is unknown about the participants and interactions that constitute this selected nematode example of intraguild predation.

Intraguild predator: *Pristionchus pacificus*

Pristionchus pacificus was first described in 1996 out of an interest to introduce an alternate nematode that can serve as a counterpoint to the established nematode *C. elegans* in comparative studies [78][79][80]. Since then, numerous studies have emerged that characterize the similarities, differences, and interactions between *P. pacificus* and *C. elegans*. *P. pacificus* and *C. elegans* are separated by an order of

100 million years of evolution [81], and yet they share a remarkable level of similarity. *P. pacificus* can be handled and cultivated using the same laboratory maintenance techniques as used for *C. elegans* [78]. On a gross morphological level, *P. pacificus* and *C. elegans* are both vermiform in shape and roughly the same size, approximately 1 mm long as young adults (Fig. 1.2.A,E). *P. pacificus*, like most nematodes, are also conveniently eutelic and have a fixed number of developmentally determined somatic cells [79]. While number, neuroanatomical positions, and processes of homologous neurons are highly conserved between the two nematodes, subtle changes in neuroanatomical features of amphid neurons and massive wiring of the pharyngeal motor system have been reported [82][83][84]. Despite having similar life cycle length, early *P. pacificus* development differs from that of *C. elegans* in that *P. pacificus* eggs hatch at the J2 stage, one full larval stage later than the corresponding *C. elegans* L1 stage. [85].

Perhaps the most striking morphological difference between *P. pacificus* and *C. elegans* relates to the predatory features of *P. pacificus*. *P. pacificus* can eat bacteria, but unlike *C. elegans*, it can also predate on other nematode larvae with the use of teeth. *P. pacificus*, as do all diplogastrids, possess a dorsal tooth and lack the pharyngeal grinder that *C. elegans* uses to grind bacteria [86]. In light of this dramatic restructuring of the buccal cavity, it therefore makes sense that drastic rewiring of the pharyngeal motor system would also occur. However, it is less certain if other aspects of the nervous system that do not directly correspond to disparate morphology would be as severely altered with respect to *C. elegans*. *P. pacificus* exhibits a developmental dimorphism in which a proportion of animals known as stenostomatous develop only the dorsal tooth, while others known as eurystomatous develop a larger dorsal tooth 1.2.B) and an additional ventral tooth 1.2.C). The relative proportions of eurystomatous and stenomatous in a population are affected by starvation, crowding,

and the sulfatase EUD-1, all of which promote the eurystomatous mouth form [87][88][89]. The eurystomatous mouth form has been shown to be adaptive for predating on nematode larvae, while the stenostomatous mouth form is unable to kill prey and is restricted to only bacteriovory [90][91]. In addition to alternate tooth forms, *P. pacficus* can also switch between feeding rhythms that vary in the respective rates of pharyngeal pumping and dorsal tooth movement [91]. While eating bacteria, pharyngeal pumping is high and tooth movements are rare. When switching to predatory feeding, pharyngeal pumping decreases to about 66% of the bacterial rate and tooth movement increases dramatically until it matches pharyngeal pumping in a 1:1 ratio. Exogenous treatment of serotonin triggers predatory rhythms in the absence of prey, while interruption of serotonin synthesis and ablation of serotonergic neurons result in uncoordinated rhythms [91][92].

In addition to phenotypic plasticity and movement of teeth, much is also known about predatory behavior of *P. pacificus*. *P. pacificus* are generalist predators of larvae of many nematode species, and use highly specific self-recognition that allows them to discriminate even between their own larvae and those of different isolates of the same species [93]. The standard *P. pacificus* laboratory strain PS312 readily bites larval *C. elegans* when it is the exclusive diet, with about 34% of bites resulting in killed larvae [91]. The same study noted that only 49% of larvae corpses were eaten, and surmised, in similar manner to the aforementioned lynx study (page 15) [74], that *P. pacificus* may be eliminating competition. Although intraguild predation is not explicitly mentioned, the metric of uneaten corpses once again implicates a non-predatory component behind the motivation for killing. It still remains to be demonstrated whether or not competition is in fact the non-predatory motivation. In absence of bacteria, *P. pacificus* bites all stages of *C. elegans* with equal rates, but seems to only be effective at killing larval stages - adults are rarely killed [91].

Therefore, adult *C. elegans* seems to be a very energetically expensive prey target, and it is perplexing why *P. pacificus* would waste effort biting adult *C. elegans* at all.

In the laboratory setting, *P. pacificus* can be cultivated on the same standard food source used for *C. elegans*, *E. coli* OP50. *P. pacificus* seems to prefer bacterial food over nematode prey. When *P. pacificus* is presented with an excess of both larval *C. elegans* bacteria, *P. pacifus* bite less often than when bacteria is absent [91]. Reduced biting of larvae on bacteria suggests that predatory drive decreases when bacteria becomes available as an alternate food. It is important to note that the convention of feeding *E. coli* OP50 to *P. pacificus* in the lab was borne out of convenience and a desire to ease the adoption of *P. pacificus* into existing *C. elegans* elegans laboratories. Several studies have surveyed the microbiomes of the *P. pacificus* collected from natural settings [94][95][96][97]. Although *P. pacificus* can also be found in rotting plant material [75], these microbiome studies focused on the bacteria present alongside *P. pacificius* in beetles. *P. pacificus* can have a necromenic association with beetles, whereby they reside exclusively as dauer larvae inside the living beetle and resume development once the beetle starts to decay [98][97]. *Enterobaceriaceae* was found in many of this studies to be the most abundant family of bacteria present *P. pacificus* harvested from beetles, although many other types of bacteria were also isolated [96][97][95]. While *E. coli* is part of the *Enterobaceriaceae* family, *Escherichia* was rarely encountered [96]. *P. pacificus* grown on *E. coli* OP50 preferred many of the bacteria isolated from beetles as well as soil over *E. coli* OP50, as measured by chemotaxis assays [94] [96][95]. Despite preference for naturally co-occuring bacteria, *E. coli* does as well or better than surveyed bacterial isolates in terms of *P. pacificus* fecundity and survival [94][95]. In fact, sometimes this preference is displayed for pathogenic bacteria, such as those of the *Serratia* genus [95]. Overall, bacterial preference does not strongly correlate with

the suitability of the food source [95].

Intraguild prey: *Caenorhabditis elegans*

The *C. elegans* response to *P. pacificus* has not been studied in depth, which is likely due to the fact that the smallest larval stage (L1) (Fig. 1.2.D) of *C. elegans* are most often used to assay *P. pacificus* predatory behavior [90][91]. Often, the larval *C. elegans* is killed immediately upon contact with *P. pacificus* nose, thereby precluding any subsequent *C. elegans* response. A recent study removed the danger of live *P. pacificus* by instead using an extract of excretions collected from live *P. pacificus* animals [99]. Interestingly, adult *C. elegans* immediately avoided this 'predator cue' when it was collected from starved *P. pacificus*, but not when the cue was collected from well-fed *P. pacificus*. This suggest that *P. pacificus* may only be a threat to *C. elegans* when bacteria is not available as an alternate food source. Thirty minutes of submerged exposure to the predator cue resulted in adult *C. elegans* laying fewer eggs in the first hour upon extrication from the predator. However, this lower number of eggs in the first hours post-removal was compensated by increased egg-laying in the second hour. This suggests that exposure to predator cue does not affect egg production. Rather, *C. elegans* can temporarily slow down egg-laying, putatively to delay deposition of eggs until the threat of predation has passed. This is consistent with previous studies that show *C. elegans* retains eggs during stressful conditions, such as starvation and hypertonic environments [100].

Much more is known about the relationship between *C. elegans* and bacteria. Importantly, this knowledge may inform hypotheses about *P. pacificus* conserved mechanisms of sensing and responding to bacteria. It is known that *P. pacificus* and *C. elegans* have disparate responses to the same set of odorants, with some odorants

that are attractive to one and repulsive to the other [101][102]. Therefore, any discussion of potential conserved bacteria responses and underlying mechanisms will have to involve direct sensation of bacteria and not of proxy odorants, such as benzaldehyde and diacetyl, that putatively represent bacteria in *C. elegans*. The first notable change in behavior that *C. elegans* exhibits upon finding a bacterial lawn is to decrease its locomotory rate [103]. This basal slowing response requires dopamine, as dopamine synthesis mutants continue moving through bacteria at the same rate as when bacteria is absent [103]. The basal slowing response seems to be mediated with a high degree of circuit redundancy - only complete ablation of all dopaminergic neurons eliminated the basal slowing response [103]. *C. elegans* exhibits the same dopamine-mediated basal slowing response when they encounter a matrix of synthetic Sephadex beads, suggesting that the sensory stimulus for slowing is mechanosensory in nature [103]. A mechanosensory, rather than olfactory, stimulus offers the advantage of precise temporal and spatial perception of the bacterial lawn. In contrast to the dopamine-mediated detection of bacteria, the absence of bacteria is associated with octopamine release [104][105]. When bacteria is present, dopaminergic cells release dopamine, which then in turn suppress octopamine signalling through D2-like receptors on both the octopaminergic RIC and the octopamine-responsive SIA neurons [105]. Absence of bacteria disinhibits octopamine signalling at these two neurons: RIC releases octopamine, and octopamine activates CREB expression SIA, with CREB accumulating with prolonged starvation [104][105].

In addition to binary detection of the presence or absence of bacteria, *C. elegans* is also able to distinguish and seek out the boundary of a bacterial lawn from its circumscribed region. Some social wild strains of *C. elegans* and *npr-1* mutants that lack the neuropeptide Y receptor naturally migrate to and aggregate at the border of a bacterial lawn, where bacteria is thickest [106]. This bordering tendency involves

oxygen sensing by guanylate cyclase, which promotes aerotaxing away from regions of higher oxygen levels towards areas of lower oxygen levels in both wildtype and *npr-1* mutants [107]. Thick *E. coli* OP50 bacterial lawns consume oxygen more quickly than can be replenished by ambient diffusion, and borders with the highest concentration of bacteria were observed to have lower effective oxygen concentrations [107]. Acute reduction of ambient oxygen levels abolished bordering behavior in *C. elegans* [107], as well as in *P. pacificus* [108]. Ablation of nociceptive sensory neurons ASH and ADL in *C. elegans* disrupt bordering behavior [109]. Therefore, *C. elegans*, as well as *P. pacificus*, may use relative lower oxygen concentrations to find and demarcate the lawn edge. The ability to detect the edge of a lawn opens up the possibility of estimating the size of the lawn. Indeed, guanylate cyclase mutants were unable to distinguish between small and large lawns of bacteria [110]. The mechanism for computing lawn size experience depends on the variability in bacteria levels that *C. elegans* senses during its exploration of the lawn [110]. The thick edge relative to the thinner interior of the lawn means that *C. elegans* will experience changing bacteria levels more often in a small lawn, where the animal will encounter the edge at a higher rate. Large bacterial variability is sensed by ASI and ASK neurons and result in downstream dopamine release [110].

C. elegans has been cultivated in the laboratory setting with *E. coli* since its debut as a model organism [111]. However, like *P. pacificus*, *C. elegans* is found in nature with a variety of other bacteria species, with *Enterobacteriaceae* and *Acetobacteraceae* species associated with high proliferation [112]. *C. elegans* also displays preference for bacterial species other than *E. coli* OP50, particularly if the other bacteria is higher quality food, as measured by growth rate [113]. Furthermore, *C. elegans* raised on higher quality bacteria leave mediocre bacteria more often [113]. One such high quality bacteria is *Comamonas sp.*, which was isolated from a

soil environment [114]. Interestingly, the list of bacteria naturally found with and preferred by *P. pacificus* also includes the *Comamonadaceae* family [96][95]. Therefore, *Comamonas sp.* may be useful in exacerbating competition between *P. pacificus* and *C. elegans*.

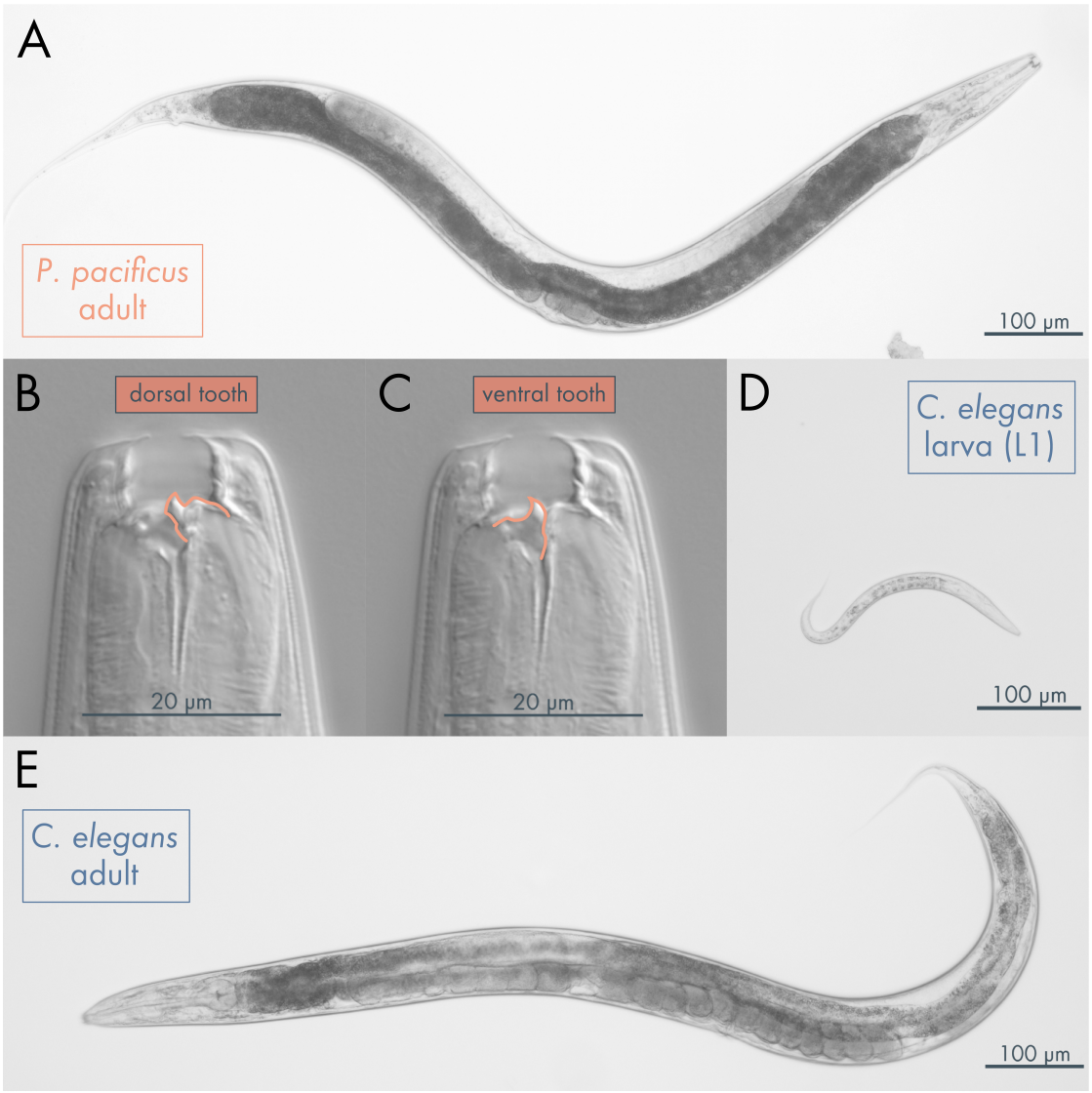

Figure 1.2: *P. pacificus* and *C. elegans* morphology. (A) Adult *P. pacificus*. (B) Dorsal tooth of eurystomatous *P. pacificus*. (C) Ventral tooth of eurystomatous *P. pacificus*. (D) L1 is the smallest larval *C. elegans* stage. (E) Adult *C. elegans*.

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Chapter 2

Scarcity of a shared bacterial resource drives interspecific territorial aggression of *Pristionchus pacificus* against competing *Caenorhabditis elegans*

Abstract

Pristionchus pacificus is a facultative predatory nematode that prefers to feed on bacteria but can also predate on other nematodes, such as *Caenorhabditis elegans*. *P. pacificus* can easily capture and consume larval *C. elegans*. However, little is known about why *P. pacificus* also bites adult *C. elegans*, which is difficult to subdue. Intriguingly, *P. pacificus* bites adult *C. elegans* even when bacteria is present. *C. elegans* also consumes bacteria and therefore competes with *P. pacificus* for a shared resource. We wondered if *P. pacificus* could intentionally bite adult *C. elegans* for competitive purposes. Here, we describe novel behavioral assays that we used to interrogate the competitive motivation and benefits of *P. pacificus* biting of adult *C.*

elegans. When bacteria is scarce, *P. pacificus* is more likely to bite adult *C. elegans* than when bacteria is abundant or completely absent, suggesting that biting is promoted by both the presence and scarcity of a shared resource. Competitive biting is territorial and is effective at prompting *C. elegans* to leave a small bacterial lawn and lay its eggs at a disadvantageous distance from food. Ablation of the putative amphid sensory neuron ADL increased competitive biting, suggesting that the neuron senses bacterial abundance. Treatment of *P. pacificus* with the D2 receptor antagonist amisulpride increased biting when a small bacterial lawn is present, but not when bacteria is absent. Combined with data from treatment with the D2 receptor agonist sumanirole, we determined that bacterial thickness activates D2 receptors. Inspired by *C. elegans* starvation signaling mediated by D2-like and octopamine receptors, we also treated *P. pacificus* with octopamine and the antagonist epinastine and found that octopamine receptors are activated by small lawn size. From these aggregate results, we formulated a model of how bacterial density and lawn size activate D2-like and octopamine receptors to influence *P. pacificus* biting tendency, with context-specific modes of action that are dependent on hunger state and whether bacteria is absent. The results of this chapter illustrate how bacterial scarcity induces *P. pacificus* territorial biting against *C. elegans* and represents a nematode model of interspecific aggression.

Introduction

P. pacificus has generated interest as a nematode model largely due to its teeth-like denticles, which *C. elegans* lack [1][2]. The single-toothed stenostomatous dimorph is unable to predate, while the eurystomatous dimorph of *P. pacificus* possesses two teeth and enables *P. pacificus* to predate on other nematode larvae

[3]. *P. pacificus* is able to effectively predate on all larval stages of *C. elegans*, but experiences precipitous difficulty in subduing adult *C. elegans* [3]. A young adult *C. elegans* is roughly the same size as a young adult *P. pacificus*, locomotes at a faster speed, and also possesses a tough cuticle [4][5]. All these features make adult *C. elegans* an exceptionally difficult prey. In spite of the severe unlikelihood of successful predation of adult *C. elegans*, *P. pacificus*, *P. pacificus* still bites adult *C. elegans*. In one study in which *P. pacificus* was presented with an exclusive diet of a specific stage of prey, *P. pacificus* did not bite adult *C. elegans* any less often than it did larval *C. elegans*, even though it was unable to kill any adult *C. elegans* [3].

If *C. elegans* is typically present in nature as a mixture of stages, then biting of adult *C. elegans* may be a tolerable inefficiency of indiscriminately biting a population of mostly edible larvae. In this case, biting of adult *C. elegans* would be nothing more than futile predation attempts. However, much of the research regarding *P. pacificus* biting has been from a predatory perspective, and alternate functions of biting have yet to be seriously considered. *C. elegans* typically emerges from a bite unscathed, with no visible damage to its ability to move. Despite minimal bodily harm, it is clear that *C. elegans* dislikes being bitten and often recoils urgently from a bite. While nonlethal biting of adult *C. elegans* is poor at killing prey, it may be sufficient to deter *C. elegans* from a shared food source. *C. elegan* has a dual trophic relationship with *P. pacificus*. In addition to being its prey, *C. elegans* competes with *P. pacificus* for bacteria. Although *P. pacificus* can eat nematode prey, it prefers to eat bacteria and produces more progeny from a bacterial diet than from a prey diet [6]. Perhaps another reason for biting adult *C. elegans*, if not for direct nutrition, is to reduce competition for bacteria.

Together, *P. pacificus*, *C. elegans*, and bacteria form a set of trophic relationships collectively called intraguild predation, which is the killing of a potential

competitor [7]. Intraguild predation simultaneously achieves nutritional and interference competition benefits. However, competition may be incidental to predation, and motivation of the intraguild predator is notoriously difficult to disentangle. Most studies of intraguild predation have focused on its effects on species coexistence and population dynamics [8][9]. Little is known about what motivates the intraguild predator on an individual level. The few studies that have researched the nature of motivation behind intraguild predation have relied on uneaten killed prey as a metric of non-predatory motivation [10]. Interestingly, *P. pacificus* was observed to have eaten only about half of all killed larval *C. elegans*. Starved *P. pacificus* fed on a higher proportion of killed larval *C. elegans* [3]. However, there are two problems with using uneaten corpses to detect competitive motivation for biting adult *C. elegans*. First, uneaten prey is not a positive demonstration of competitive motivation - it only indicates the presence of non-predatory component but has limited ability to identify what that other component is. Second, adult *C. elegans* are so difficult to kill that the number of corpses is effectively zero.

To address these issues, I designed novel behavioral assays in which I meticulously manipulated bacterial abundance and measured how probability of biting adult *C. elegans* responds to changes in the bacterial environment. The use of a prohibitively difficult prey permits each bite to be treated independently, since *P. pacificus* satiety changes from prey ingestion are precluded. If *P. pacificus* biting is only motivated by nutrition, then biting probability should be highest when bacteria is absent and adult *C. elegans* is the only food source. On the other hand, if biting is also competitively motivated, then biting of adult *C. elegans* should be highest when bacteria is most scarce. The results of this chapter vindicated the latter model. Well-fed *P. pacificus* was most likely to bite adult *C. elegans* when bacteria was most scarce, specifically when the bacterial lawn was small and just barely

perceptible. Biting diminished to its lowest levels when bacteria was either completely absent or abundant. This demonstrates that biting was deployed against adult *C. elegans* primarily to defend bacteria when it was in limited supply. Starvation induces high biting when bacteria is absent, which indicates that predatory biting is turned on by starvation. Increasing thickness and size of the lawn accordingly decreased biting probability. Ablation of the amphid sensory neuron ADL increased territorial biting on a small lawn of intermediate thickness, which suggested that ADL senses lawn thickness. Nonlethal biting was sufficient to drive adult *C. elegans* out of the bacterial lawn and coerce *C. elegans* to spend less time on the lawn and lay eggs in unfavorable locations away from bacteria. Therefore, competitive biting is an effective form of territorial aggression.

A drug screen revealed the D2 receptor antagonist amisulpride as a candidate drug for increasing competitive biting. Amisulpride increased biting only when a small lawn was present, but not when bacteria was absent. Amisulpride maintained this effect even when *P. pacificus* was starved. Treatment of *P. pacificus* with the D2 agonist sumanirole provided additional support that D2-like receptors are activated by bacterial thickness. Treatments with octopamine and its antagonist epinastine further revealed that lawn size is communicated via octopamine receptor signaling. By combining data from untreated and treated *P. pacificus*, we were able to construct a model of how *P. pacificus* signal bacterial information via D2-like and octopamine receptors to modulate biting probability in a manner that is context- and hunger-specific.

To the best of our knowledge, these results represent the first description of interspecific territorial aggression in nematodes. More importantly, they demonstrate how the intertwined competitive and predatory motivations of intraguild predation can be unraveled to reveal subtle forms of competition. What originally seemed like failed

predation of adult *C. elegans* may now be more accurately characterized as an effective form of territorial aggression.

Results

The *P. pacificus* wild isolate RS5194 reliably latches onto, but not kill, adult *C. elegans*

In order to maximize the nonlethal harmfulness and visibility of a bites, I tested different isolates of *P. pacificus* for their ability to bite, latch onto, and kill *C. elegans*. We tested three *P. pacificus* strains PS312, RS5275 and RS5194, to see select the best biter. We modified an existing assay that was previously used to classify the predatory efficacy of *P. pacificus* PS312 bites [3][11]. In a 3.2 mm diameter arena, I placed a single adult *P. pacificus* with either ~100x L1 larval *C. elegans* or 8x adult *C. elegans* and recorded biting events for 30 minutes. To maximize visibility of the most subtle bites, the arena was placed on an empty agar plate with no bacteria that may obscure the *P. pacificus* mouth. We classified three types of escalating bites events: 1) bite, 2) bites that latch onto the prey body, and 3) bites that latch onto and kill prey. A 'bite' is defined as the *P. pacificus* mouth fully contacting the *C. elegans* body at a perpendicular orientation while exhibiting predatory pumping rhythms. Bites elicit an escape response from *C. elegans*. The most basic and least effective bites are ones in which *C. elegans* escapes without any resistance. Bites that 'latch' onto the *C. elegans* body display at least a momentary attachment of the *P. pacificus* mouth to the *C. elegans* body. This attachment slows down *C. elegans* escape, disrupts normal sinusoid locomotion *C. elegans*, or cause the *P. pacificus* head to be pulled along as *C. elegans* escapes 2.1.C-D). Therefore, the visual indicators of latching

bites go beyond the *P. pacificus* mouth and are amplified with irregular body postures of *P. pacificus* and/or *C. elegans*. Finally, bites that 'kill' cause penetration of the cuticle as evidenced by the leaking of pseudocoelomic fluid 2.1.A-B). All three *P. pacificus* strains were more successful in biting larval *C. elegans* than adult *C. elegans*. *P. pacificus* strains RS5275 and RS5194 were superior to PS312 in biting both larval *C. elegans*. Most RS5275 and RS5194 bites of L1 *C. elegans* resulted in kills, while only 14.2% of PS312 bites of L1 *C. elegans* resulted in kills. RS5194 was better than both PS312 and RS5275 at latching onto adult *C. elegans*. While RS5194 could latch onto the majority of adult prey, bites virtually never culminated in feeding within the 30 minute assay period (Fig. 2.1.E).

The crowded nature of the previous assay biased scoring of biting success toward strategies that rely on killing prey with a single bite. Prey that escape can quickly get lost in a large crowd of other prey. Predation that depends on accumulating damage onto the same prey would be unfavored. Therefore, we limited the predator-prey interaction to a single *P. pacificus* and a single adult *C. elegans* within a small arena (∅ 3.2 mm) and allowed 24 hours for killing to occur. Under these conditions, both RS5275 and RS5194 were more successful than PS312 at killing adult *C. elegans*. By 24 hours, only 50% of PS312 killed adult *C. elegans*. This halfway benchmark occurred by four hours for both RS5275 and RS5194. By 24 hours, 100% of both RS5275 and RS5194 killed (Fig. 2.1.F). Therefore, the effort to kill an adult *C. elegans* far exceeds the near-instantaneous kill of L1 *C. elegans*. Since RS5194 outperform the other two tested *P. pacificus* strains in biting adult *C. elegans*, we selected RS5194 as the *P. pacificus* strain for the remainder of this chapter. The high visibility of RS5194's latching bites allows for more confident detection of biting, even when they occur on bacteria.

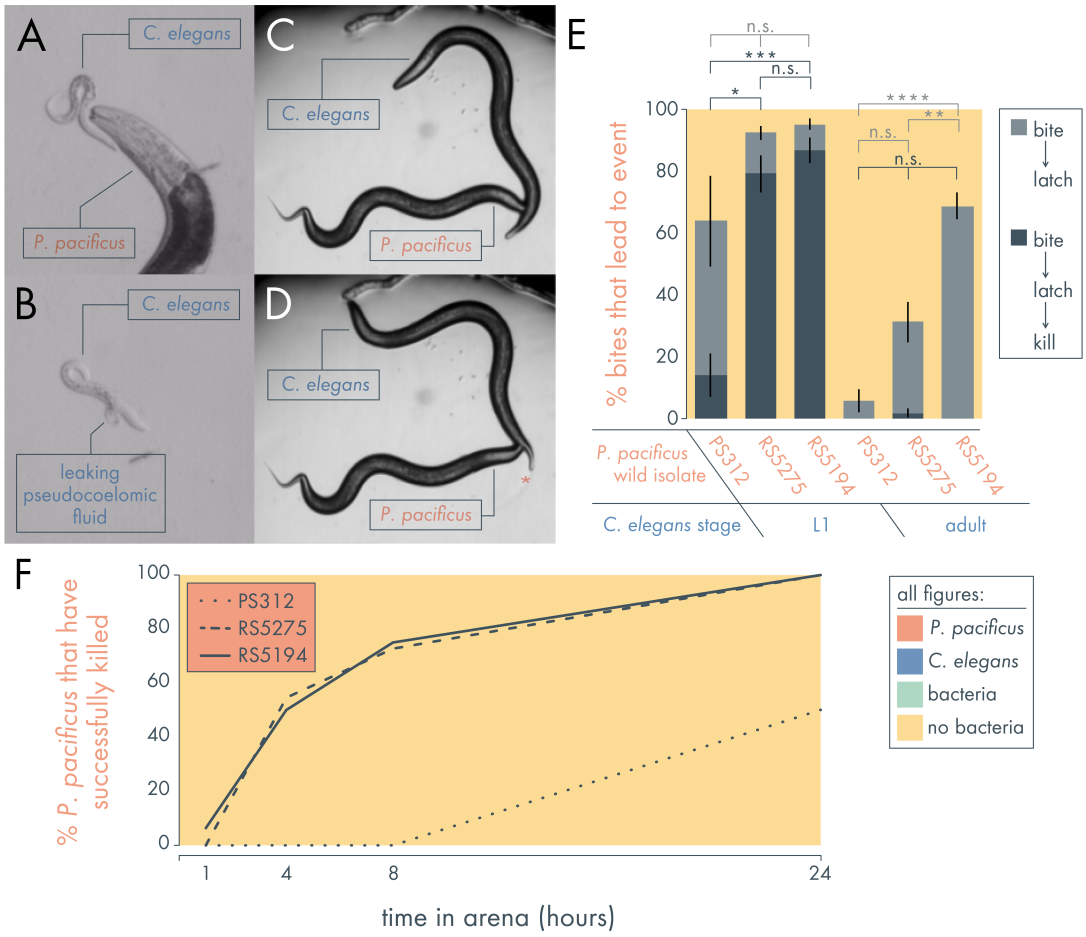

Figure 2.1: Biting ability of three *P. pacificus* strains. (A) *P. pacificus* biting larval *C. elegans*. (B) Larval *C. elegans* after being bitten and killed. (C) *P. pacificus* bite that has successfully latched onto adult *C. elegans* body. (D) Latched *P. pacificus* mouth is dragged by escaping *C. elegans*. Asterisk indicates original position of *P. pacificus* mouth upon biting. (E) *P. pacificus* strains RS5275 and RS5194 were better than PS312 at killing L1 *C. elegans*, while RS5194 was better than both RS5275 and PS312 at latching onto adult *C. elegans* (Dunn's test: *p<0.05, **p<0.01, ***p<.001, ****p<0.0001). Almost none of the bites by any strain resulted in killed adult *C. elegans*. (F) RS5194 and RS5275 were better than PS312 at killing adult *C. elegans* when allowed to focus biting on a single prey for 24 hours.

Bacteria has more value as food than C.*elegans* prey

I next investigated the nutritional value of and preference for three different food sources for *P. pacificus* : bacteria, L1 *C. elegans*, and adult *C. elegans*. In order for competitive biting to occur, *P. pacificus* must value bacteria more than *C. elegans* prey as food. For bacteria, I chose the laboratory strain *E. coli* OP50, which is used in normal laboratory cultivation of *C. elegans* and *P. pacificus* [12][13]. *E. coli* OP50 has the advantage of being easy to grow into transparent, thin, and small lawns that maintain shape. Compared to the sessile *E. coli* OP50, both L1 and adult *C. elegans* prey are more difficult to attain as food. However, I wondered about the nutritional value of each food source regardless of how energetically expensive it may be reach the feeding stage. For example, it is plausible that long time investment towards hunting large adult *C. elegans* prey may come with commensurate nutrition dividends. Pack hunting may also overcome some of the difficulty of hunting adult *C. elegans*. Previous studies have utilized the fat-soluble dye Oil Red O (ORO) to assess fat stores in nematodes [14],[15]. In preparation for ORO staining, we set up feeding plates consisting of ~300 *P. pacificus* that fed ad libitum for 6 hours on an exclusive diet of an excess of *E. coli* OP50, L1 *C. elegans*, or adult *C. elegans*. First, We selected 6 hours as the feeding period for several reasons. Secondly, our previous results indicated that 50% of RS5194 can kill adult *C. elegans* within 4 hours, given that predator and prey are in close proximity for a long period of time. Accordingly, we set up dense clumps of *P. pacificus* mixed with prey to limit *C. elegans* ability to escape bites. Lastly, we wanted to collect *P. pacificus* for staining before eggs laid by adult *C. elegans* prey hatch, about 9 hours at 22°C. Hatched eggs would contaminate the exclusive adult *C. elegans* prey diet with L1 *C. elegans*. The results of ORO staining revealed that *P. pacificus* accumulated more fat stores from a diet of *E. coli* OP50 than from a *C. elegans* prey diet. L1 *C. elegans* and adult *C. elegans* diets are comparable to each

other, although adult C. *elegans* is significantly higher in nutritional value (Fig. 2.2.A).

P. pacificus preference for food may not necessarily correlate with nutritious value. In order to construct a hierarchy of food preference, we posed pairwise choices of food to *P. pacificus*. In order to account for the possibility that *E. coli* OP50 may emit more salient odors than prey, we desinged an 'easy' food choice assay by placing small spots separated by 2 mm. By having these food spots only 2 body lengths apart, *P. pacificus* can easily switch between the two food sources. We used C. *elegans* motility mutants so that we would not have to use a paralytic that would also affect *P. pacificus* and prevent switching. To be sure that the final location of *P. pacificus* was due to a decision to stay or remain, rather than just being the first food source it encountered, we placed a single well-fed *P. pacificus* directly in one of the two food spots. After one hour, we checked to see whether or not *P. pacificus* switched to the neighboring food spot. We found that food switching was most likely to occur, in descending order, from an adult C. *elegans* spot to OP50, from adult C. *elegans* to L1 C. *elegans*, and from L1 C. *elegans* to OP50 (Fig. 2.2.B). The inverse of these switches had low probabilities of occurring. By calculating the probability difference between inverse switches (+0.342 A $\rightarrow$ L1, +.406 L1 $\rightarrow$ OP50, +0.615 A $\rightarrow$ OP50) , we determined the preference hierarchy as *E. coli* OP50 > L1 C. *elegans* > adult C. *elegans*.

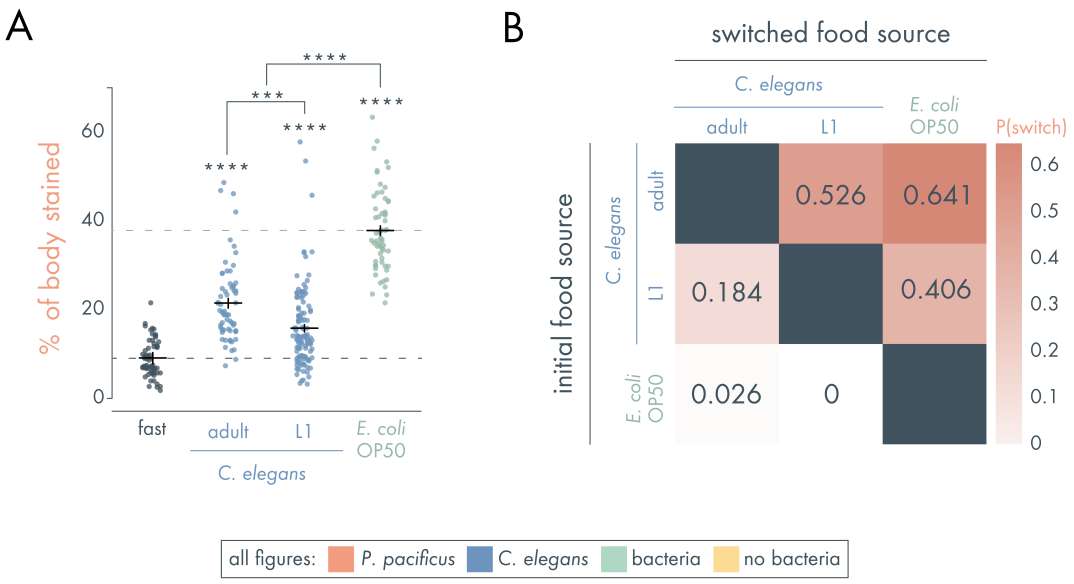

Figure 2.2: Nutritional value of and preference for prey and bacteria. (A) Oil Red O staining of *P. pacificus* after 6 hours of feeding on exclusive diets of adult *C. elegans*, larval *C. elegans*, or *E. coli* OP50. *P. pacificus* was fasted for 6 hours as a control. *P. pacificus* obtained less nutrition from both stages of *C. elegans* prey than from bacteria (Dunnett T3 test: ***p<0.001, ****p<0.0001). (B) Probability of switching between pairs of adult *C. elegans*, larval *C. elegans*, and *E. coli* OP50. *P. pacificus* switched most often when the initial food source is adult *C. elegans* and when the alternate food source is OP50.

C. elegans consumes bacteria faster and produces more progeny than *P. pacificus*

After characterizing *C. elegans* as a prey, I next assesses its potential as a competitor to *P. pacificus*. Compared to *P. pacificus*, *C. elegans* is an obligate bacterivore and possesses a specialized structure in its pharynx that mechanically lyses bacteria. *P. pacificus*, on the other hand, lacks this grinder and carries live bacteria in its gut [1]. It has been speculated that this leads to longer digestion time in *P. pacificus* and why *P. pacificus* has a longer defecation cycle than *C. elegans* when fed the same bacterial strain [16]. In addition to perhaps consuming bacteria more quickly than *P. pacificus*, adult *C. elegans* presents a secondary future threat to a bacterial supply by producing more progeny than *P. pacificus*. Previous studies report that the average brood size of *C. elegans* is 300 per self-fertilizing hermaphrodite, whereas that of *P. pacificus* is 200 per self-fertilizing hermaphrodite [4]. The shorter ex utero development of *C. elegans* eggs further amplifies the collective competitive threat of *C. elegans* progeny [17]. *C. elegans* takes about 9 hours for laid eggs to hatch, whereas *P. pacificus* requires about 25 hours of development before hatching. To see if textitC. elegans can deplete a small bacterial food source faster than *P. pacificus*, I placed a single *P. pacificus* or adult *C. elegans* on a small (∅ 2 mm) lawn of GFP-labelled *E. coli* OP50 and inspected the lawn every 12 hours for remaining bacteria and number of progeny. Adult *C. elegans* consumes bacteria faster than adult *P. pacificus*. At 12 hours, when almost no *C. elegans* and *P. pacificus* eggs have hatched yet, adult *C. elegans* consumed 50% more bacteria than *P. pacificus*. 44% of *C. elegans* depleted their bacteria lawns (<5%) within 24 hours, increasing to 100% by 36 hours. Meanwhile, *P. pacificus* exhibited a 12 hour lag in achieving the same level of bacterial consumption as *C. elegans* (Fig. 2.3.A). At all timepoints, *C. elegans* lays nearly twice as many eggs than *P. pacificus* (Fig. 2.3.B). As expected, we

observed *C. elegans* larvae at 24 hours, but only found *P. pacificus* larvae at 36 hours (Fig. 2.3.C). The lack of accelerated bacterial depletion post-hatching suggests that adult *C. elegans* is the primary force of bacterial consumption, and that its pool of young progeny are only threatening in terms of their impending adulthood (about 48 hours from hatching to adulthood). Taken together, *C. elegans* is more efficient than *P. pacificus* at consuming bacteria and producing progeny, and thus can seriously impinge upon *P. pacificus* access to bacteria resources.

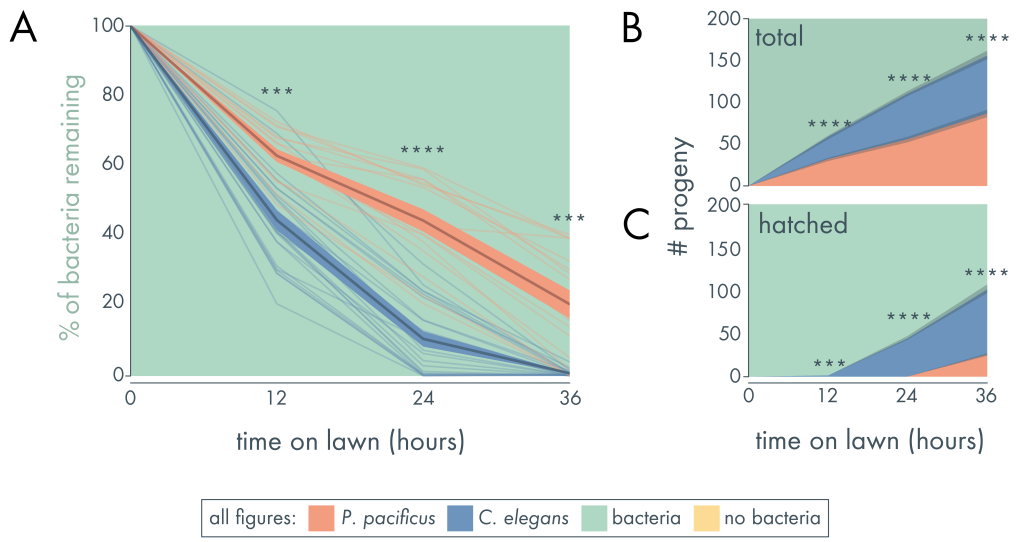

Figure 2.3: *C. elegans* is a more efficient than *P. pacificus* at exploiting bacteria. (A) Adult *C. elegans* consumed OP50 at a faster rate than *P. pacificus* (Mann-Whitney U test: ***p<0.001, ****p<0.0001). (B) Adult *C. elegans* laid eggs at a faster rate than *P. pacificus* (Mann-Whitney U test: ****p<0.0001). (C) *C. elegans* eggs hatched sooner than *P. pacificus* eggs (Mann-Whitney U test: ***p<0.001, ****p<0.0001).

P. pacificus competitive biting on bacteria is dependent on bacterial density, lawn size, and detectability of lawn

The results above suggest that *P. pacficus* could retain more of its bacterial supply if it were able to expel adult *C. elegans* from a shared bacterial resource. *P. pacificus* may be able to achieve this through biting. However, it is possible that biting can achieve this competitive benefit as an incidental side effect of failed predatory attempt. In order to determine whether biting of *C. elegans* on bacteria can be intentionally motivated by interference competition and resource protection, I designed a competitive biting assay in which a bacterial lawn can be adjusted in both size and density (Fig. 2.4.A). More specifically, I designed the competitive assay with the goal of distinguishing between two behavioral models of biting motivation. In the predatory model, biting is driven solely by nutrition and expected to be highest when bacteria is completely absent and prey is the only food option. As bacteria supply increases, biting would gradually decrease as alternate food becomes available (Fig. 2.4.E). In the competitive model, biting is primarily motivated by interference competition and is expected to be high when bacteria is scarce. Biting would be low when bacteria is absent if *P. pacificus* values bacteria considerably more than prey. Starvation is known to increase biting of larval prey on bacteria [3] and may have an equalizing effect on preference for bacteria and prey. In order to preserve a strong preference for bacteria over prey, I only used well-fed *P. pacificus* for the competitive biting assay. A critical prediction of the competitive model concerns how *P. pacificus* behaves at the lower extreme of its ability to detect bacteria. In this edge case, I expected biting to be highest when bacteria is barely detectable, and thus most scarce on the perceptible scale, and drop precipitously when bacteria becomes barely undetectable(Fig. 2.4.E). Notably, the predatory and competitive models have the same shape when bacteria is present; the two models are only distinguishable by

the difference in biting between when bacteria is absent and when bacteria is present (Fig. 2.4.E-F).

In order to elicit competitive biting, I prepared bacterial lawns that can range from scarce to abundant. It is easy to produce an excess of bacteria, but few studies have described presenting nematodes with very small or very thin lawns. I reasoned that a 'small' lawn would be just wide enough to fit a single *P. pacificus*, so the smallest lawn diameter I used was 1 mm, the length of an adult *P. pacificus*. This size of lawn could also be practically defended by a single *P. pacificus*. In order to create a lawn with a density than might be considered scarce by *P. pacificus*, I diluted a liquid bacterial culture of *E. coli* OP50 and used it to seed and grow a lawn for 20 hours at 20°C. I found that a liquid culture with OD_{600}=0.01 produced a lawn that is patchy and has an interrupted border (Fig. 2.4.B). In contrast, lawns seeded with higher optical densities of liquid bacterial cultures produced lawns with homogeneous interior area and markedly thicker boundary relative to the interior (Fig. 2.4.C-D). Lawns grown thicker than the one in (Fig. 2.4.D) distorted visibility of *P. pacificus* mouth too much and were thus not used.

In the arena (∅ 3.2 mm) of the competitive assay, I placed a single *P. pacificus* and a single adult*C. elegans* with a bacterial lawn of various sizes and densities. I then recorded the arena for 30 minutes and identified encounters and which of those encounters resulted in a bite. To account for differences in number of encounters across conditions and within condition, I calculated biting probability as number of bites divided by number of encounters. The results of the competitive assay vindicate the competitive model of biting motivation (Fig. 2.4.H-I). Biting was highest on the smallest (∅ 1 mm) and thinnest lawn (OD_{600}=0.01), and lowest when bacteria was absent or undetected. The OD_{600}=0.01 lawn represented a bacterial density that is the 'detection limit' for *P. pacificus*. *P. pacificus* usually dwelled on and rarely left

OD_{600}=0.30 and OD_{600}=1.00 lawns. However, on OD_{600}=0.01 lawns, *P. pacificus* often fluctuated between periods of dwelling on the lawn and periods of leaving or crawling right through without slowing down. In order to determine respective biting probabilities for barely detected and barely undetected bacteria, I segregated encounter events on OD_{600}=0.01 lawns into two categories: encounters that occurred on the thin lawn and encounters that occurred off the lawn. Encounters that occurred on the thin lawn exhibited the highest biting probability, while encounters that occurred off the thin lawn had low biting probability that was similar to when bacteria is absent. Increasing the density of a small lawn accordingly reduces the biting probability (Fig. 2.4.H). Increasing the size of an OD_{600}=0.3 lawn also incrementally decreased biting probability, with a ∅ 3 mm lawn able to diminish biting probability to the same level as when bacteria is absent (Fig. 2.4.I). In summary, the results of the competitive biting assay provide evidence in support of the competitive model of biting motivation. It is important note that this does not mean that biting of adult *C. elegans* is exclusively competitive; rather, the model only purports that competition is a major contributing factor to biting.

I next assessed how effective competitive biting is at protecting a scarce bacterial territory. Using data from all tested small lawns, I looked at the likelihood that adult *C. elegans* immediately exited the lawn given that it was bitten. This conditional probability of exiting does not differ significantly across densities of small lawns, so I pooled all densities together and found the general exiting probability given a bite to be 0.700 (Fig. 2.4.G). Therefore, *P. pacificus* biting has a 70% success rate in removing *C. elegans* intruders from a bacterial territory, and competitive biting can be considered as an effective form of territorial aggression.

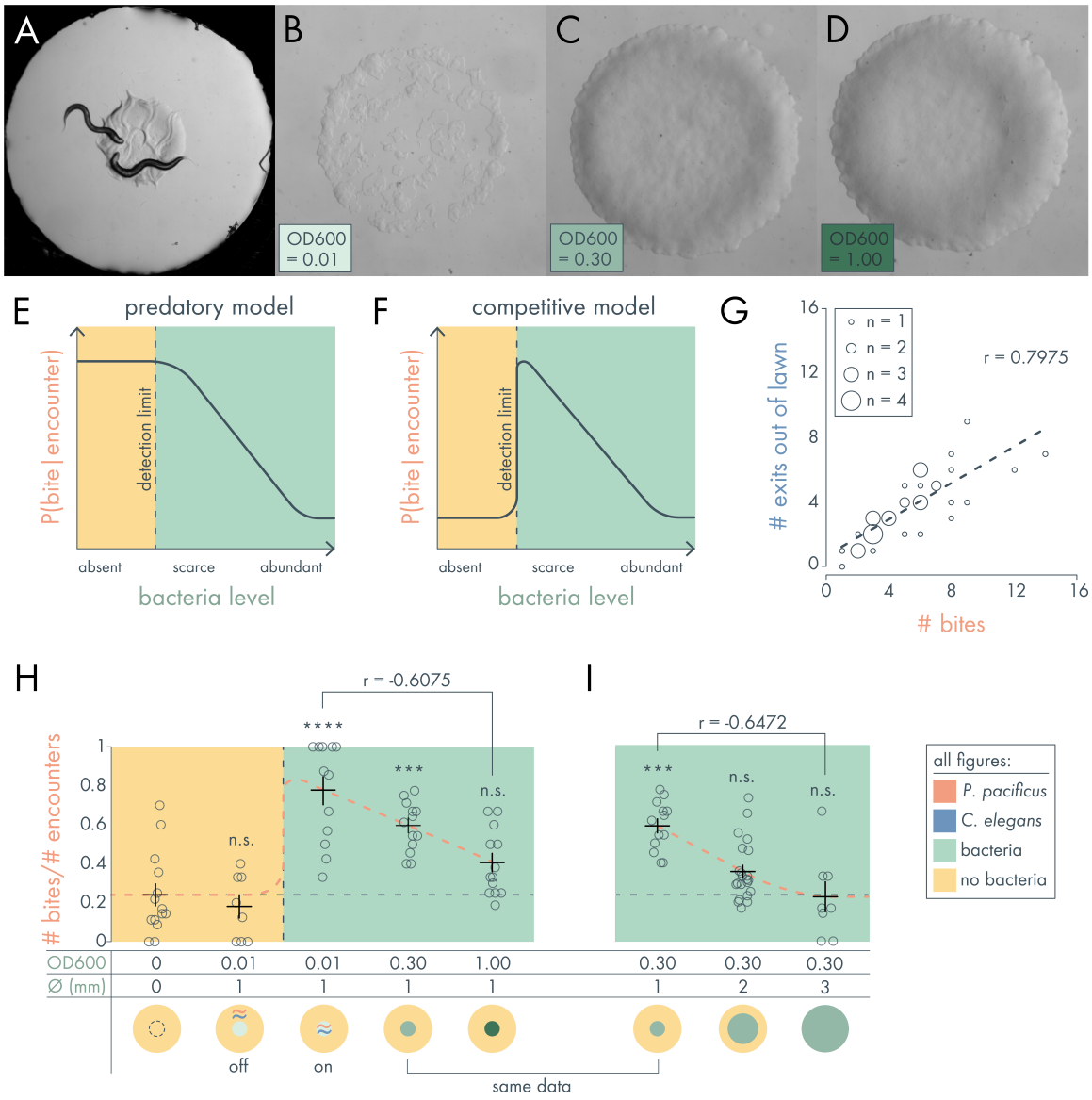

Figure 2.4: Competition motivates biting of adult *C. elegans* on scarce bacteria. (A) Setup of competitive biting assay. (B-D) Small lawns seeded with varying optical densities (OD_{600}) of liquid *E. coli* OP50 culture. (E) Predatory model of biting motivation. (F) Competitive model of biting motivation. (G) Incidences of adult *C. elegans* exiting the lawn is correlated with the number of bites received. Larger dots represent multiple data points (Pearson r = 0.7975, p<0.0001). (H) Biting probabilities on Ø 1 mm lawns with different bacterial densities. (Dunn's test: ***p<0.001, ****p<0.0001). Biting probability and log(OD_{600}) have a negative correlation (Pearson r=-0.6075, p<0.0001). (I) Biting probabilities on an OD_{600}=0.3 lawn with different lawn diameters. Data points for small OD_{600}=0.3 lawn are reused from (H). Biting probability and lawn diameter have a negative correlation (Pearson r=-0.6472, p<0.0001).

Ablation of the amphid sensory neuron ADL increases competitive biting

I next sought to identify which sensory neurons are involved in scarcity-induced increase in biting probability. *P. pacificus* has few known neuron-specific promoters, but are amenable to to lipophilic dye tracing of ciliated amphid sensory neurons [18][19][20]. By staining *P. pacificus* with the lipophilic dye DiO, I was able to identify *7* pairs of amphid neurons with ciliated nerve endings that are exposed at the *P. pacificus* nose (Fig. 2.5.A). I used a focused laser microbeam to target and ablate corresponding pairs of stained amphid neurons in larval *P. pacificus*. I then tested ablated animals two days later at the adult stage in the competitive biting assay with a ∅ 1 mm OD_{600}=0.3 lawn. While most ablated pairs of amphid neurons showed no difference from mock ablated animals, ablation of neuron VII resulted in increased biting probability 2.5.A). Neuron VII has been recently identified as the homolog to *C. elegans* amphid neuron ADL [20]. All ablated *P. pacificus* were able to detect bacteria and dwell within the bacterial lawn, so the biting increase seen with ablated ADL (VII) is not caused by a complete disabling of bacteria perception. Therefore, ADL likely does not sense the the binary presence absence of bacteria. Rather, ADL may sense a more subtle graded quality such as bacterial density, and ablation of ADL may cause for bacteria density to seem more scarce. Previous *C. elegans* studies on the role of ADL in sensing hypoxia and the association of hypoxia to bacterial oxygen consumption suggest that perhaps ADL senses hypoxia as an indicator of thick bacterial density [21][22].

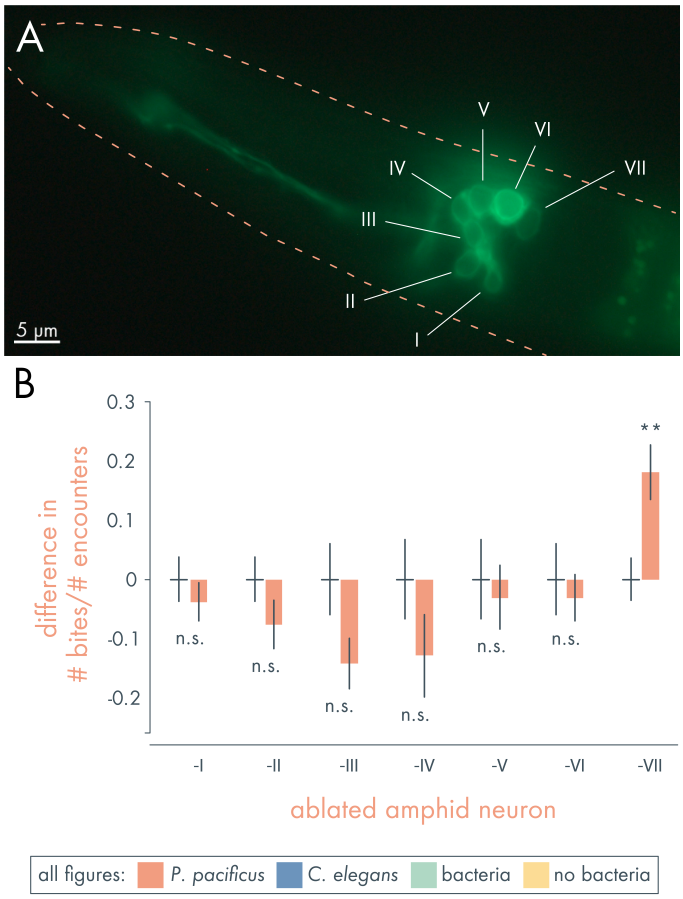

Figure 2.5: Ablation of ADL amphid sensory neuron increases competitive biting. (A) Fluorescent image of *P. pacificus* J2 larvae stained with DiO. Seven symmetric pairs of amphid neurons are stained. (B) Biting probability increases when the amphid neuron ADL (VII) is ablated (t test: **p<0.01).

C. elegans lays eggs off bacteria after prolonged exposure to *P. pacificus*

The above results suggests territorial biting as a means for *P. pacificus* to oust *C. elegans* from a limited bacterial resource, but it remains to be shown if territorial biting has meaningful and lasting consequences for *C. elegans* fitness. Previous research has shown that starvation inhibits egg-laying in *C. elegans* [23][24][25]. One possible way for *P. pacificus* to curtail *C. elegans* fitness is to bite adult *C. elegans* to a degree that *C. elegans* is sufficiently starved to trigger inhibition of egg-laying. However, this explanation is unlikely, since *P. pacificus* bites do not always lead to immediate expulsion of *C. elegans*, and even minute and intermittent access to bacteria may be adequate to sustain normal egg-laying. I hypothesized that a more subtle way of affecting *C. elegans* fitness is to influence where *C. elegans* lays its eggs, rather affecting than the number of eggs laid. In order to determine if *P. pacificus* can influence *C. elegans* egg-laying behavior, I modified the arena from the competitive biting assay to have a larger diameter (∅ 9.5 mm) to expand the empty space where *C. elegans* may leave a lawn and lay eggs. I used a ∅ 2mm to allow space to fit four adult nematodes, which is the minimal number of nematodes that allows mixtures of equal and imbalanced proportions of *P. pacificus* and *C. elegans* (Fig. 2.6.A). For this egg distribution assay, I used a bacterial density of OD_{600}=0.3, which maintains its integrity after *7* hours (Fig. 2.8.B). To discriminate between *P. pacificus* and *C. elegans* eggs, I used a strain of *C. elegans* (CX7389) with integrated GFP expression that is visible at the egg stage (Fig. 2.6.C,D). A combination of 4 *P. pacificus* and/or *C. elegans* are placed in the egg distribution arena for *7* hours, after which the adults are removed and the arena is analyzed egg counts and location (Fig. 2.6.B). I limited egg-laying time to *7* hours to preclude eggs from hatching into larvae, which are motile and thus obscure the original location of

the laid egg. Results show that the distribution of *C. elegans* eggs changes when *P. pacificus* is present (Fig. 2.7). As anticipated, the numbers of eggs laid by *C. elegans* and *P. pacificus* do not change across varying ratios of both nematode species (Fig. 2.8.A). Instead, *C. elegans* changes where it lays its eggs when *P. pacificus* is present. In a group consisting of only conspecifics, both *C. elegans* and *P. pacificus* preferred to lay almost all eggs on the bacterial lawn. In a mixture of the two nematode species, *C. elegans*, lays more eggs off the bacterial lawn when any proportion of *P. pacificus* are present (Fig. 2.8.B). The median distance from the center of the lawn of *C. elegans* eggs lies within the lawn when *C. elegans* is in a conspecific group, but increases to be outside of the lawn when adult *P. pacificus* outnumbers adult *C. elegans* (Fig. 2.8.C). The presence of *P. pacificus* also increases standard deviation of *C. elegans* egg distances from the center of the lawn (Fig. 2.8.D). Meanwhile, *P. pacificus* maintains an egg distribution that is highly confined to the bacterial lawn in all tested mixtures of nematodes (Fig. 2.7). Therefore, prolonged *P. pacificus* competitive biting is an effective form of territorial aggression that alters both location and dispersion of *C. elegans* egg distributions in a way that prevents *C. elegans* from concentrating its eggs on the bacterial lawn.

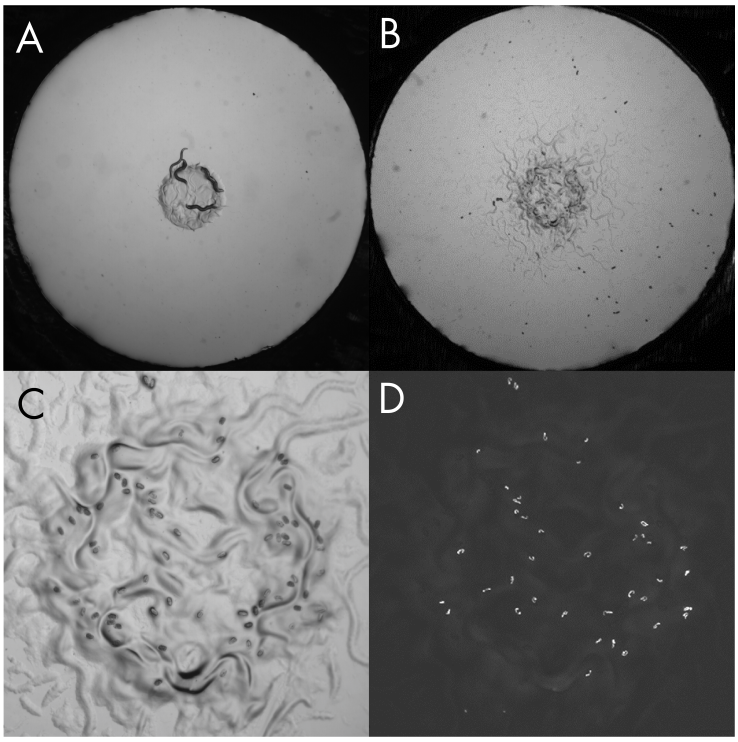

Figure 2.6: Egg distribution assay. (A) Setup of egg distribution assay. A mix of four adult *P. pacificus* and/or *C. elegans* are placed in the arena. (B) *7* hours into the egg distribution assay, adult nematodes removed. (C) Zoomed-in brightfield view of a small lawn that contains both *P. pacificus* and *C. elegans* eggs. (D) *C. elegans* eggs are identified using integrated *elt-2::GFP* fluorescent reporter that expresses in the egg stage.

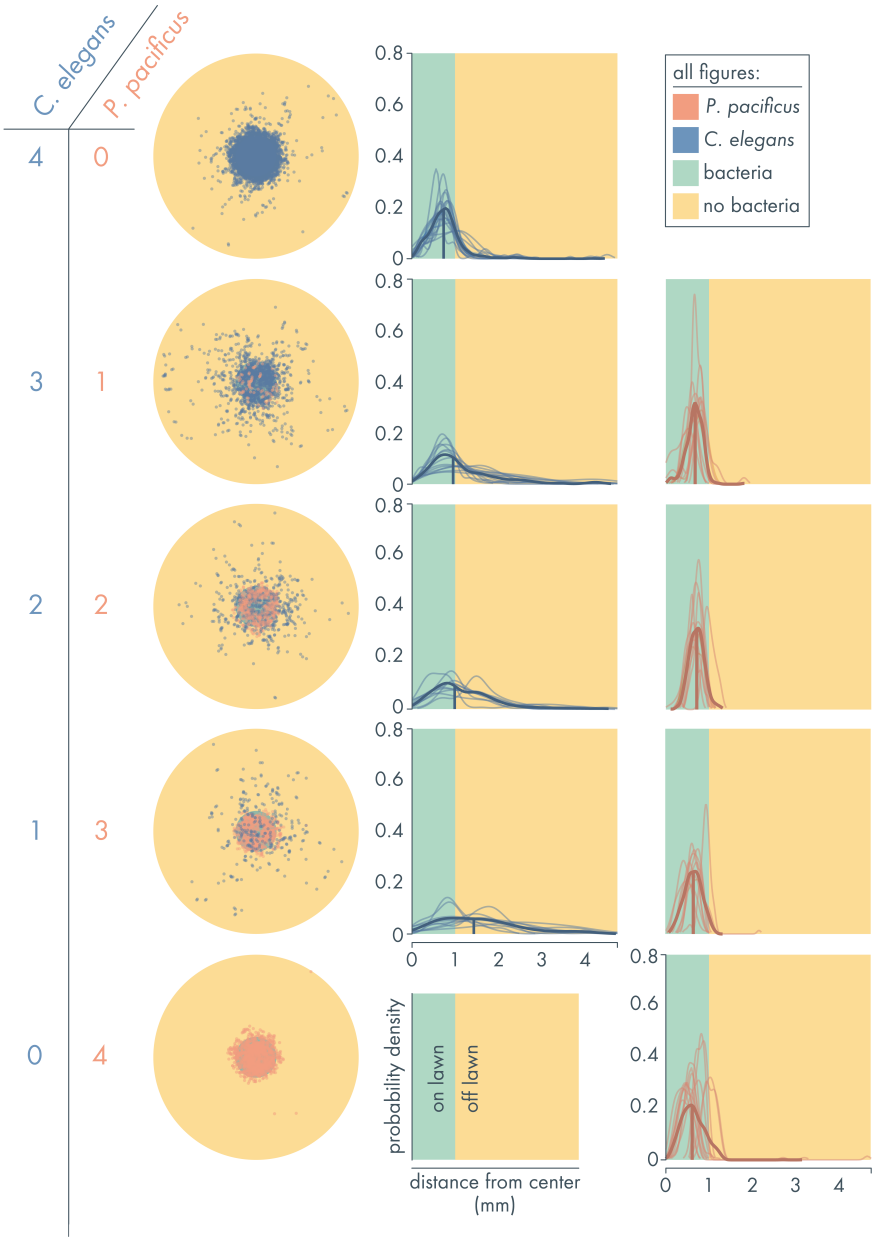

Figure 2.7: Distributions of *C. elegans* and *P. pacificus* eggs in various mixtures of adult *P. pacificus* and/or *C. elegans*. All actual two-dimensional locations of eggs within the arena are visually represented in the circles on the left. Middle and right columns show probability densities of one-dimensional distances of eggs from the center of the lawn. Middle column shows *C. elegans* egg distributions. Right column shows *P. pacificus* egg distributions.

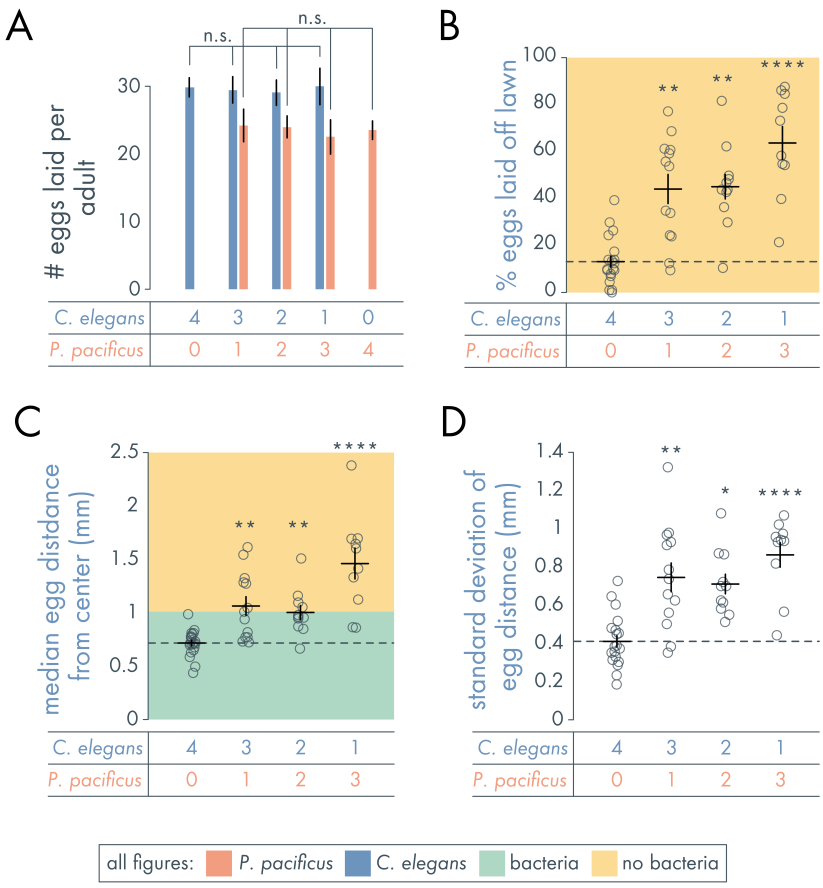

Figure 2.8: *C. elegans* lays eggs off bacteria when *P. pacificus* are present. (A) Number of eggs laid by *C. elegans* and *P. pacificus* adults across different mixtures (Tukey's test). (B) Percentage of *C. elegans* eggs laid off the lawn for different mixtures of *P. pacificus* and/or *C. elegans* adults (Dunn's test: **p<0.01, ****p<0.0001). (C) Median distance from center of lawn of *C. elegans* eggs (Dunn's test: **p<0.01, ****p<0.0001). (D) Standard deviation of *C. elegans* egg distances from center of lawn (Dunn's test: *p<0.5, **p<0.01, ****p<0.0001).

C. elegans avoids entering bacteria lawn after prolonged exposure to *P. pacificus*

At the end of 7 hours of the egg distribution assay with 3 adult *P. pacificus* and 1 adult *C. elegans*, we often noticed *C. elegans* strangely splayed just outside of the lawn (Fig. 2.9.B). We did not observe this behavior early in the egg distribution assay, during which adult *C. elegans* freely entered and dwelled on the lawn (Fig. 2.9.A). To see if *C. elegans* changes its exploration behavior after long term exposure to *P. pacificus*, we recorded for 30 minutes at the beginning and at 6 hours of the egg distribution assay, for 0:4 and 3:1 ratios of *P. pacificus* to *C. elegans*. When a single *C. elegans* shares the arena with three *P. pacificus*, *C. elegans* initially spent most of its time fully within the lawn. *C. elegans* exits the lawn immediately after most bites, but usually re-entered the lawn after a short latency. However, after 6 hours of exposure to *P. pacificus*, *C. elegans* spent almost no time on the lawn, preferring instead to linger at the edge of the lawn. At the edge, *C. elegans* inserted only its nose into the lawn, while the rest of its body remained outstretched away from the lawn. In a group of conspecifics, *C. elegans* spent most of its time with its body completely on the bacterial lawn at both 0 and 6 hours. The slight increase in time spent off the lawn and at the edge may be due to the dense accumulation of eggs that accumulated by 6 hours (Fig. 2.9.C). *P. pacificus* can still bite *C. elegans* at the lawn edge and cause *C. elegans* to retreat fully off the lawn. Interestingly, *C. elegans* received less bites at 6 hours than at 0 hours (Fig. 2.9.D). *P. pacificus*, which spends almost all of its time on the bacterial lawn, is less likely to encounter the minimal protrusion of *C. elegans* head into the lawn. From the perspective of *P. pacificus*, initial investment of frequent biting pays off later when *C. elegans* becomes conditioned to avoid the lawn. Thus, *P. pacificus* more efficiently guards its territory at 6 hours and expends less energy to keep *C. elegans* out of the lawn.

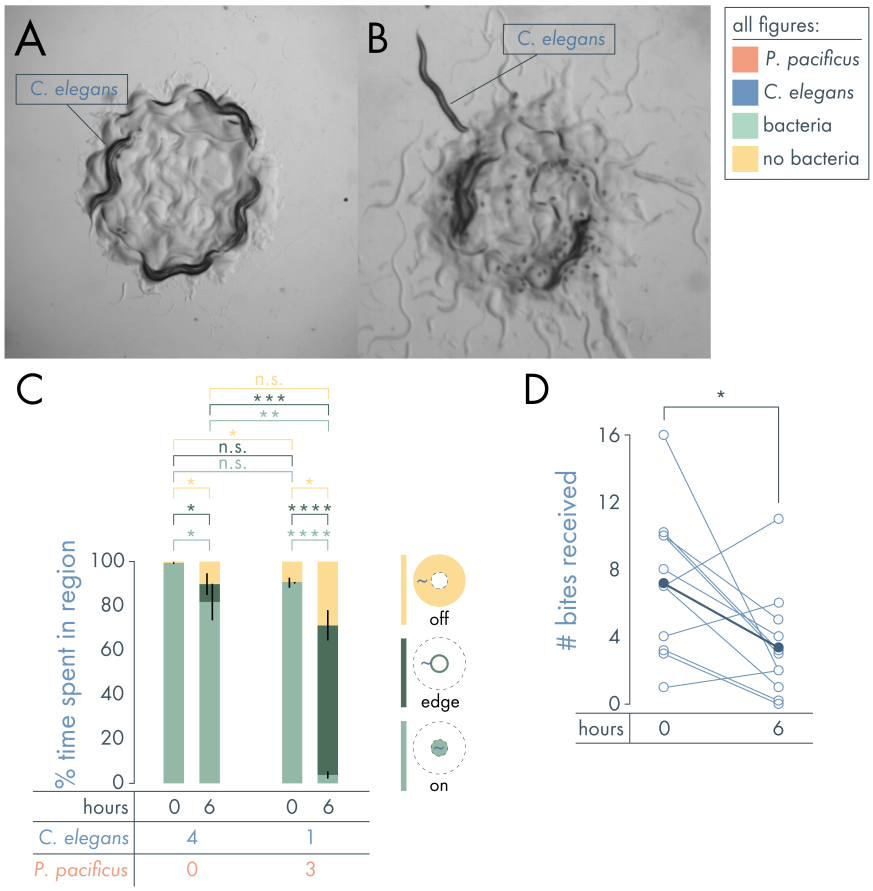

Figure 2.9: Adult C. *elegans* spends more time outside of the lawn when *P. pacificus* is present. (A) Upon initial exposure to adult *P. pacificus* on a small lawn, adult C. *elegan* freely enters and dwells on the lawn. (B) After 6 hours of exposure to *P. pacificus* on a small lawn, adult C. *elegan* lingers outside of the lawn at the edge. (C) Adult C. *elegans* spends less time on the lawn and more time on the edge after prolonged exposure to *P. pacificus* on a small lawn (Dunn's test: *p<0.05, **p<0.01, **p<0.001,****p<0.0001). (D) Adult C. *elegans* receives less bites at 6 hours than at the onset of exposure with *P. pacificus* (paired t test: *p<0.05).

C. elegans progeny laid off bacteria have difficulty finding bacteria

Our above results suggest that *P. pacificus* territorial biting can pressure *C. elegans* to spend more time off the lawn and lay eggs away from bacteria. However, it is possible that larvae hatched away from the bacterial lawn can overcome this distance, in which case, *C. elegans* does not suffer substantial loss to fitness. For *C. elegans* larvae to experience a meaningful disadvantage, newly hatched L1 *C. elegans* would have to be unable to find bacteria by 34 hours, at which point L1 enters the dauer diapause. *C. elegans* development is arrested and cannot reproduce while in the dauer state [26]. Therefore, *C. elegans* fitness can be effectively stunted if *P. pacificus* can cause *C. elegans* eggs to be laid sufficiently far from the bacteria that it will never be able to chemotax to and find the small lawn. To identify which distances away from a small lawn can induce dauer formation, I placed 10 newly hatched L1 *C. elegans* at various distances from a ∅ 2 mm lawn centered on a ∅ 100 mm agar plate and counted how many find the bacterial lawn at the end of 36 hours. At 10 mm away from the bacterial lawn, half (52.22%) of L1 *C. elegans* find the lawn. Attrition of larvae increases as L1 *C. elegans* are placed further from the lawn, such that only 20% of L1 *C. elegans* find the lawn when placed 30 mm away and beyond (Fig. 2.10.A).

It should be noted that the maximum distance from the lawn (3.76 mm) in the egg distribution arena is less than 5 mm, which the above results indicate allows most L1 *C. elegans* to find the lawn. However, the size of the egg distribution arena was an artifice imposed for convenient imaging of all eggs within the same field of view. It may be that *C. elegans* would have laid its eggs farther away if not impeded by the barrier. In order to obtain a more valid measure of *C. elegans* fitness, we extended the egg distribution assay in both time and space. The ∅ 2 mm lawn bacterial lawn was placed in the center of a ∅ 100 mm agar plate, which provided 43 mm of

explorable radial distance from the perimeter of the lawn. I placed either 0:4 or 3:1 ratio of *P. pacificus* to *C. elegans* adults on the lawn and checked 36 hours later to count the number of fluorescent *C. elegans* larvae that were within a 10 mm square area around the lawn. More larval *C. elegans* per adult *C. elegans* were found within 10 mm of the lawn when a conspecific group of adult *C. elegans* was placed on the lawn than when a single adult *C. elegans* was accompanied by 3 *P. pacificus*. Interestingly, larval *C. elegans* counts with 3:1 *P. pacificus* to *C. elegans* adults exhibited a bimodal distribution. One subgroup (n=16) had 10 or less larvae within 10 mm. Another subgroup (n=14) exhibited 50 or more larvae and had a subgroup mean centered at 80.64 larvae, which is still only 63.43% of larval count per adult *C. elegans* in a conspecific group (Fig. 2.10.B). This clustering suggests that about half of adult *C. elegans* largely resided at the extreme edge of the explorable space, where its progeny are unlikely to find the lawn or adult *C. elegans* egg-laying is inhibited by starvation.

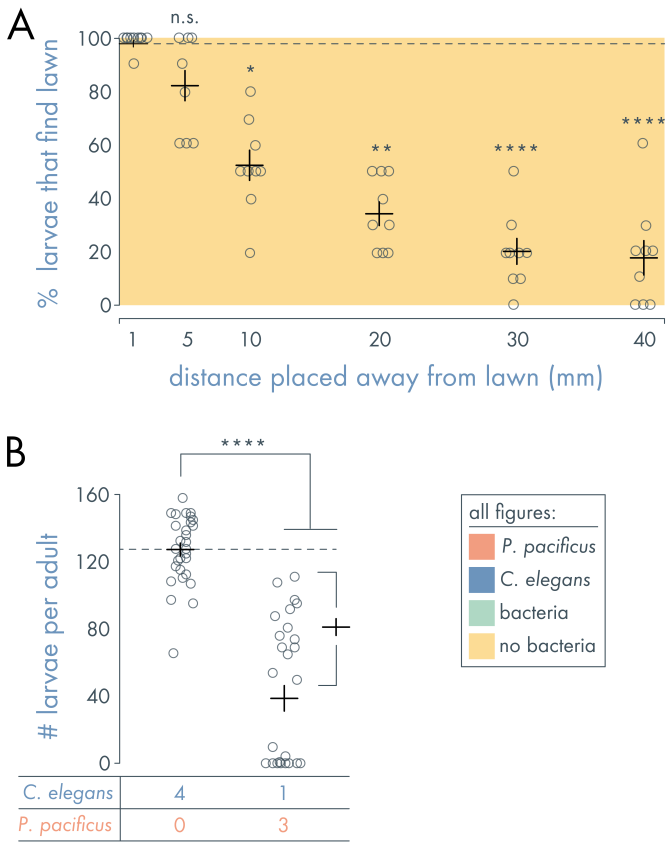

Figure 2.10: Changes in *C. elegans* egg distribution reduce *C. elegans* fitness. (A) Larval *C. elegans* are less likely to find bacteria before dauer transition when placed far away from a small lawn (Dunn's test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). (B) Less *C. elegans* progeny survive when *P. pacificus* are present with a small lawn (Mann-Whitney U test: ****p<0.0001).

The D2 receptor antagonist amisulpride increases biting only on a small lawn

I next used the egg distribution as a high throughput behavioral assay to screen drugs that affect competitive biting. Since few neuronal mutants are available in *P. pacificus*, I decided that it would be more expedient to conduct a drug screen as a first attempt at gaining insight into the signaling that underlies territorial biting. I pruned a library of pharmolocologically active compounds (LOPAC^{1280}) to include only drugs with documented biological action in neurotransmission, which produced a list of 680 compounds to test. To make the drug screen maximally sensitive and prevent attenuation of drug effect, I directly dosed the egg distribution lawn with 2 ul of 1 M of the compound instead of treating *P. pacificus* separately beforehand. However, there was a trade-off for continuous treatment of *P. pacificus* throughout the 7-hour duration of the egg distribution. *C. elegans* was also exposed to the compound, which by itself could have affected egg-laying behavior. 3 *P. pacificus* and 1 *C. elegans* adults were used for all egg distribution assays in all passes of the drug screen. Successive validation iterations were conducted on only those drugs whose median distance surpassed 2.5 mm. These second and third passes replaced the treated lawn with a separate 2-hour treatment of *P. pacificus* immediately prior to the egg distribution assay. The drug screen revealed the D2 receptor antagonist amisulpride (LOPAC ID: 15B04) as the highest performing candidate drugs. When *P. pacificus* was treated with amisulpride, adult *C. elegans* laid eggs far from the bacterial lawn (Fig. 2.11).

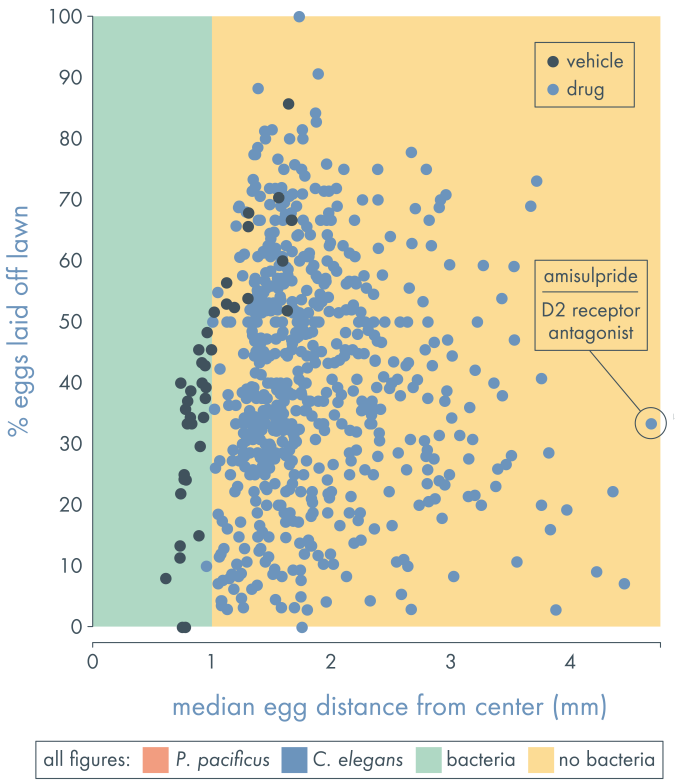

Figure 2.11: Drug screen of pharmocologically active drugs with neural action. Dark grey dots are untreated controls. Blue dots are all drugs with egg distributions that were centered off the lawn. The dashed vertical line indicates the boundary of the lawn at 1 mm radius from the center. Treatment of *P. pacificus* with D2 receptor antagonist amisulpride resulted in *C. elegans* laying eggs far from the lawn.

C. elegans egg distribution is an indirect measure of *P. pacificus* biting. In order to directly validate amisulpride's effect on *P. pacificus* behavior and to determine whether amisulpride increases biting generally, I recorded 30 minutes of 3 pre-treated *P. pacificus* and 1 *C. elegans* in various conditions. To establish points of reference, we first measured biting in untreated *P. pacificus* that were either starved or well-fed, and placed on either a small lawn or a bacteria-free arena. For the small lawn, we judiciously selected an intermediate size (∅ 2 mm) and intermediate density (OD_{600}=0.3) to allow for competitive biting on a small lawn to increase or decrease in both directions, and to permit inferences about which bacterial lawn trait is being affected by drug treatments. In untreated *P. pacificus*, the greatest differential in biting probability occured when *P. pacificus* was well-fed: *P. pacificus* bit significantly more on a small lawn than on a bacteria-free arena. When *P. pacificus* was starved, biting equalized such that biting probability was the same on both a small lawn and a bacteria-free arena. Biting probability on an empty arena was higher when *P. pacificus* was starved than when well-fed (Fig. 2.12). These results suggests that starvation promotes predatory biting on both small lawns and bacteria-free arenas, while competitive biting only occurs when *P. pacificus* is well-fed and on a small lawn.

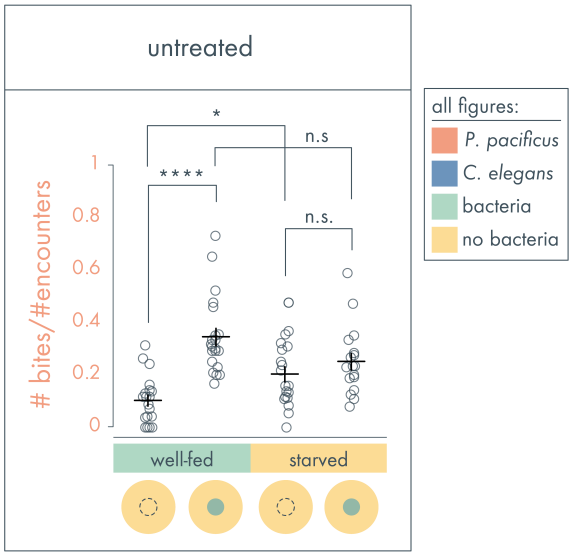

Figure 2.12: Effect of hunger state and biting probability. Untreated well-fed *P. pacificus* displays competitive biting that differs between when bacteria is absent and when a small lawn is present. Untreated starved *P. pacificus* displays predatory biting that is the same when bacteria is absent and when a small lawn is present (Dunn's test: *p<0.05, ****p<0.0001).

P. pacificus treated with amisulpride showed increased biting probability only on a small lawn, but not on a bacteria-free arena . This specificity holds for both well-fed and starved *P. pacificus*. If amisulpride were acting by increasing hunger, then I would expect well-fed *P. pacificus* treated with amisulpride to phenocopy starved untreated *P. pacificus*. However, this is not observed; rather, starved *P. pacificus* treated with amisulpride presented behavior that is characteristic of well-fed untreated *P. pacificus* (Fig. 2.13.A). To further explore the involvement of D2 receptor signalling in competitive biting, I treated *P. pacificus* with the D2 agonist sumanirole. Sumanirole treatment significantly decreased *P. pacificus* biting only in starved *P. pacificus* on a bacteria-free arena. The fact that the change induced by sumanirole is in the direction of decreasing biting probability suggests that D2-like receptor activation may corresponds with lawn thickness. Sumanirole-treated well-fed *P. pacificus* did not show any change on a bacteria-free arena (Fig. 2.13.B).

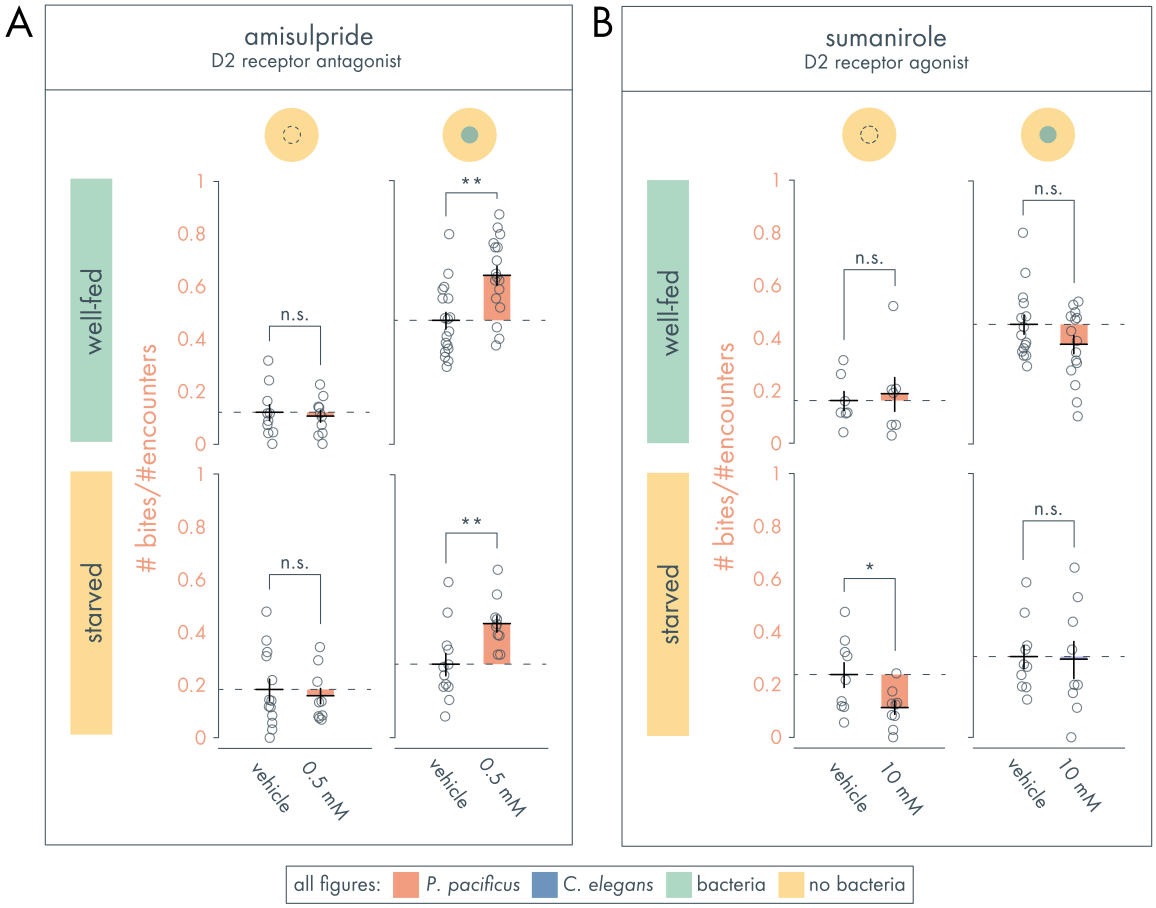

Figure 2.13: D2 receptor drugs affect competitive biting. (A) Amisulpride-treated well-fed and starved *P. pacificus* only increase biting on a small lawn, but not when bacteria is absent or abundant (t test: **p<0.01). (B) Sumanirole decreases biting only when bacteria is absent and *P. pacificus* is starved (t test: *p<0.05).

The combined results of amisulpride and sumanirole treatments and the conditions in they do or not have effects suggest that D2-like receptors are activated by lawn thickness. The effects of exogenous amisulpride and sumanirole treatments on D2-like receptor activation are co-occuring with endogenous D2-like receptor activity, which may explain presence or lack of a drug effect. The D2 receptor antagonist amisulpride has an effect when a small lawn is present, which indicates that D2-like receptors must be endogenously activated in this condition. Sumanirole has no effect on a small, which suggests that D2-like receptors are mostly fully activated. When bacteria is absent, it may be possible that D2-like receptors are not endogenously activated, which means that amisulpride would have no additional effect in that context. Under the same logic, sumanirole treatment would change biting only in a bacteria-free arena but not on a small lawn. Based on these results, I outlined a basic model of endogenous D2-like receptor activation, which provide support that D2-like receptor activation encodes lawn density rather than lawn size (Fig. 2.14). Based on this model, amisulpride increases biting on a small lawn because or perceived thinner bacteria, while sumanirole decreases biting on an empty arena because of perceived thick bacteria.

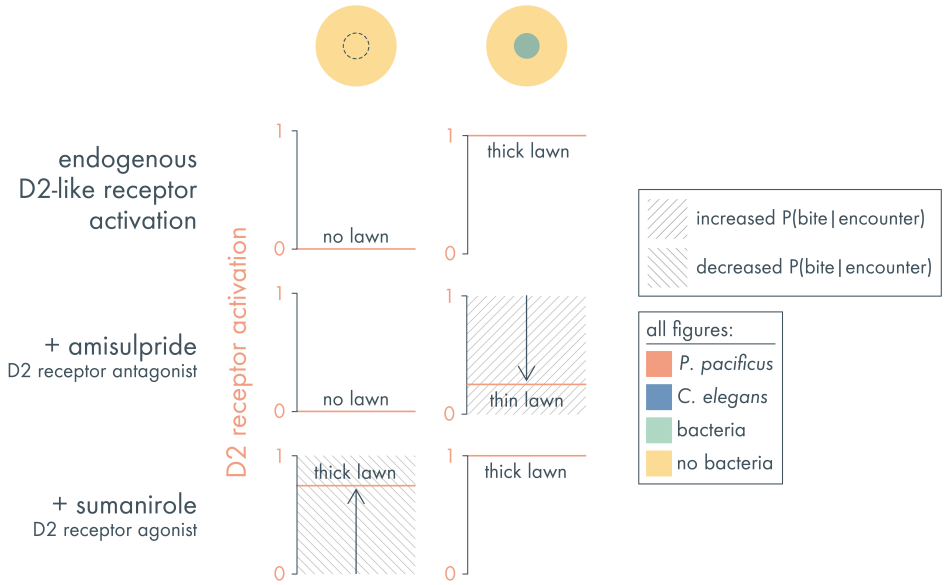

Figure 2.14: Model of amisulpride and sumanirole effects on endogenous D2-like receptor activation. D2-like receptors are endogenously activated when a thick lawn is present, while no activation occurs when bacteria is absent. Amisulpride can only decrease D2-like receptor activation when endogenous activation is sufficiently high, such as when bacteria is present. Sumanirole can only increase D2-like receptor activation when endogenous activation is sufficiently low, such as when bacteria is absent.

The octopamine antagonist epinastine produces opposite effects on biting probability in different bacterial contexts

In *C. elegans*, the presence of bacteria triggers CEP neurons to release dopamine, which in turn activates D2-like receptors on two octopamine-related targets. First, activation of D2-like receptors DOP-3 inhibits octopamine release from RIC neurons. Second, activation of D2-like receptors DOP-2 and DOP-3 on SIA neurons inhibit activation of CREB expression, which is activated by octapamine from RIC neurons (Fig. 2.15.A). In the absence of bacteria, octopamine signaling from RIC neurons is disinhibited, as well as the disinhibition of CREB expression in SIA neurons, leading to starvation-induced activation of (Fig. 2.15.B) [27]. Since the D2 receptor antagonist amisulpride is involved in detecting bacteria and subsequently modulating biting probability in *P. pacificus*, I wondered if octopamine could also be involved. In *C. elegans*, exogenous octopamine treatment elicits behaviors that are similar to those seen during starvation [28]. Therefore, I expected exogenous octopamine treatment to phenocopy the biting behavior of starved *P. pacificus* and promote predatory biting. Octopamine treatment of *P. pacificus* increased biting probability only in well-fed *P. pacificus* on a small lawn. Unlike amisulpride treatment, octopamine treatment did not affect starved *P. pacificus* biting (Fig. 2.16.A). If octopamine were simply simulating starvation, then I would expect biting of well-fed *P. pacificus* to also increase on a bacteria-free arena. However, this was not observed, suggesting that octopamine signaling in *P. pacificus* is still used for processing bacterial availability, but does not lead directly to starvation-induced signals as it does in *C. elegans*.

I next treated *P. pacificus* with epinastine, a highly specific antagonist of octopamine [29]. Epinastine treatment of well-fed *P. pacificus* decreased biting probability on a small lawn, as well as increased biting probability on a bacteria-free arena (Fig. 2.16.B). The latter result of increased biting on a bacteria-free arena

suggests that perhaps octopamine treatment would have had the opposite effect in the same condition, but that the untreated biting probability was already too low to decrease further. Compared with amisulpride and octopamine, which increased biting in particular conditions and had no effect in others, epinastine both increased and decreased biting depending on bacterial context.

The results of octopamine and epinastine treatment are consistent with a model of endogenous octopamine levels that correspond to lawn size. In this model, octopamine receptor activation is highest on a bacteria-free lawn and lowest on a large lawn (Fig. 2.17). Increased octopamine activation is associated with the perception of a smaller lawn. Importantly, this model provides a mechanism for how epinastine can have opposite effects on a small lawn and on a bacteria-free arena. Epinastine treatment on a small lawn acts to make the lawn seem larger, thereby decreasing biting probability. Conversely, epinastine treatment on a bacteria-free lawn may result in perception of a small lawn where there is none, thereby increasing biting. Since D2 receptor activation is minimal on a bacteria-free lawn, epinastine-induced appearance of a small lawn on an empty arena should also appear infinitesimally thin.

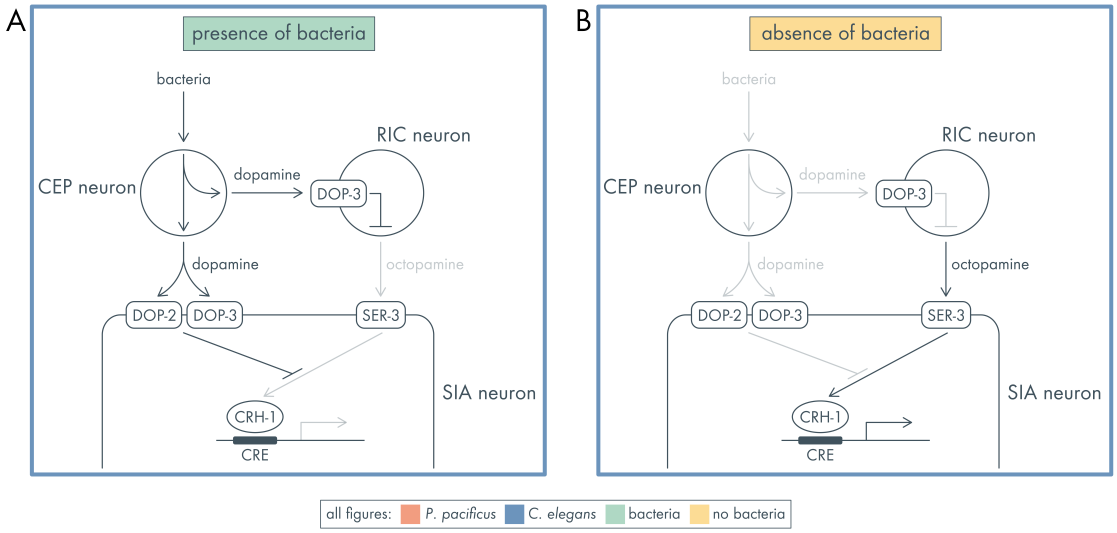

Figure 2.15: *C. elegans* circuit for detecting food absence. Adapted from Suo et al., 2009. (A) The presence of bacteria activates D2 receptors, which in turn inhibit octopamine release. (B) The absence of bacteria disinhibits octopamine release, activates octopamine receptors, and activates CREB expression. Prolonged absence of bacteria in results in starvation signaling.

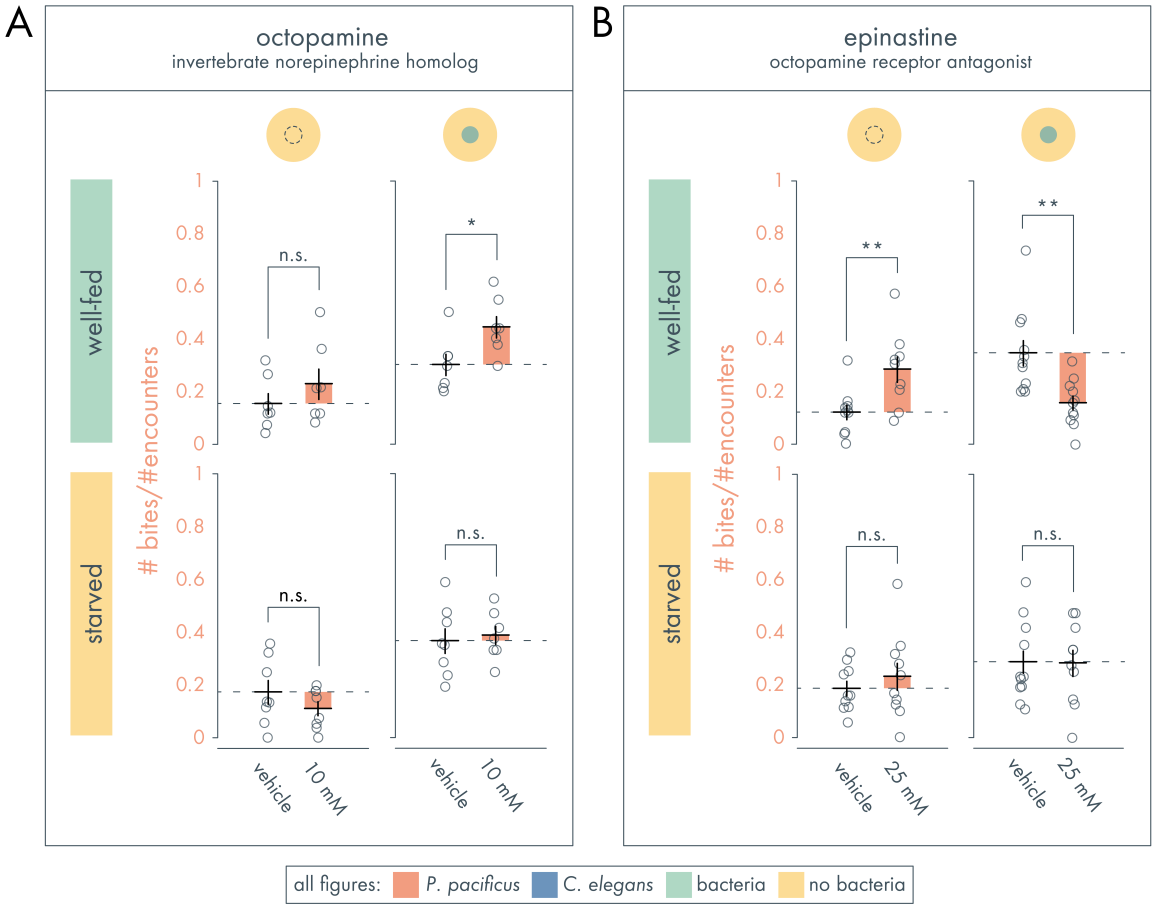

Figure 2.16: Octopamine receptor drugs affect competitive biting. (A) Octopamine increases biting only in well-fed *P. pacificus* on a small lawn (t test: *p<0.05). (B) Well-fed *P. pacificus* treated with epinastine increaes biting on a bacteria-free arena, but decreases it on a small lawn (t test: **p<0.01).

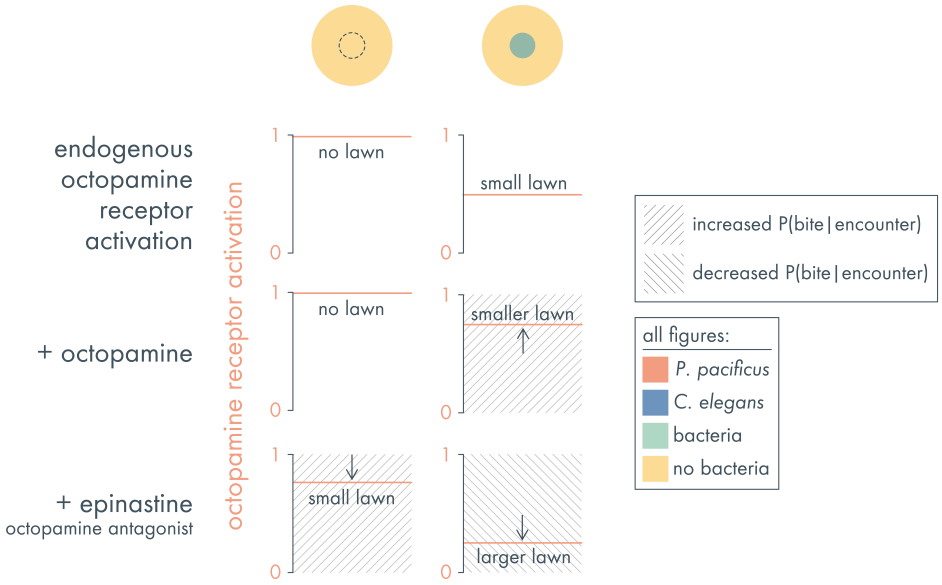

Figure 2.17: Model of octopamine and epinastine effects on endogenous octopamine receptor activation. Octopamine receptors are endogenously activated when a lawn is small, treating the smallest possible lawn as occurring when bacteria is absent. Octopamine receptors are not endogenously activated when a lawn is sufficiently large. Octopamine can only increase octopamine receptor activation when endogenous activation is sufficiently low, such as when a small lawn is present. Epinastine can only decrease octopamine receptor activation when endogenous activatoin is sufficiently high, such as when bacteria is absent or a small lawn is present.

A model of how D2-like and octopamine receptors signal bacterial information to influence biting probability

We next sought to describe a mechanistic model of competitive biting that can explain how bacterial information is integrated to influence biting probability. We considered four different types of bacterial information. The first type of bacterial information is whether bacteria is immediately present or not. The absence of bacteria serves as a binary switch to turn off scarcity-induced competitive biting when *P. pacificus* is well-fed and bacteria is absent. Well-fed biting on an empty lawn has the lowest biting probability that I observed. Well-fed biting on a large lawn diminishes competitive biting back down to the same level, but not lower. Therefore, I defined the basal biting probability to the probability present when *P. pacificus* is well-fed and on a bacteria-free environment. Various bacterial conditions either increase or not increase biting above this basal biting probability. The second parameter is whether bacteria has been absent for a prolonged time and *P. pacificus* is starved. Ours results indicate that starvation leads to a pattern of biting that is concordant with the predatory model of biting motivation. Specifically, biting is high in a bacteria-free environment when *P. pacificus* is starved. It is as if starvation turned off the suppression of biting in bacteria-free environments that is itself turned on when *P. pacificus* is well-fed (Fig. 2.18.A). Finally the last two parameters are bacterial density and lawn size. The results of exogenous treatments show that D2-like activation is low and octopamine receptor activation is high when a bacterial lawn is scarce. D2 receptors are likely activated by thick lawns, while octopamine receptors are likely activated by small lawns. Therefore, both D2-like receptor and octopamine receptor activation respond to bacterial abundance in a monotonic fashion (Fig. 2.18.B-C). We also included the ADL amphid neuron homolog, which we determined likely detects bacterial thickness.

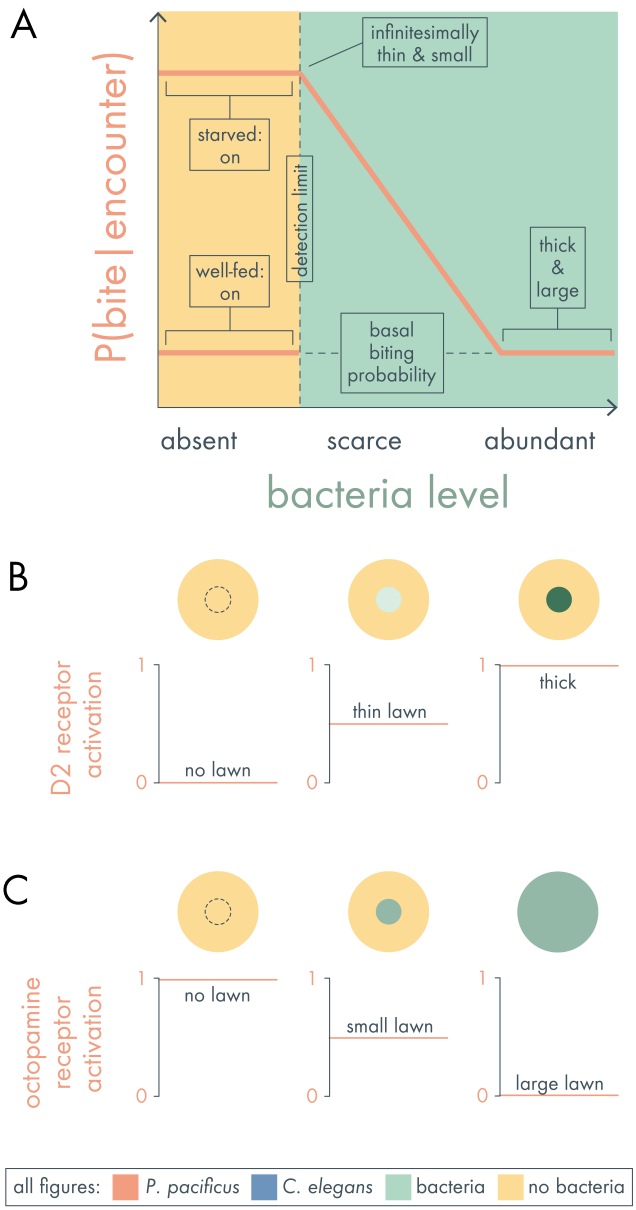

Figure 2.18: Relation of endogenous receptor activation models to behavioral models of biting probability. (A) The competitive model of biting is turned on by well-fed hunger state. The predatory model of biting is turned on by starvation. The switch between competitive and predatory biting occurs when bacteria is absent. (B) Endogenous D2-like receptor activation increases as bacteria becomes more abundant in terms of density. (C) Endogenous octopamine receptor activation decreases as bacteria becomes more abundant in terms of lawn size.

I incorporated all of this into a model that explains how multi-faceted bacterial information flows through D2-like and octopamine receptors to influence biting probability. We found the best-fitting parsimonious model to explain 90.5% of data. Better-fitting models could be obtained with more connections and addition types of bacterial input. In our selected parsimonious model of untreated animals, biting is lowest when *P. pacificus* is well-fed and bacteria is absent, and increases when *P. pacificus* is well-fed on a small lawn or starved in either a small lawn or bacteria-free condition . Biting when starved and in a bacteria free environment is the highest biting probability in the model, higher than either well-fed or starved biting on a small lawn. This is due to my characterization of starvation in the model as complete, although starvation may be a cumulative signal that escalates over time. In our experiments, we starved *P. pacificus* for 2 hours. If this model is true, then we expect that longer starvation periods would further increase biting in a bacteria-free arena to be higher than when a small lawn is present. Regardless, the directions of biting change matches that of untreated data (Fig. 2.19).

Our model also accurately explains when drug treatments induce changes in biting probability compared to untreated controls. In simulated injection of either D2 receptor antagonist amisulpride or agonist sumanirole, the model accurately explains increases in biting when amisulpride is added to either well-fed or starved *P. pacificus* on a small lawn, as well as decreased biting when sumanirole is added to starved *P. pacificus* on a bacteria-free environment (Fig. 2.20). The model also similarly explains changes in biting caused by octopamine or octopamine antagonist epinastine(Fig. 2.21). The model explains the effect of exogenous octopamine to increase biting of well-fed *P. pacificus* on a small lawn. Most importantly, the model accurately characterizes that epinastine treatment of well-fed *P. pacificus* increases biting on an empty arena and has the opposite direction of effect a on small lawn.

Taken together, the model has considerable explanatory power to describe how multiply types of bacterial information gets applied in a context-dependent manner to affect biting probability in a way that is most appropriate to the current available resources and hunger state.

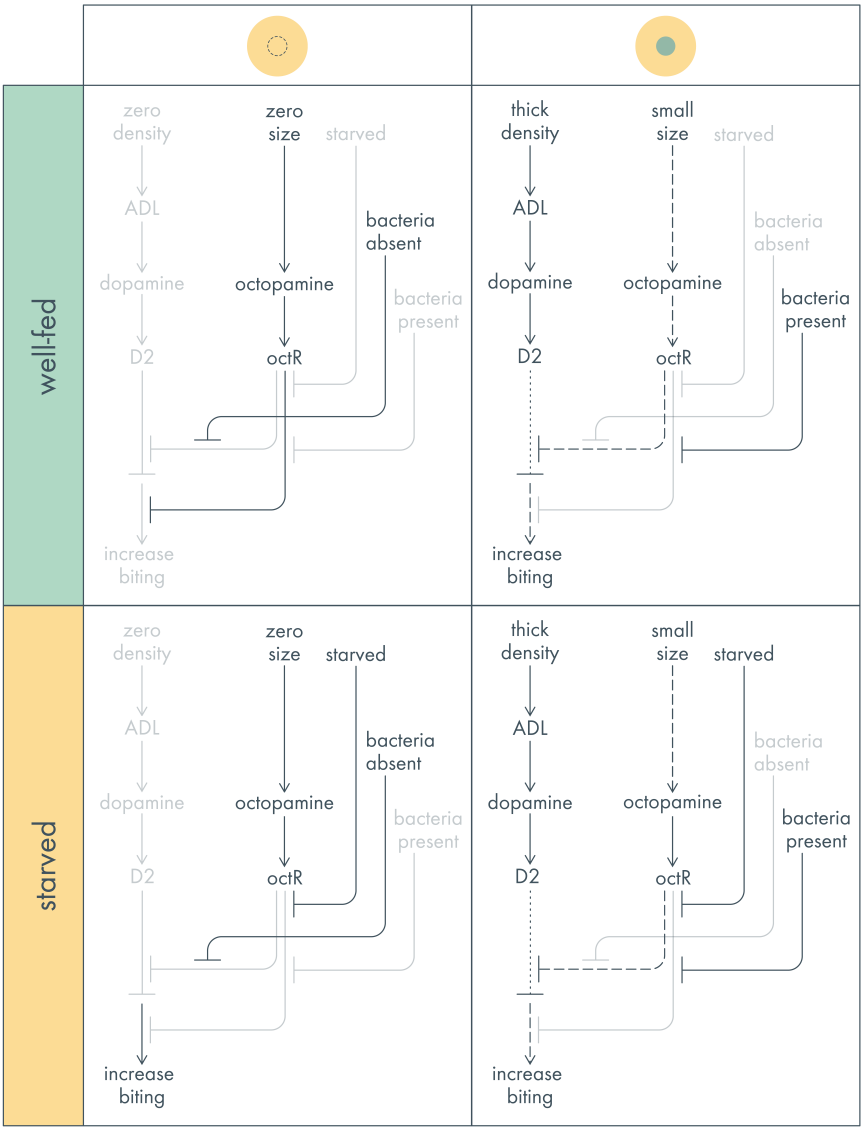

Figure 2.19: A model of how D2-like and octopamine receptors signal bacterial information to influence biting probability. The model explains context- and hunger-specific increases in biting probability.

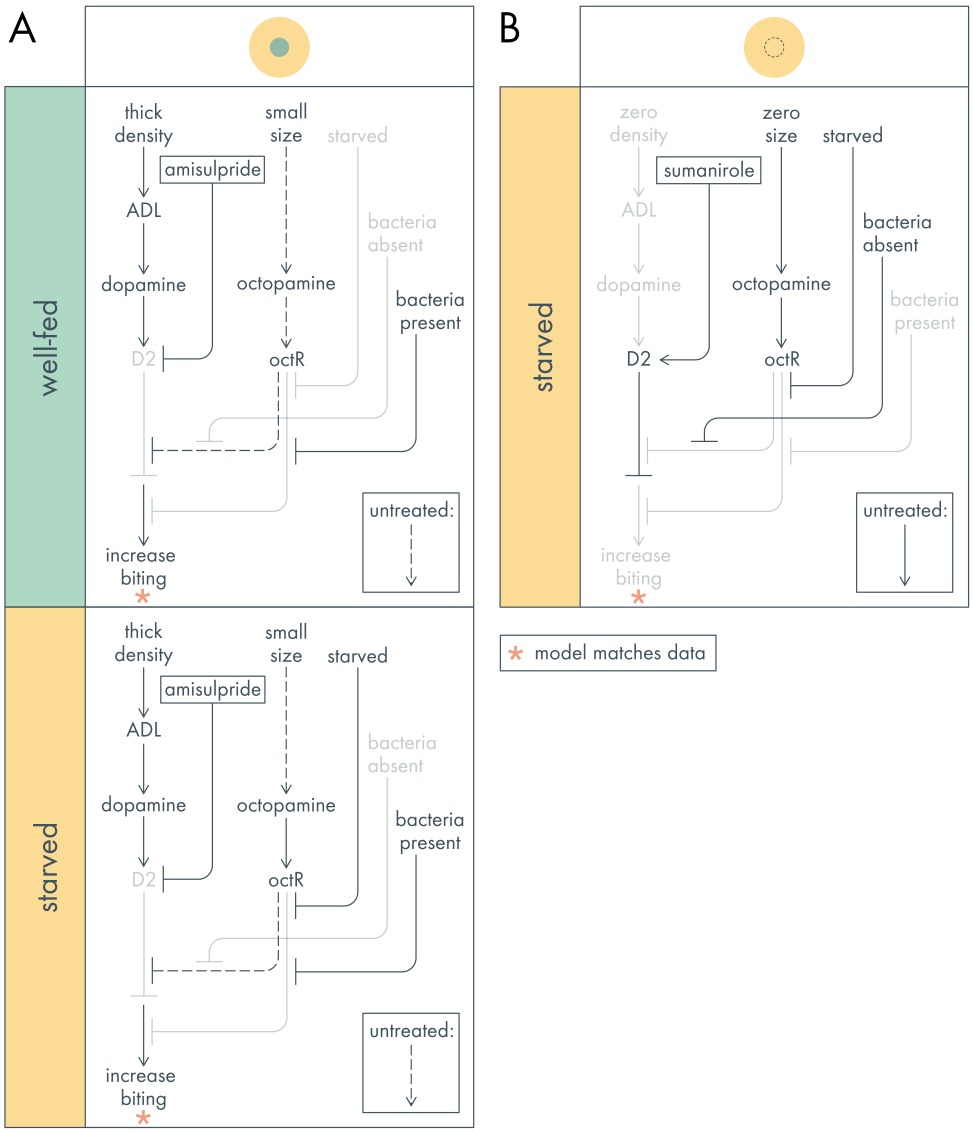

Figure 2.20: The signaling model explains changes in biting probability caused by D2 receptor drugs. (A) The model explains how amisulpride increases biting on a small lawn in both well-fed and starved *P. pacificus*. (B) The model also explains how sumanirole decreases biting of well-fed *P. pacificus* on a bacteria-free arena.

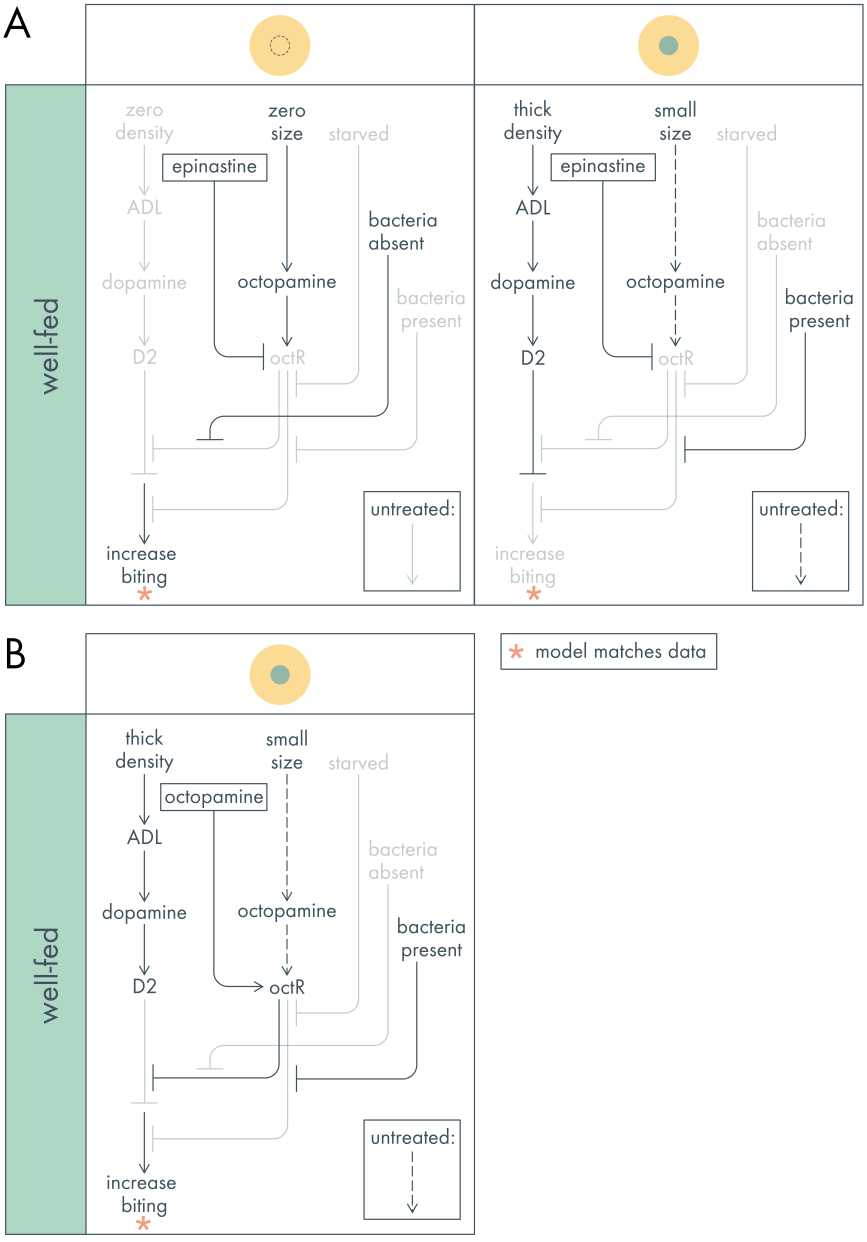

Figure 2.21: The signaling model explains changes in biting probability caused by D2 receptor drugs. (A) The model also explains how epinastine increases well-fed biting on an empty lawn and decreases it on a small lawn. (B) The model explains how octopamine increases biting when *P. pacificus* is well-fed and on a small lawn.

Discussion

Our results show that *P. pacificus* senses bacterial scarcity and uses that information to adjust how much it bites adult *C. elegans* that invades its bacterial territory. These results reveal novel and nuanced competitive motivation and aggressive aspect of *P. pacificus* biting, which has this far studied been studied in a predatory capacity [6][3][30]. Given our observation that predatory biting motivation is only engaged upon starvation, we suggest that competition is the default biting motivation when *P. pacificus* is well-fed and can afford to prefer bacteria more than difficult prey food. These results are consistent with a previous finding that well-fed *P. pacificus* only consumes half of all successfully killed larval prey, which suggested a nonpredatory motivational component to biting [3].

We found that when bacteria is present, *P. pacificus* increases its probability of biting upon encounter with *C. elegans* as bacteria becomes more scarce. Bacterial scarcity is determined by a combination of density and lawn size. Our results suggest that lawn density is conveyed via D2-like receptor activation, while lawn size is represented by octopamine receptor activation. Our results suggest that thick bacteria and large lawn size saturate D2 receptor activation and deprive octopamine receptor activation, respectively. D2-like and octopamine receptor signaling converge to maintain biting probability at a low basal level when bacteria is abundant. However, when bacteria is scarce, D2-like and octopamine receptor activation reverse. We suggest that thin bacteria and small lawn size diminishes D2 receptor activation and elevates octopamine receptor activation, respectively. The confluence of D2-like and octopamine receptor signaling then triggers an increase in biting probability. When bacteria is absent, scarcity-induced biting is suppressed when *P. pacificus* is well-fed and unsuppressed by starvation (Fig. 2.19).

A simple switch regulates the transition between predatory and competitive biting

Our behavioral models of biting motivation allowed us to use biting probability in the presence and absence of bacteria to determine whether competition or predation was the dominant motivation driving biting of adult *C. elegans*. Our predatory and competitive models of biting motivation are identical when bacteria is present. The critical point of difference is when bacteria is absent. In the absence of bacteria, competitive *P. pacificus* exhibit the lowest biting probability, while predatory *P. pacificus* exhibit the highest biting probability. Predatory biting in the absence of bacteria was very similar to biting on scarce bacteria. Therefore, predatory biting in the absence of bacteria is a continuation of scarcity-induced biting. Conversely, competitive biting switches off scarcity-induced biting in the absence of bacteria. We found that hunger state regulates whether or not this switch is turned on (Fig. 2.4.E-F). Well-fed *P. pacificus* biting conforms to the competitive model and exhibits a sudden drop off of biting when bacteria is no longer detectable (Fig. 2.4.H). Starvation turns on predatory biting in the absence of bacteria back on (Fig. 2.12). We propose that this simple starvation-mediated switch may have been one way that early forms of aggression may have arisen. The form of territorial aggression that we discovered relies on existing predatory machinery and circuitry, with only one simple modification.

Our selection of a prohibitively difficult prey was critical to our ability to definitively demonstrate competitive biting. We present for the first time a scenario in which *P. pacificus* bites more when bacteria is present than when it is absent. A prior report showed that well-fed *P. pacificus* bit larval *C. elegans* less when on bacteria [3]. This suggests that *P. pacificus* natively treats adult *C. elegans* more as a competitor and easily killed larval *C. elegans* more as a prey. Previous attempts to disentangle predatory and competitive motivations in intraguild predation relied on the proportion

of uneaten corpses as an indicator of a potentially competitive motivation [10]. In contrast, we selected a readout that did not rely on the lack of feeding motivation, but instead focused on the attack itself. The use of a difficult prey was critical to permitting the use of biting probability as our primary biting metric. Feeding on prey may affect subsequent biting probability as satiety increases, especially when *P. pacificus* is starved. Since no biting of adult *C. elegans* in our competitive biting assays resulted in feeding, we were able to effectively treat all bites as independent events.

Nonlethal biting of adult *C. elegans* is an effective form of territorial aggression

Our studies show that while nonlethal *P. pacificus* biting of adult *C. elegans* is poor for obtaining nutritional value, it is sufficiently harmful to chase away adult *C. elegans* from exploiting a small bacterial lawn. A key prediction of stable intraguild predation that the intraguild prey is better at exploiting a shared resource than the intraguild predator [7]. Our results confirm this prediction. Compared to *P. pacificus*, *C. elegans* consumes bacteria faster, lays more eggs, and its progeny hatch sooner (Fig. 2.3.A-C). Since *P. pacificus* is inferior at exploiting bacteria than *C. elegans*, it is critical that interference competition strategies are deployed to limit *C. elegans* access to bacteria. We show that territorial biting has immediate and long term effects. Any single bite has a strong likelihood of inducing an escape response from *C. elegans*, thus prompting it to urgently leave the lawn (Fig. 2.4.G). However, *C. elegans* eventually returns to the lawn after a short latency. Prolonged territorial biting induces a shift in *C. elegans* exploration behavior such that it spends very little time inside the lawn. Rather, *C. elegans* that has experienced territorial biting for 6 hours will linger just outside of the lawn so that its nose barely contacts the edge of the lawn, but the rest of its body is splayed outward and away from the lawn (Fig. 2.9.C). This

behavior may represent an ecologically relevant example of fear conditioning that is adaptive for *P. pacificus*. We show that the decreased time spent by the *C. elegans* on the bacterial lawn has negative fitness consequences. While *C. elegans* prefers to lay its eggs on bacteria, territorial biting compels *C. elegans* to lay eggs away from bacteria (Fig. 2.8.B-D). Our results from the spatial and temporal extension of the egg distribution assay indicate that territorial biting induces *C. elegans* to lay eggs far enough from a small lawn that hatched larvae are unable to find it (Fig. 2.10.B). This is consistent with previous reports that larval *C. elegans* is poor at chemotaxing toward food-associated odors [31]. Therefore, *P. pacificus* territorial biting is effective at keeping both adult *C. elegans* and its progeny from accessing a limited bacterial resources. Unlike most other studied forms of interspecific territorial aggression, *P. paciificus* does not exert territorial aggression against members of its own species. This is in contrast to animals such as birds, damselfish, and damselflies that display high degrees of interspecific territorial aggression, but also show abundant if not more frequent intraspecific territoriality [32][33][34]. This has led to conclusions that interspecific aggression is misdirected intraspecific aggression [32] [34]. However, in the case of *P. pacificus*, intraspecific territoriality was not observed, which provides more support that its breed of territoriality was borne out of a co-opted predation rather than intraspecific aggression.

D2-like and octopamine receptor signaling has been reconfigured to convey bacterial information to influence biting instead of starvation

We suggest that the circuitry involved in producing starvation-induced signals in *C. elegans* has been rewired in *P. pacificus* to regulate biting probability in accordance with bacterial abundance. *In C. elegans*, the presence of bacteria activates D2-like receptors, which in turns suppresses an octopaminergic neuron. In the

absence of bacteria, lack of D2-like receptor activation disinhibits octopamine release. Subsequent octopamine receptor activation then leads to starvation-induced response [27]. Our results suggest that D2-like receptor signaling is also involved in *P. pacificus* signaling of bacteria availability, specifically bacterial density. Ablation results suggest that bacterial density is sensed by the putative ADL amphid neuron homolog. Unlike in *C. elegans*, octopamine receptor activation in *P. pacificus* seems to be activated by a separate bacterial feature, lawn size, rather than just a directly downstream conduit of D2-like receptor-mediated inhibition of octopamine release. Therefore, D2-like and octopamine receptors convey different bacterial information in *P. pacificus*. However, bacterial density and lawn size are not totally independent traits of any natural bacterial lawn. As bacteria becomes very thin and barely detectable, the lawn size becomes difficult to ascertain. If our model of receptor activation is correct, then we expect biting to be highest when a lawn is small and bacterial density is effectively zero. Experiments that are currently in progress suggest that 'lawns' made with Sephadex beads can achieve this unnatural lawn configuration. Sephadex beads possesses a texture that mimics the mechanosensation of bacteria [35]. It is unlikely that mechanosensation is used to detect lawn density, since previous *C. elegans* studies have shown that ADL neurons sense oxygen [21], although this has yet to be confirmed in *P. pacificus*. While we were unable to identify any *P. pacificus* sensory neurons involved in determining lawn size, a previous study from our lab showed that ASK and ASI neurons in *C. elegans* together sense variability in bacterial environment to determine lawn size [36]. Future experiments will have to pinpoint the nature of lawn size determination in *P. pacificus*. None of our ablation or drug treatments obliterated *P. pacificus* ability to find and dwell on a bacterial lawn, which matches *C. elegans* literature that bacteria is sensed in many ways, by chemosensation, mechanosensation, oxygen sensation, and ingestion [37][35][22][36].

Previous studies have shown that exogenous octopamine induced starvation phenotypes in *C. elegans* [28]. However, octopamine receptor activation did not induce starvation phenotypes in *P. pacificus* (Fig. 2.16.A). Since *P. pacificus* is a facultative predator and not an obligate bacteriovore like *C. elegans*, it makes sense that starvation should not be tied directly to bacteria sensing. Starvation in *P. pacificus* should be detected by a prolonged deprivation of nutrients, regardless of whether those nutrients came from bacteria or nematode prey. Rather than transmitting bacterial information to affect starvation, bacterial scarcity signaled by D2-like and octopamine receptor activity is instead rerouted to either increase or not increase biting probability in *P. pacificus*. Previous comparitive studiy of *C. elegans* also showed dramatic system-wide rewiring in *P. pacificus* compared to *C. elegans*. In that example, rewiring was observed in pharyngeal nervous system and likely reflects the presence of teeth in *P. pacificus* in place of a grinder that *C. elegans* uses to lyse bacteria [38]. Similarly, the rewiring that our model implicates may also reflect dietary differences between *P. pacificus* and *C. elegans*.

None of our drug treatments increased biting generally across all conditions, which makes us confident that D2-like and octopamine receptor signaling affects biting proclivity upstream of biting motor programs. This is consistent with previous report that exogenous serotonin triggered pharyngeal pumping and tooth movement that are characteristic of biting, but exogenous dopamine and octopamine did not [3]. Further work will have to be done to figure out how dopamine and octopamine-mediated biting probability interfaces with serotonin-mediated biting movements.

Methods

Animals

The standard *C. elegans* N2 strain was used for all experiments involving *C. elegans*, except for food switching and egg distributrion assays. *C. elegans* CB81 (unc-18(e81)X) was used for L1 food spots in the food switching assay. *C. elegans* IV95 (ueEx46; Is[ida-1p::IDA-1::GFP]) was used for adult food spots in the food switching assay. *C. elegans* CX7389 (kyIs392) with integrated elt-2::GFP was used in egg distribution assays to visualize *C. elegans* eggs. The standard *P. pacificus* PS312 strain and two other wild isolates were used for comparison of biting ability. *P. pacificus* RS5194 was used for all other experiments. Both *C. elegans* and *P. pacificus* strains were grown and maintained under standard conditions at 20°C [12][13].

Biting ability

Short term biting ability was assessed by placing a single young adult *P. pacificus* with either 8 young adult *C. elegans* N2 or 100 L1 *C. elegans* N2 in a 1/8" inch copper corral on an empty NGM agar plate. A 30 minute was recording using a QImaging CCD camera. The video was scored for bites, latches, and kills. Non-latching bites were identified by *P. pacificus* head contractions and *C. elegans* escape response. Latches were identified by attachment of the *P. pacficus* to the *C. elegans* body that impedes *C. elegans* locomotion or drags *P. pacificus* as *C. elegans* escapes. Kills were identified by a breached cuticle and leakage of pseudocoelomic fluid. Long term biting ability was assessed using the same corral, but with only a single young adult *P. pacificus* and a single young adult *C. elegans*. The corral was checked at 1, 4, 8, and 24 hours for dead *C. elegans*.

Oil Red O lipid staining

Oil Red O staining of *P. pacificus* RS5194 was carried out as described in [39] and [40]. Briefly, 200-300 young adult *P. pacificus* were washed 4x with PBST with 560xg centrifugation in between. Animals were rocked with 600 ul 40% isopropanol for three minutes, spun down at 560xg, then supernatant removed. Animals were stained with filtered 3 mg/ml ORO in 60% isopropanol for 2 hours at room temperature, then washed with PBST for 30 minutes, spun down at 560xg, then supernatant removed. Resuspended animals were placed on slides and imaged immediately with a color camera. Color deconvolution was done in ImageJ to separate ORO, background, and body colors. Contours were drawn around each worm body and ORO pixels quantified as a percentage of worm body area.

Food switching

Pairs of food spots were placed 2 mm apart on a 35 mm NGM plate. *E. coli* OP50 spots were made by seeding 0.3 ul of OD_{600}=0.4 liquid culture and grown for 2 days. Adult *C. elegans* spots were made by placing 20x young adult roller mutants (IV95) in a clump. Larval *C. elegans* spots were made by placing 500 L1 *unc-18* mutants with kinky phenotype in a clump. A single young adult *P. pacificus* RS5194 was placed in one food spot of a pair and check one hour later for which spot it settled on.

Bacteria consumption rate and progeny

0.3 ul of OP50-GFP liquid culture (OD_{600}=0.7) was seeded on 3% agar NGM 35 mm plates that had peptone omitted to minimize bacterial growth. The

lawn was grown for 2 days until lawn growth stunts. OP50-GFP lawns were imaged with a Zeiss Axio Zoom.V16. Lawns were imaged prior to adding animals to get initial GFP luminance reading. A single young adult *P. pacificus* RS5194 or *C. elegans* N2 was placed on a OP50-GFP lawn and imaged every 12 hours for 36 hours. Eggs, hatched larvae, and luminance were recorded from all timepoints.

Competitive biting assay

Lawns were prepared by using specific optical densities *E. coli* OP50 liquid culture, as measured by a spectrophotometer at 600 nm. A Ø 1 mm lawn was seeded by dipping a P10 pipette tip into cold liquid culture and lightly contacting the tip onto a cold 3% agar NGM plate. Ø 2 mm and Ø 3 mm lawns were seeded by aspirating and dispensing 0.3 ul and 1 ul of cold liquid culture onto cold plates using low retention tips. All lawns were grown for 20 hours at 20°C. Corrals were centered around the lawn. A single young adult *P. pacificus* RS5194 and a single young adult *C. elegans* N2 were cleaned of bacteria and then placed into a Ø 1/8" copper corral with an eyelash pick. Once the *P. pacificus* mouth touched the lawn, a 30 minute video was recorded using a QImaging CCD camera. Videos were scored for encounters and bites. Encounters began when *P. pacificus* mouth contacted the *C. elegans* body. Transient mouth touches were not counted. Encounters ended when the *P. pacificus* mouth separated from *C. elegans* for more than 10 seconds or more than a third of a body length away. Bites were scored when a latch or a combination of mouth contraction and *C. elegans* escape response was observed. Conditional biting probility was calculated as the number of bites divided by the number of encounters.

Amphid neuron ablation

J2 *P. pacificus* RS5194 were stained for two hours in 15 ng/ml Fast DiO (ThermoFisher) liquid agar-free NGM on a nutator. Animals were then de-stained on an empty NGM plate for 1 hour. An agar plug was used to gently transfer stained J2s onto a 2% agarose in M9 pad, infused with 20 mM NaN3 paralytic. Pairs of amphid neurons were ablated using an Andor Micropoint focused laser microbeam system. Cell death was confirmed immediately by looking for a bubble contained within the cell, and again after behavior by re-staining. Ablated J2 were transferred using an agar plug by onto a lawn to recover and used two days later in the competitive biting assay.

Egg distribution assay

∅ 2 mm OD_{600}=0.3 lawns were prepared the same way as described for the competitive biting assay. A ∅ 3/8" inch was centered around the lawn on a 3% agar NGM plate. A combination of 4 nematodes of young adult *P. pacificus* RS5194 and/or young adult *C. elegans* CX7389 were cleaned of bacteria and then placed around the bacterial lawn using an eyelash pick. The egg distribution assay was run for 7 hours, at which point all adult animals were removed. The plate was incubated at RT for one hour and then at 4°C for 2 days to allow GFP expression while preventing hatching. Arenas were then imaged under bright-field and fluorescence microscopy using a Zeiss Axio Zoom.V16. For measuring *C. elegans* exploration, 30 minute recordings were taken at 0 and 6 hours using a QImaging CCD camera.

Drug screen

We conducted a drug screen using the library of pharmolocologically active compounds (LOPAC^{1280}). We only tested drugs that had documented biological effect on neurotransmission, a total of 680 drugs. For the first pass of the drug screen, 2 ul of 1 M of the compound was directly placed on the ∅ 2 mm lawn of the egg distribution arena and allowed to dry. 3 young adult *P. pacificus* RS5194 and 1 *C. elegans* CX7389 were placed on the drugged lawn and conducted a normal egg distribution assay. For second and third passes of the drug screen, *P. pacificus* was treated separately using the same drugged lawn for 2 hours immediately prior to use in the egg distribution assay.

Receptor drug treatment

Treatment of well-fed *P. pacificus* receptor drugs was done using the same drugged lawn as in the drug screen. Treatment of starved *P. pacificus* was done by submerging *P. pacificus* in a drug solution with M9 as diluent. In order to match drug concentrations across liquid and bacteria media, epinastine, which kills *P. pacificus* at high concentrations, was gradually increased until *P. pacificus* started to die after two hour treatments. For the same effect, the 2 ul droplet for drugged lawn was found to be 10x that of the liquid drug solution used for starved treatment. This conversion factor was used for all D2 and octopamine receptor drugs.

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dissertation author will be the primary author of the prospective paper.

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Chapter 3

Conclusions and Future directions

Ecology is all-encompassing field that concerns all interactions between organisms and their environment. Some of these types of interactions, such as intraguild predation, are fascinating because they represent a mixture of simpler interactions that produce complex and less predictable effects on the population dynamics of an ecosystem. On an individual level, intraguild predator behavior is less straightforward than exclusively competitive or predatory behavior. In the preceding chapter, we illustrated that *P. pacificus* has a more complex and nuanced system of sensing bacteria scarcity than *C. elegans* in order to deal with the complicated relationship that *P. pacificus* has with its food. However, we have only just begun to understand the circuitry underlying *P. pacificus* biting behavior. In this chapter, I highlight several lines of further investigation that would paint a more satisfying picture of the peculiar triangle-shaped relationship between *P. pacificus*, *C. elegans*, and bacteria.

Refinement of signaling model

The work in Chapter 2 provided an initial insight into how *P. pacificus* integrates various types of bacterial information and hunger state to change biting probability in a way that is appropriate for whether *C. elegans* should be treated as a prey or a competitor. However, our signaling model has many missing pieces. First, we do not have a complete picture of the sensory input layer. Through ablation experiments, we were able to identify ADL as the neuron that is most likely sensing bacterial density. However, we have no identities of the sensory neurons responsible for sensing lawn size and presence/absence of bacteria. Since we only ablated 7 of the 12 pairs of amphid neurons, perhaps ablation of the remaining of amphid neurons would reveal the sensory neurons that we seek. However, a better method of identification would be to express GCaMP in all amphid neurons. In C. elegans, the *osm-6* promoter expresses in amphid neurons, and the P. pacificus homologous promoter may also similarly express in its own set of amphids [1]. If successful, then a microfluidic imaging chip can be used to flow liquid bacteria past the nose of P. pacificus [2]. To confirm ASL as the neuron that responds to thick bacteria via oxygen sensing, high concentrations of bacteria as well as hypoxic liquid media should elicit ADL neuronal activity. Flow of highly variable bacteria may elicit activity in neurons that sense small lawns. This method of stimulation is borrowed from a previous paper that showed that ASI and ASK neurons in *C. elegans* detect a small lawn by sensing high variability in bacterial levels that correspond to encountering the lawn edge more often [3]. Next, there should also be a sensory neuron that is responsible for sensing the binary value of whether bacteria is present or absent. We expect this neuron to be activated across all types of bacterial stimuli, and not activated in all conditions where bacteria is absent. Starvation is last piece of sensory information necessary for a full

understanding of biting. Downstream of the sensory input layer, the signaling model lacks exact identification and locations of the D2-like and octopamine receptors involved. Exogenous drug treatment is limited by its lack of specificity. The first step would be to use *cat-2* and *tbh-1* promoters to identify dopaminergic and octopaminergic neurons. Next, D2-like and octopamine receptor promoters, such orthologs *dop-2, dop-3* and *ser-3* [4][5][6], would identify potential targets that were acted upon by the drug treatments of the preceding chapter. Once these neurons can be fluorescently visualized, ablation experiments can inform whether and where they are involved in the biting circuit. Knockout and overexpression mutants should match results seen with receptor drug treatments.

Patrolling

We noticed that *P. pacificus* spent less time than *C. elegans* exploring the interior of a small lawn. Specifically, *P. pacificus* spent most of its time patrolling the perimeter of the small lawn, moving in forward motion with small turns to stay on the curving path. Patrolling may be a form of sentry behavior in which *P. pacificus* guards the boundary of its territory. Preliminary results show that *P. pacificus* exhibit more patrolling behavior when *C. elegans* is also present on the lawn than when another *P. pacificus* is present. Furthermore, *P. pacificus* raised in *Comamonas sp.* bacteria patrol an *E. coli* OP50 lawn more than when raised on *E. coli* OP50. Surprisingly, we found that biting probability is very high on *Comamonas sp.* lawns, even when bacterial density is high. *Comamonas sp.* has been previously described to be more nutritious than *E. coli* OP50 for *C. elegans* [7]. Bacteria of the *Comamonadaceae* family have also been found in guts of wild *P. pacificus* [8][9]. Perhaps the nutrient richness and/or ecological relevance of *Comamonas sp.* imparts additional value to the bacteria as

resource to be protected from competitors. If this is true, then enhanced patrolling of *P. pacificus* raised on *Comamonas sp.* may be related to changes in how *P. pacificus* appraises the value of its bacterial territory. However, an extensive investigation with a variety of other ecologically relevant bacteria will be required to understand the nature of *Comamonas sp.* effect of *P. pacificus* behavior. The use of bacteria naturally found in *P. pacificus* in the wild would lend more ecological validity to laboratory findings.

Another interesting aspect of patrolling is how it can shape *C. elegans* response to prolonged biting. I reported in Chapter 2 that *C. elegans* exits a small when it is bit by *P. pacificus*. After hours of this territorial biting, *C. elegans* avoids the lawn, preferring to linger at the edge rather than enter the lawn. Preliminary results hint that *C. elegans* that has been exposed to *P. pacificus biting* on a larger lawn that fills a corral will instead retreat to the center of the lawn. In this arena, there is no empty space outside of the lawn. *P. pacificus* still patrols the edge of the lawn, which in this case is also the edge of the arena. In this case, the center of the lawn is the region where *C. elegans* has the lowest probability of being bit, since it is the farthest point from the edge where *P. pacificus* spends most of its time. Therefore, *P. pacificus* patrolling may be an interesting tool for studying fear learning in *C. elegans*. In different contexts, *C. elegans* may learn different avoidance strategies that are best suited to avoid encountering *P. pacificus* in that particular environment.

Intraspecific territoriality

While I did not observe any intraspecific biting between *P. pacificus*, a recent study reported that *P. pacificus* will bite larvae of other wild isolates of the same species. *P. pacificus* relies on a high-specific cuticle SELF-1 peptide to discriminate between self-progeny and other nematode larvae [10]. Inspired by this finding, I am

eager to see if *P. pacificus* will exhibit territorial biting against other strains of *P. pacificus*. The use of a *P. pacificus* competitor, rather than *C. elegans*, would be moving away from studying intraguild predation. However, it would allow for investigations about whether *P. pacificus* would also be territorial over larval *C. elegans* food. In the food switching assay used in Chapter 2, an *unc-18* mutant of *C. elegans* CB81 was used to form a stable food spot of larval *C. elegans*. CB81 larvae are defective in locomotion in way that a clump larvae will tighten over time and cause the food spot to shrink in size. Therefore, CB81 can be used to form very small spots of larvae and used to assess if prey scarcity will induce *P. pacificus* to bite other *P. pacificus*.

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