Host Seeking and the Genomic Architecture of Parasitism

Among Entomopathogenic Nematodes

Thesis by

Adler Ray Dillman

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2013

(Defended November 5, 2012)

To my father Ray, my mother Annette, my stepfather Wallace, and my loving wife Beth, for nurturing my passion and enthusiasm for the natural world, and to those who have gone before, lighting the way with the knowledge of their discoveries.

"Doing what little one can to increase the general stock of knowledge is as respectable an object of life, as one can in any likelihood pursue."

—Charles Darwin

"To seek, to strive, to find, and not to yield."

—Lord Alfred Tennyson

Acknowledgments

The longer a person lives, the more friends, family, peers, acquaintances, and strangers affect their life. I have too many people to possibly thank for helping me get to this point and seeing it through successfully. While I certainly am pleased to be finishing my doctoral studies, by no means do I suppose that this is a task I have undertaken alone nor would I contend that this degree has been earned solely by my own work, but with considerable aid. I would like to thank all the teachers I have had over my many years in education, especially those who helped nurture my love of nature and biology, whether by teaching biology or science directly, or by teaching other subjects, thus helping highlight for me the importance and allure of biology. Although one might expect the acknowledgments of a doctoral thesis to focus primarily on the graduate school years, I feel it necessary to reach back into the recesses of the past and thank not only those who have helped me through these last five years, but also all those who have fed the insatiable flames of my passion for biology, thus helping me get to where I am now. Though I cannot remember all the teachers who have influenced me, I would like to begin by thanking Robert Mower and Dan Miller for teaching high school biology with such infectious enthusiasm that my burgeoning interest was greatly encouraged.

Gratitude is owed to the many college professors who taught with inspiring skill and dedication and who continued to add fuel to my passion for the natural sciences. I want to thank Kim O’Neill for teaching the singular greatest biology course of my college career: Introduction to Molecular Biology. In connection with that course, I want to thank Mike Proudfoot for inviting me to be in a study group and showing me how to really learn biology as a diligent student. I also need to thank Lindzie Head, Jessica

Plowman, Joel Mathews, Shauna Clayton, Jay Jevne, and other members of that study group for their participation, camaraderie, and friendship during those long study sessions. Other amazing professors who influenced my college education include: William Bradshaw for teaching me how classroom biology applies to scientific research; Alan Harker for teaching me the basics of ecology; Richard Robison for increasing my love of bacteria and viruses; William McCleary for deepening my understanding of bacterial physiology; Jerry Johnson for encouraging me to take my first graduate-level course and for making speciation and evolution so accessible; Duane Jeffery for championing evolution and working diligently to dispel imaginary conflicts between evolution and religion; Keith Clearwater, Michael Whiting, and Jack Sites for their friendship, encouragement, example, and welcome advice.

I would like to offer special thanks to Byron J. Adams for taking an interest in me when I needed it most. Byron invited me to join his lab, where I first learned about nematodes and why they matter. I cannot say enough good about Byron, how he encouraged me in my personal and professional life, how he allowed me to attend numerous scientific conferences as an undergraduate, and how he has continued to be my friend and mentor. As a member of the Nematode Evolution Good Lab, I became acquainted with some great friends and mentors, many of whom remain friends and collaborators. They include John and Sheena Chaston, TJ Bliss, Tim Davie, Dana Blackburn, Scott Peat, and Bishwo Adhikari. I would like to thank them and all the other members of the Adams lab for making science so much fun. I am grateful to Byron Adams, Jerry Johnson, and Gregory Burton for helping me decide where to go to graduate school, for writing many letters on my behalf, and for their continued friendship

and mentoring over the years. In addition to my colleagues at Brigham Young University, I must thank many other colleagues I was privileged to meet through conferences and various other academic pursuits. Byron included me on the soil ecology team that was part of the long-term ecological research project at McMurdo Base, Antarctica, during the ’05–’06 season. As part of this research effort I became acquainted with such scientific giants as Diana Wall, Berry Lyons, Ross Virginia, and Jeb Barrett, who helped me realize what scientific pursuit can be and continue to inspire me through their own achievements and efforts. I am grateful to Seymour Van Gundy and Ed Platzer for including me in their biodiversity research excursion to Moldova. I have learned a lot from them about science and foreign relations and continue to enjoy their friendship and advice. I am also grateful to James Baldwin for encouraging my work every step of the way and continually acting as my advocate when any scientific or academic opportunity comes my way.

Deciding where to go to graduate school and who to work for can be a very difficult decision. I do not think I could have chosen a better place than Caltech nor a better graduate advisor and mentor than Paul W. Sternberg. Most of my graduate school applications had already been sent off by the time Todd Ciche, then a postdoctoral researcher in the Sternberg lab, came to speak at BYU. He told his story about insect parasitic nematodes and what he was doing with them, and my imagination was sent into overdrive with the possibilities. Todd visited the Adams lab and I got to spend a couple of hours talking about his project and what else he was planning to do. Immediately following Todd’s visit, I researched more information about Caltech and Paul. I made up my mind to apply to Caltech and knew I wanted to work for Paul. Whereas all my

previous graduate applications had been to graduate schools, this application was different, because, although Caltech is among the best universities in the world, I was really only applying to work with Paul. When I came to interview I met with Paul and with several of his colleagues, including Sarkis Mazmanian and Judith Campbell. Of course they all approached the interview formally, as they might with any prospective graduate student. But when I made it clear that my intentions were to work for Paul Sternberg, the interviews became much less formal and they told me what a great person Paul was, how kind and considerate and genuinely interested in his students and postdocs. I thought it odd that none of his colleagues mentioned his scientific accomplishments and prowess but instead talked about what a charitable and genuinely kind person Paul is. I came to understand later that Paul’s professional work speaks for itself but that what is remarkable about him is his seemingly endless reservoir of enthusiasm and excitement for research and his unassuming and approachable kindness. His door is always open, his scientific insight is unmatched, and his sincere kindness and concern for others is apparent to all who know him. It has been a pleasure and a privilege to work with such a brilliant and magnanimous man, and I am honored to have him as a mentor and colleague. He has nurtured my intellect, provided seemingly unlimited resources for my research, and helped to focus my enthusiasm and penchant for taking on too many projects.

Along the way I have met and befriended many graduate students and postdocs during my time at Caltech and would like to thank all of them for their friendship, encouragement, and advice. I want to thank the following individuals: Ted Ririe for helping me get started at Caltech and for his patience in showing me numerous molecular techniques; Cheryl Van Buskirk for her friendship, advice, and listening ear; Jagan

Srinivasan for his focus, friendship, and many good conversations; Elissa Hallem for her example of truly brilliant science, for helping me see how incredibly precise and clear scientific writing can be, and for a wonderfully fruitful collaboration that I hope continues for many years; L. Ryan Baugh for helping me see the value of asking piercing questions, for demonstrating over and over again how a brilliant mind can dissect overinflated science, and for his help and friendship over the years; Nathan Pierce for sharing his contagious enthusiasm for our craft, for his example of uncompromisingly high scientific standards, and for many excellent lunchtime conversations at Señor Fish and other choice restaurants; Aadel Chaudhuri for his encouragement and conversation; Michael Rome for being a great sounding board for ideas and for many pleasant business lunches where I learned about proteins and tried to preach the value of nematodes; Paul Minor, my good friend, for introducing me to a catalogue of great music, for empathizing with the stresses of graduate school, for his entertaining company during countless lunches, for helping me stay grounded and realistic, and for providing much needed comic relief so as to avoid taking myself or the work too seriously; Andrea Choe for encouraging the breadth of my interests and sharing her creative genius with me; Margaret Ho for her willingness to read and provide excellent feedback on many manuscripts and for her cheerful attitude and quick smile; Hillel Schwartz for sharing the immense archive of collected literature and knowledge that his mind has amassed over the years, making him a veritable encyclopedia of nematology; Erich Schwarz for his patience in helping me understand computational biology and his constant encouragement and kind words; Ali Mortazavi for broadening and sharpening my informatics skills and paving the way for many collaborative genome projects both active and planned; John DeModena for his listening ear, his molecular skill, his taste for Asian cuisine, and his advice and friendship. I would like to thank all of the many other graduate students, postdocs, faculty, and staff that have befriended me and shared their knowledge, insights, and camaraderie including: Steven Kuntz, Natalie Kolawa, Liz Wang, Janna Nawroth, Jon Valencia, Eric Erkenbrack, Chris Cronin, Julie Cho, Srimoyee Ghosh, Daniel Leighton, Collin Yu, Mihoko Kato, Ray Deshaies, Michael Dickinson, Victoria Orphan, Wyatt Korff, Eric Matson, Ping Hsueh, Megan Dobro, Alon Zaslaver, and many others who have helped me through word and deed, even if only by example.

I owe many thanks to the members of my thesis committee, Michael Dickinson, Bruce Hay, David Prober, Jared Leadbetter, James Baldwin, and Paul Sternberg for taking time out of their busy schedules to meet with me, read my work, and offer their constructive criticism and advice. I offer my thanks and gratitude to the Howard Hughes Medical Institute and the National Institutes of Health for generously funding much of the research that takes place in Paul's lab, making it easy to focus on the research and worry less about how to pay for it. I thank Caltech for allowing me to be part of such an amazing institution and for the wonderful research experience they have provided. I am convinced that I will never again find the unbelievable atmosphere of collaboration, scholarship, abundance of resources, and free-flowing creativity that is present in the Caltech community, and I am sad to see this chapter of my life close. What a truly marvelous place.

My family has been amazingly supportive of the time and effort I have put in to this program, and I am grateful to have their love, encouragement, and support, without which none of this work would have been possible. My parents, siblings, nephews, uncles, aunts, cousins, grandparents, and wife have been there with encouragement and advice in all my trials, both professional and personal. What an invaluable blessing it is to have the uninhibited and unconditional support of family. It has made this journey so much easier and more enjoyable. I hope to be there for each of them when they need it most. Lastly, I offer a sincere and heartfelt acknowledgement to God, our Heavenly Father, the creator of all things, and His son and our Savior Jesus Christ, for all that I have and am. I am particularly grateful for the beauty and wonder of the natural world and our ability to understand what little of it we can. I have come to believe that scientific research at the highest level can draw one as close to God as many religious pursuits and that it can deepen one's reverence and awe for the Creator rather than dispel it.

Preface

The diversity of life on planet Earth is astounding, making it easy to become enthralled by the myriad different forms of all the organisms one encounters. However, when asked to define a narrowly focused research project, this wondrous diversity becomes challenging—even when a burgeoning biologist has some idea of the kinds of questions that pique his interest. How does one decide in which organism to study those questions? There are, of course, practical considerations such as the culturability of different organisms in the lab, how long they take to reproduce and develop, the cost of obtaining and growing them, the amount of space and resources they require, governmental regulations requiring specific care and treatment of some organisms (i.e., vertebrates), and many others. But there is also the matter of personal taste, passion, and interest. Some biologists absolutely love the organism they work with, while others see the organism as a tool or means to an end. As an undergraduate, I was counseled to become a 'question driven' biologist, meaning that I should not get too attached to any particular organism or technique, but that it should be the biological questions that drive the research, and with the questions well formed, one can then decide which organism and techniques are best suited to address those questions. While I appreciate the value of this counsel, I confess that I am completely enamored by nematodes. I have come to see in them an amazing model system where nearly any aspect of biology can be studied. They are particularly well suited as a model system of behavior, neurobiology, and genomics. Their central nervous system is relatively simple, their genomes are compact, and they are still capable of tremendously interesting behaviors, detailed in the thesis that follows.

Abstract

Nematodes represent an especially abundant and species-rich phylum, with many free-living and parasitic species. Among the diversity of parasitic species is a guild of specialists known as entomopathogenic nematodes due to their unusual ability to quickly kill their hosts with the aid of pathogenic bacteria. Herein I discuss in detail the hallmarks of entomopathogenic nematodes and how they are different from other insect parasites. Further I explore their host-seeking behaviors, demonstrating their ability to detect insect hosts in complex soil environments and assess their odor preference profiles. I show that CO_2 is a major driver of host seeking and that entomopathogenic nematodes detect CO_2 using the same pair of conserved neurons that the fruit-dwelling *Caenorhabditis elegans* uses to detect and respond to CO_2. I demonstrate dramatic differences in odor preference profiles and virulence capabilities, even between closely related nematodes. I discuss the role of genomic sequencing generally and more specifically in nematology, including how genomes are sequenced and analyzed and the types of characteristics that are most prominently assessed. This thesis concludes with a discussion of the genomic sequencing of entomopathogenic nematodes in the genus *Steinernema* and the clues these genomes provide regarding the genomic architecture of parasitism.

Contents

Chapter 1:

An Introduction to Nematodes and Entomopathogenic Nematodes*

* This chapter includes a quick guide first published in *Current Biology* in 2012 that was written by Adler R. Dillman and Paul W. Sternberg.

Abstract

Nematodes are amazing animals, both ancient and diverse. Among their diversity are many plant and animal parasites, many of which negatively affect humans. However, not all parasitic nematodes are bad and some are currently being used as organic alternatives to chemical pesticides for controlling damaging insect pests. Although there are many insect-parasitic nematodes, the entomopathogenic nematodes are the best studied of these and are remarkably different in their lifestyle and in their particular parasitism. Herein I discuss the difference between entomopathogenic nematodes and other insect parasites and what makes them so interesting and useful.

Introduction

In an effort to discern order amid the astounding diversity of life, humans have classified life into the following taxonomic rankings, in descending order: Domain, kingdom, phylum, class, order, family, genus, and species. Modern taxonomists and systematists use this conceptual hierarchy genealogically, grouping closely related species (singular: species) into genera (singular: genus), closely related genera into families, families into orders, orders into classes, classes into phyla (singular: phylum), phyla into kingdoms, and kingdoms into domains [1]. This classification scheme, or genealogy of life, was originally established by Carolus Linnaeus in the 1700s and has been modified to its current form by a host of scientists, reshaping this scheme according to newer findings, as our understanding of the relationships between organisms has increased. For instance, the ranking of domain was not introduced until 1990, and currently there only three recognized domains of life: Archaea, Bacteria, and Eukaryota

[2]. At present, there are at 35 recognized phyla in the animal kingdom, though this number may fluctuate with new discoveries and as our understanding of animal relationships increases. Most people are only familiar with a handful of these phyla, such as Chordata, which includes all vertebrates, encompassing virtually anything you would see at a zoo. Other more commonly known phyla include Arthopoda and Mollusca, which are made up of insects, crustaceans, arachnids, and cephalopods (e.g., squid and octopuses) and gastropods (e.g., snails and slugs). Nematoda is a phylum of roundworms that originated during the Precambrian or Cambrian explosion over 500 million years ago [3, 4]. Although fewer than 30,000 species of nematodes have been described, there are thought to be between 1 and 10 million species of nematodes on Earth, making Nematoda the most speciose (alluding to both their beauty and species-richness) phylum on the planet, even more so than Arthopoda [5–8]. This abundance of evolutionary time and their relatively simple body plan has allowed nematodes to adapt and occupy virtually every ecological niche and climate imaginable. Nematodes occupy marine, freshwater, and terrestrial environments from tropical and temperate environments to extremely dry and restrictively cold environments. Nathan A. Cobb, often considered the father of modern nematology, has written: "[Nematodes] occur in arid deserts and at the bottom of lakes and rivers, in the waters of hot springs and in the polar seas where the temperature is constantly below the freezing point of fresh water. They were thawed out alive from Antarctic ice in the far south by members of Shackleton's expedition. They occur at enormous depths in Alpine lakes and in the ocean" [8]. To borrow another famous quote of his: "If all matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then

investigate it, we should find its mountains, hills, vales, rivers, lakes and oceans represented by a film of nematodes" [9].

Most nematodes are microscopic, varying from 0.5–2 mm in length, with the most heavily studied nematode, *C. elegans*, averaging 1 mm (Figure 1.1). Though rare, there are larger nematodes. The largest, *Placentonema gigantissima*, is a whale parasite that was recorded at over 8 meters in length. Their general body plan is highly conserved among species and relatively simple, essentially consisting of a round tubular body with a mouth on one end and an anus on the other, a digestive tract, and reproductive system [10]. Nematodes also have an excretory-secretory system and a complex nervous system, but no circulatory system. Though this general body plan is conserved, there is extensive morphological diversity of the mouth and cephalic appendages among many species, generally relating to feeding, habitat, and ecology.

While most species of nematodes are "free-living", there are also many parasites of plants and vertebrates. Most of these parasites are devastating and cause many well-known diseases, including elephantiasis, trichinosis, and river blindness. The World Health Organization estimates that more than two billion people are infected with nematodes (http://www.who.int/wormcontrol/statistics/). Though many parasites affect humans directly by causing disease, it is important to emphasize that vertebrate parasitic nematodes also affect humans indirectly by infecting livestock and pets [11]. There are many devastating plant-parasitic nematodes as well, causing an estimated 12.3% annual crop loss worldwide, effectively causing more than 77 billion dollars annually in lost crops [12].

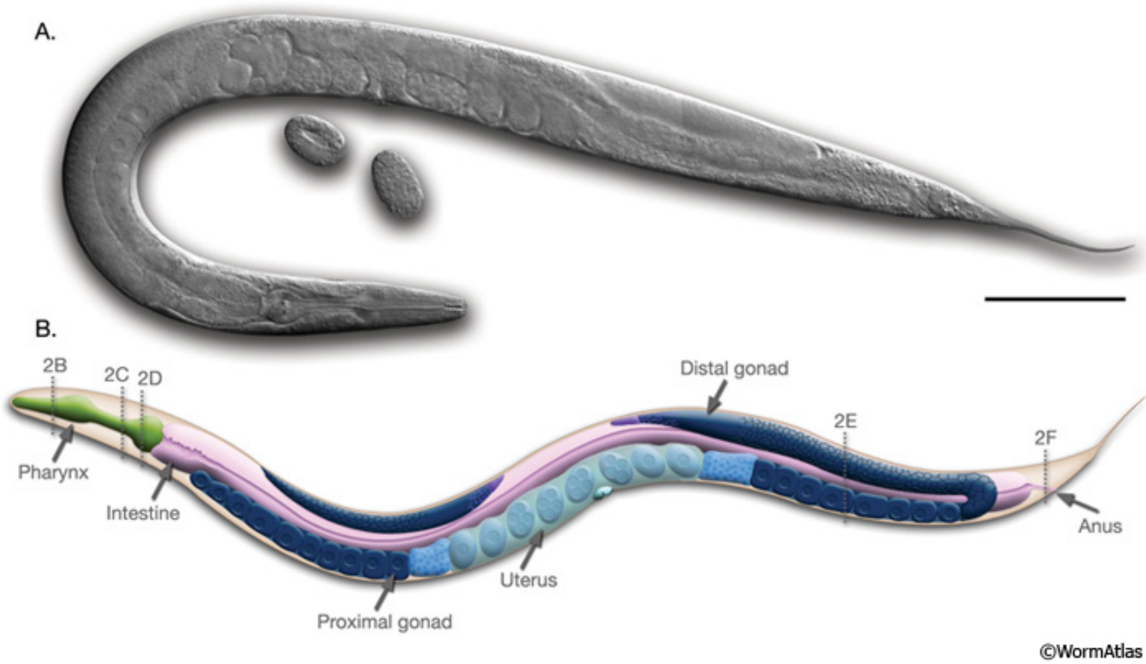

Figure 1.1 | Anatomy of an adult hermaphrodite *C. elegans*. A. DIC image of an adult hermaphrodite *C. elegans*, left lateral side. Scale bar is 0.1 mm. The two round shapes in the middle are recently laid eggs. **B.** Schematic drawing of anatomical structures. Dotted lines and numbers mark areas of additional detailed anatomical information that can be found at http://www.wormatlas.org.

While it is true that most parasitic nematodes affecting humans either directly or indirectly tend to have negative effects, there are some beneficial parasitic nematodes. Many insect-parasitic nematodes have been explored as potential alternatives to chemical pesticides for controlling harmful insect pests. Among these insect parasites, the entomopathogenic nematodes have been the most studied. What follows is taken from a "quick guide" published in *Current Biology* (see footnote in chapter heading), as a brief introduction to entomopathogenic nematodes.

What are entomopathogenic nematodes? Nematodes seem to have evolved to occupy nearly every niche imaginable, including a wide diversity of parasitic niches. Among the vast variety of parasitic nematodes, some have evolved an association with insect pathogenic bacteria. Together the bacteria and nematode are a lethal duo. These nematodes are called 'entomopathogenic nematodes' or EPNs for short. Essentially the nematodes serve as mobile vectors for their insect-pathogenic bacteria cargo, like little Typhoid Marys. The nematodes seek out and invade potential hosts and release their pathogenic payload into the nutrient-rich hemolymph. Infected insect hosts die quickly, the bacteria proliferate, and the nematodes feed on bacteria and insect tissues, and reproduce. When the host cadaver is depleted of resources, nematodes associated with pathogenic bacteria emerge and search for new hosts to infect (Figure 1.2). The cooperation with bacteria and the speed with which they kill sets EPNs apart from other nematode parasites.

How do they kill? The nematode and the pathogenic bacteria they carry contribute to varying degrees, depending on the combination. The known bacterial associates of EPNs, species of *Photorhabdus* and *Xenorhabdus,* are known to produce a toxic cocktail of secondary metabolites that are not only lethal to the insect hosts, but that prevent opportunistic bacteria and fungi from utilizing the nutrient rich cadaver, sequestering the resources for themselves and their nematode partners. The bacteria always contribute to the virulence of the duo, and usually contribute the lion's share. Some species of nematodes are thought merely to shuttle the bacteria, contributing very little to host death, while others are known to be lethal in their own right, producing a variety of secreted protein products that degrade and digest host tissues, in addition to short-circuiting the host immune system. Even though some nematodes appear lethal on their own, no non-bacterial associated EPNs are known to exist.

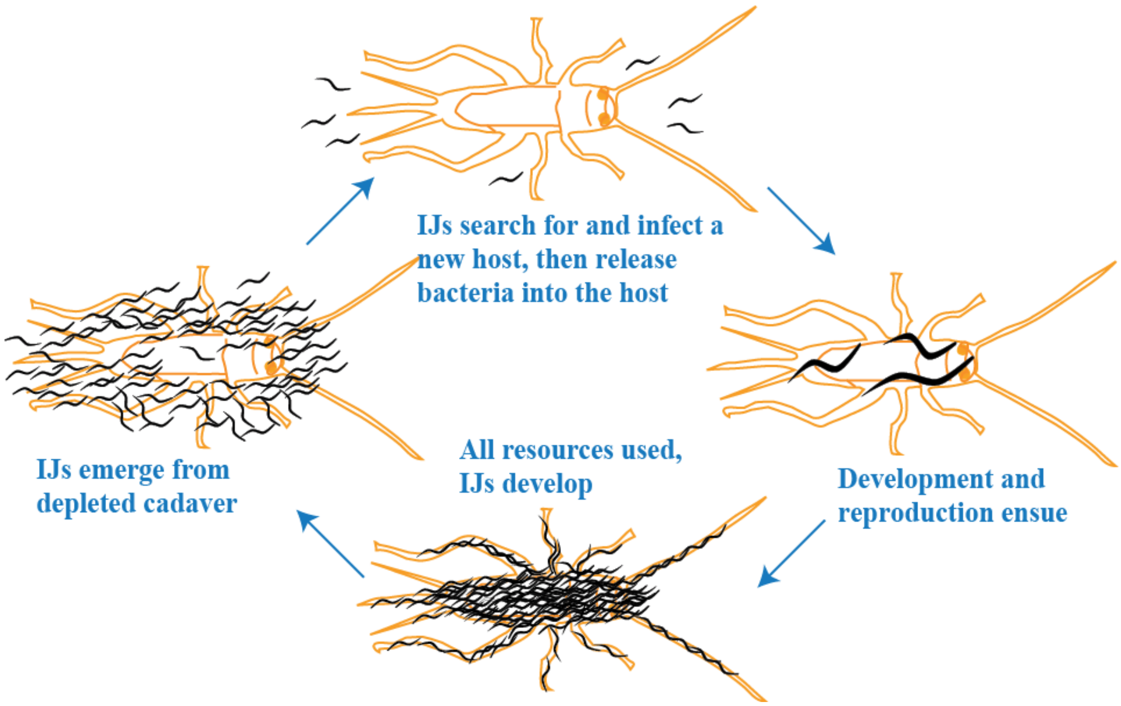

Figure 1.2 | Life cycle of entomopathogenic nematodes. The infective juvenile (IJ) stage seeks out a new host to infect, penetrating into the hemolymph and releasing the pathogenic bacteria it carries. The nematodes develop and reproduce in the nutrient-rich insect, going through several rounds of reproduction, depending on the size of the insect host. As resources deplete, a new generation of infective juveniles form and emerge, seeking new hosts to infect with the pathogenic bacteria they carry.

Are all stages infectious? The short answer is no. Only a modified third larval stage called the infective juvenile, analogous to the dauer juvenile stage in *Caenorhabditis elegans*, is infectious (Figure 1.3). In fact, infective juveniles are the only free-living stage of known EPNs, while all other developmental stages are only found inside infected hosts. The infective juvenile is a stress tolerant, non-feeding, bacterial vectoring stage that seeks out insects to infect and kill.

Figure 1.3 | Entomopathogenic nematodes emerging from insects. Pictures showing entomopathogenic nematode infective juveniles emerging from *Galleria mellonella* waxworm larvae on the left and *Acheta domestica* crickets on the right

How did they get their name? The first entomopathogenic nematode was described by Gotthold Steiner in 1923; since then more than 75 species have been described, with more species being described every year. Most studies focus on EPNs from two genera: *Steinernema* and *Heterorhabditis*. It is through their association with insect pathogenic bacteria that they began to be called entomopathogenic nematodes. First the nematodes' bacterial partners were called entomopathogenic bacteria, because these bacteria have a median lethal dose or LD_{50} of ten thousand cells or less. This means that an inoculum of ten thousand bacterial cells or less, into the hemolymph, kills half of a tested population of insects. The term 'entomopathogenic' began to be applied to the nematodes themselves in the late 1980's and reinforces the link between nematology and insect pathology. It is a useful technical epithet that differentiates them other types of parasitic nematodes, of which there are many.

Are they harmful to humans? While most parasitic nematodes might be seen as harmful, EPNs are beneficial to humans. Their potential as alternatives to chemical pesticides for controlling pesky insects was recognized early on and they have been subjected to extensive laboratory and field-testing. EPNs have been used in biological control since the 1930s and are currently used worldwide. For example, they have been used with high levels of success to control invasive species of mole crickets in Florida and continue to be used in orange groves in both Florida and California to control the citrus root weevil and other damaging crop pests. EPNs are even commercially available for pest control in home gardens and are commonly marketed as 'beneficial nematodes.'

Why are EPNs being studied? For starters, the symbiotic association with bacteria is highly specific in most cases and provides an excellent model for understanding the development and evolution of symbiosis. EPNs' potential as biological control agents continues to be evaluated with studies focusing on selection of desirable traits such as virulence, heat and stress tolerance, persistence, etc. Because at least two distantly related genera have evolved this specific type of parasitism (*Heterorhabditis* and *Steinernema)*, EPNs are an interesting system for the study of convergent and parallel evolution. Also, since they are odd intermediates between predators and parasitoids, there are many studies regarding their host-seeking behavior. They rely primarily on chemoreception for host seeking and some of them are capable of jumping, which is an extraordinary behavior in nematodes that is unique to some *Steinernema*. Imagine, a 0.5–1 mm worm with no legs or hard body parts, and yet it is capable of jumping up to 9 times its body length.

What remains to be explored? There is much that remains unknown about EPNs, including: their global abundance and diversity, the extent of their host range and whether or not other arthropods or even non-arthropods are also infected, what has led to the specialization of some for certain hosts and not others, what drives niche partitioning within this guild, the molecular underpinnings of their symbiosis and parasitism, how they can survive carrying highly pathogenic bacteria, how they suppress or avoid host immunity, or just how genetically similar disparate species that have converged on this very particular lifestyle are. These and other questions remain underexplored, providing plenty of room for studying these fascinating, useful, and delightful worms.

References

1. Margulis, L., and Schwartz, K.V. (2000). Introduction. In Five Kingdoms, J. Tannenbaum and G.L. Hadler, eds. (New York: W. H. Freeman and Company), pp. 1–35.

2. Woese, C.R., Kandler, O., and Wheelis, M.L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A *87*, 4576–4579.

3. Ayala, F.J., Rzhetsky, A., and Ayala, F.J. (1998). Origin of the metazoan phyla: molecular clocks confirm paleontological estimates. Proc Natl Acad Sci U S A *95*, 606–611.

4. Rodriguez-Trelles, F., Tarrio, R., and Ayala, F.J. (2002). A methodological bias toward overestimation of molecular evolutionary time scales. Proc Natl Acad Sci U S A *99*, 8112–8115.

5. Hart, M.W. (2008). Speciose versus species-rich. Trends in ecology & evolution *23*, 660–661.

6. Hugot, J.P., Baujard, P., and Morand, S. (2001). Biodiversity in helminths and nematodes as a field of study: an overview. Nematology *3*, 199–208.

7. Lambshead (1993). Recent developments in marine benthic biodiversity research. Oceanis *19*, 5–24.

8. Lambshead, P.J. (2004). Marine nematode biodiversity. In Nematode morphology, physiology, and ecology, Z.X. Chen, Y. Chen, S.Y. Chen and D.W. Dickson, eds. (CABI), pp. 4554–4558.

9. Cobb, N.A. (1914). Nematodes and their relationships. U.D.o.A. Yearbook, ed.

10. Gibbons, L.M. (2002). General organization. In The Biology of Nematodes, D.W. Lee, ed. (Boca Raton, FL: CRC Press), pp. 31–60.

11. Holden-Dye, L., and Walker, R.J. (2007). Anthelmintic drugs. WormBook, 1–13.

12. Sasser, J.N., and Freckman, D.W. (1986). A World Perspective on Nematology—The Role of the Society. J. Nematol. *18*, 596–596.