Title
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Journal
Molecular and Biochemical Parasitology, 208(1)

ISSN
0166-6851

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Publication Date
2016-07-01

DOI
10.1016/j.molbiopara.2016.05.007

Peer reviewed

HHS Public Access

Author manuscript

Mol Biochem Parasitol. Author manuscript; available in PMC 2017 July 01.

Published in final edited form as:

Mol Biochem Parasitol. 2016 July ; 208(1): 23–32. doi:10.1016/j.molbiopara.2016.05.007.

Mechanisms of host seeking by parasitic nematodes

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Abstract

The phylum Nematoda comprises a diverse group of roundworms that includes parasites of vertebrates, invertebrates, and plants. Human-parasitic nematodes infect approximately one billion people worldwide and cause some of the most common neglected tropical diseases, particularly in low-resource countries [1]. Parasitic nematodes of livestock and crops result in billions of dollars in losses each year [1]. Many nematode infections are treatable with low-cost anthelmintic drugs, but repeated infections are common in endemic areas and drug resistance is a growing concern with increasing therapeutic and agricultural administration [1]. Many parasitic nematodes have an environmental infective larval stage that engages in host seeking, a process whereby the infective larvae use sensory cues to search for hosts. Host seeking is a complex behavior that involves multiple sensory modalities, including olfaction, gustation, thermosensation, and humidity sensation. As the initial step of the parasite-host interaction, host seeking could be a powerful target for preventative intervention. However, host-seeking behavior remains poorly understood. Here we review what is currently known about the host-seeking behaviors of different parasitic nematodes, including insect-parasitic nematodes, mammalian-parasitic nematodes, and plant-parasitic nematodes. We also discuss the neural bases of these behaviors.

Graphical Abstract

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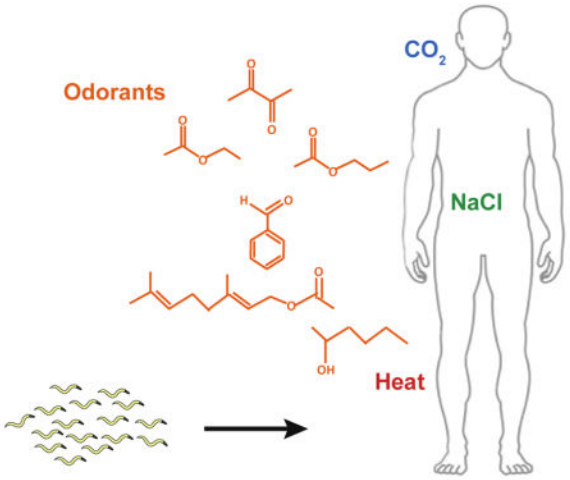

Keywords

parasitic nematodes; parasitic helminths; host-seeking behavior; olfactory behavior; skin-penetrating nematodes; entomopathogenic nematodes

Host seeking by entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) in the genera *Heterorhabditis* and *Steinernema* are parasites that infect and kill insects. EPNs are known as “beneficial nematodes” because they infect a wide variety of insect pests and disease vectors, and are used commercially throughout the world for biocontrol. EPNs are of interest not only as biocontrol agents, but also as models for understanding human-parasitic nematodes. EPNs are broadly distributed geographically, having been found on every continent except Antarctica [2]. Most EPNs, including many species commonly used for biocontrol such as *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*, are generalists that are capable of infecting and killing many different insect species (Figure 1A). However, some EPNs are specialists that primarily target a single type of insect [2]. For example, *Steinernema scapterisci* targets mole crickets [3] and *Steinernema diaprepesi* targets the larval stages of the root weevil *Diaprepes abbreviates*, a citrus pest [4].

Life cycle of EPNs

EPNs are infective during a particular life stage called the infective juvenile (IJ), or alternatively the infective third-stage larva (L3i). IJs invade insect hosts by entering through an orifice such as the mouth or spiracles, or by penetrating through the cuticle [5]. The IJs contain a bacterial endosymbiont in their gut, and upon host entry they deposit their symbiotic bacteria into the insect. The worms and bacteria rapidly kill the insect, typically within 48 hours of host entry. The worms grow and reproduce inside the insect cadaver, feeding off bacteria and cadaver tissue until food sources are depleted. New IJs then form and disperse into the environment to search for new hosts (Figure 1B) [5].

Host-seeking strategies of EPNs

The host-seeking strategies of EPN species are typically described as varying along a continuum ranging from ambushing, in which the IJs remain relatively stationary and latch on to passing hosts, to cruising, in which the IJs disperse in search of hosts [6]. Ambushers often nictate, where the IJ stands on its tail and waves to facilitate attachment to passing hosts. Some ambushing *Steinernema* species also jump, where the IJ stands on its tail, curls, and propels itself into the air [6]. In general, ambushers are most effective at targeting motile hosts, while cruisers are most effective at targeting non-motile hosts [7]. However, recent studies suggest that many species are capable of engaging in either ambushing or cruising depending on the environmental context. For example, although *Ste. carpocapsae* is generally considered a classical ambusher, it moves more in peat than sandy soil, suggesting that it can ambush or cruise depending on its environment [8]. The extent to which *Ste. carpocapsae* moves in the soil also depends on which insect hosts are present [7, 9]. In addition, all EPN species examined so far exhibit robust chemotaxis in the presence of insect-derived odorants [10, 11]. Thus, most EPNs appear to be capable of cruising toward host-emitted sensory cues under at least some conditions.

Responses of EPNs to olfactory cues

A number of studies have demonstrated that EPNs use olfactory cues to locate hosts to infect. IJs are attracted to the odor blends emitted by live insects and to a diverse array of insect-emitted odorants, including carbon dioxide (CO_2) (Figure 2A–B) [10–15]. CO_2 is an essential host cue for EPNs: attraction to insect odor blends is greatly reduced or eliminated when CO_2 is removed (Figure 2C) [11, 12]. Jumping in *Steinernema* is stimulated by insect odor blends, CO_2, and host-specific odorants [10, 11, 16]. EPNs are also attracted to volatile components of insect feces [17]. A large-scale comparative analysis of olfactory behaviors across species revealed that different EPN species respond differently to odorants [10, 11]; thus, EPNs appear to have specialized olfactory systems that contribute to host selection.

EPNs also respond to odorants emitted by insect-damaged plants [18]. For example, the odorant (E)-β-caryophyllene is released by maize roots in response to insect feeding and attracts the EPN *Heterorhabditis megidis* [19]. Similarly, *Ste. diaprepesi* is attracted to volatiles released by plant roots that have been damaged by its host *D. abbreviates* [4]. CO_2 acts synergistically with root volatiles to attract EPNs [18]. Thus, EPNs appear to use CO_2, insect odorants, and plant odorants to find insects to infect.

Responses of EPNs to other sensory cues

In addition to responding to olfactory cues, EPNs respond to a number of other sensory cues that may contribute to host seeking. For example, EPNs have been shown to aggregate at temperatures that approximate insect body temperature, which is slightly (<1°C) above ambient temperature due to insect metabolic processes [20]. EPNs also respond to salt gradients. *Ste. carpocapsae* IJs can navigate in gradients of Na^+, Mg^{2+}, Ca^{2+}, CO_3^{2-}, and Cl^- and accumulate at different preferred concentrations for each ion [21]. EPNs also respond to electric fields, magnetic fields, vibration, and mechanical stimulation [22–26]. These other sensory responses are presumed to facilitate environmental navigation and/or host finding.

Host seeking by mammalian-parasitic nematodes

Nematode parasites of humans are widespread and pose dangerous health risks. Approximately one billion people worldwide harbor at least one nematode infection, mostly in low-income tropical and sub-tropical regions of the world [27]. Many parasitic nematode species are co-endemic and mixed infections are frequently observed. Parasitic nematode infections can cause chronic gastrointestinal distress, anorexia, anemia, and stunted physical and cognitive development in children. Select nematode species can cause severe symptoms such as permanent disfigurement and blindness, and some can even be fatal for infants and the immunocompromised [27]. In addition, nematode parasites of non-human animals are widespread, and preventing or controlling nematode infections in commercial livestock and household pets costs billions of dollars annually [1].

Many mammalian-parasitic nematodes infect only one or a limited number of host species. For example, *Strongyloides fulleborni kellyi* is a human parasite, while *Strongyloides stercoralis* has a limited host range that includes humans, primates, and dogs (Figure 1A) [28]. Mammalian-parasitic nematodes can infect hosts by skin penetration, passive ingestion, or direct transmission via intermediate vectors [1]. Vector-borne parasitic nematodes such as *Wuchereria bancrofti* and *Onchocerca volvulus*, the causative agents of lymphatic filariasis and onchocerciasis, respectively, rely on the host-seeking capabilities of their intermediate insect vectors to infect their definitive hosts [27]. By contrast, skin-penetrating nematodes and passively ingested nematodes use environmental and host-emitted stimuli to seek out nearby hosts or position themselves in advantageous locations for host ingestion. We focus here on skin-penetrating and passively ingested nematodes.

Life cycles of mammalian-parasitic nematodes

Skin-penetrating nematodes such as the human hookworms *Ancylostoma duodenale* and *Necator americanus,* and the human threadworm *Str. stercoralis,* have similar life cycles inside the host (Figure 1A, C). Parasitic adults colonize the mucosa of the host intestine and shed eggs that are passed with feces. The nematodes develop on the host feces to the IJ stage, and the IJs then find and infect new hosts. The soil-dwelling IJs infect by skin penetration, commonly through the feet [28, 29]. The IJs typically migrate through the circulatory system to the lungs, where they penetrate the alveoli and cause irritation and a dry cough. The IJs are coughed up and swallowed, and then pass through the stomach into the intestine, where they resume development into parasitic adults. Hookworms can also infect orally [30]. *Strongyloides* species can undergo one or a limited number of free-living generations outside of the host, and *Str. stercoralis* can cycle through multiple generations inside the same host (Figure 1C) [28, 29].

Passively ingested nematodes vary in their life cycles. For example, the human-parasitic giant roundworm *Ascaris lumbricoides* colonizes the host intestine and eggs are passed in the feces. *As. lumbricoides* larvae developmentally arrest while still inside the egg, and development resumes when the host ingests infective eggs [31]. In contrast, the ruminant parasite *Haemonchus contortus* is passively ingested as IJs and has a life cycle outside the host that is similar to that of skin-penetrating hookworms (Figure 1A, C) [32]. However, unlike hookworms, the in-host life cycle of *Ha. contortus* is constrained to the gut. *Ha.*

contortus IJs exsheath in the rumen and travel to the abomasum, where they develop into parasitic adults [33].

Host-seeking strategies of mammalian-parasitic nematodes

Like EPNs, mammalian-parasitic nematodes vary in their host-seeking strategies. In the absence of stimulation, the dog hookworm *Ancylostoma caninum* and the human hookworms *An. duodenale* and *Ne. americanus* have been described as ambushers that remain relatively motionless [34, 35]. In the presence of host-emitted cues, the IJs crawl or nictate [34, 35]. By contrast, the *Strongyloides* species appear to be cruisers that spend more time crawling than nictating in the absence of stimulation [26, 36]. Human-parasitic *Str. stercoralis* IJs crawl faster than rat-parasitic skin-penetrating IJs and EPN IJs in the absence of sensory stimulation, suggesting that *Str. stercoralis* may have evolved longer-distance dispersal mechanisms to accommodate for more motile hosts [26]. In contrast, passively ingested *Ha. contortus* is less motile than the skin-penetrating species and appears to be an ambusher [26]. As with EPNs, some mammalian-parasitic nematodes have foraging behaviors that are intermediate between traditionally classified cruising and ambushing behaviors. For example, the rat hookworm *Nippostrongylus brasiliensis* is capable of crawling at a speed comparable to that of the skin-penetrating rat parasite *Strongyloides ratti,* yet unlike *Str. ratti, Ni. brasiliensis* prefers to nictate on certain surfaces [26]. Thus, like EPNs, mammalian-parasitic nematodes appear to modulate their foraging strategy depending on environmental conditions.

Responses of mammalian-parasitic nematodes to CO_2

There is now substantial evidence that mammalian-parasitic nematodes use host-emitted chemosensory cues to identify potential hosts. One important host cue for some mammalian-parasitic nematodes is CO_2, which is exhaled by mammals at concentrations of 4–5% during respiration (compared to 0.04% in air) [37]. CO_2 induces nictation behavior in *An. caninum* [34]. It also increases random crawling in *Str. stercoralis* and *An. caninum,* but decreases random crawling in *Ha. contortus* [36].

In the presence of a CO_2 gradient, the skin-penetrating nematodes *Str. stercoralis, Str. ratti,* and *Ni. brasiliensis* are repelled by high CO_2 but neutral to low CO_2 (Figure 2D) [26]. Avoidance of high CO_2 by skin-penetrating nematodes is consistent with the fact that they typically infect around the feet and lower extremities, where CO_2 concentrations are less than 1% [38]. However, whether CO_2 is attractive in combination with other host cues has not yet been investigated. In contrast to the skin-penetrating nematodes, passively ingested *Ha. contortus* is attracted to CO_2 concentrations at or above 2.5% (Figure 2D) [26]. *Ha. contortus* infects grazing animals and has been shown to migrate vertically between herbage and soil in response to changes in environmental factors such as temperature and humidity [39, 40]. Attraction to host-emitted CO_2 may stimulate *Ha. contortus* to migrate up the herbage toward the mouths of grazing ruminants and thereby increase the chances of a successful infection.

Responses of mammalian-parasitic nematodes to soluble host cues

Mammalian-parasitic nematodes respond to a number of chemicals present in mammalian skin, sweat, and serum. For example, *An. caninum* is attracted to hydrophilic components extracted from dog skin [34]. In addition, *An. duodenale* and *Ne. americanus* show increased random crawling speeds and skin-penetration behaviors in the presence of human skin extract [35]. More quantitative studies are needed to determine if human hookworms are attracted to human skin extracts. In the case of threadworms, *Str. stercoralis* is attracted to dog skin extracts, human serum, and human sweat, while *Str. ratti* is attracted to mammalian serum components [41–43]. Furthermore, both *Str. stercoralis* and *Str. ratti* are capable of navigating in sodium chloride gradients and accumulate at concentrations comparable to the concentrations found in sweat [44, 45].

Responses of mammalian-parasitic nematodes to volatile host cues

Humans and other mammals emit hundreds of odorants from skin, sweat, skin microbiota, and breath [46, 47]. Since host-emitted odor blends are species-specific, olfaction is likely to contribute to the species-specificity of many parasites, including parasitic nematodes [48]. One of the first skin odorants identified as an attractant for mammalian-parasitic nematodes was urocanic acid. *Str. stercoralis* IJs are robustly attracted to urocanic acid even at low concentrations (Figure 3A–B) [42]. Urocanic acid is found on the skin of many mammals, but in humans it is especially abundant on the feet, where *Str. stercoralis* infections commonly occur [42]. Thus, urocanic acid is likely to be an important host-seeking cue for *Str. stercoralis*.

More recently, *Str. stercoralis*, *Str. ratti*, *Ni. brasiliensis*, and *Ha. contortus* IJs were tested in chemotaxis assays to assess their responses to a large panel of human-emitted odorants (Figure 3C–D) [26]. Each of the species tested showed a unique odor response profile, demonstrating that like EPNs, mammalian-parasitic nematodes have species-specific olfactory preferences (Figure 3D) [26]. In the case of *Str. stercoralis,* nearly all of the strongest attractants identified are also known attractants for anthropophilic mosquitoes, suggesting that mosquitoes and nematodes may utilize similar olfactory cues to locate human hosts (Figure 3D). Two of the attractive odorants identified, 7-octanoic acid and 6-methyl-5-hepten-2-one (sulcatone), are thought to be highly enriched in human body odor relative to the body odor of other mammals [49, 50]. In addition, sulcatone response in certain *Aedes aegypti* mosquito populations was recently associated with preference for human hosts [50]. The finding that *Str. stercoralis* is attracted to sulcatone raises the possibility that it also uses sulcatone to preferentially target humans [26]. We note that sulcatone is also an insect pheromone [51, 52], perhaps explaining why it is an attractant for EPNs as well as *Str. stercoralis*. In contrast to the skin-penetrating nematodes, passively ingested *Ha. contortus* was repelled by most skin and sweat odorants tested. However, *Ha. contortus* was attracted to fresh grass extracts, as well as methyl myristate and myristic acid, known components of cow and goat milk (Figure 3D) [26]. These responses may allow *Ha. contortus* IJs to position themselves in grazing areas frequented by ruminants.

A quantitative comparison of olfactory behavior in skin-penetrating IJs, passively ingested IJs, EPN IJs, and dauer larvae of the free-living nematode *Caenorhabditis elegans* revealed

that species with similar host ranges responded more similarly to odorants, even when phylogenetically distant (Figure 3E–F) [26]. For example, *Str. ratti* and *Ni. brasiliensis* are distantly related but share a rodent host, and they respond more similarly to odorants than *Str. ratti* and *Str. stercoralis*. Thus, the olfactory preferences of mammalian-parasitic nematodes reflect their host specificity rather than their phylogenetic relationships. These results suggest that mammalian-parasitic nematodes have specialized olfactory systems that support host finding and host selection.

Thermosensory behaviors of mammalian-parasitic nematodes

Skin-penetrating nematodes respond robustly to thermal stimulation. Both hookworms and *Strongyloides* species can navigate through thermal gradients and accumulate at temperatures approximating mammalian body temperature (Figure 4A) [34, 35, 53, 54]. In addition, *Str. ratti* and *Str. stercoralis* display relatively straight crawling trajectories at room temperature but increased crawling speeds and highly curved trajectories at 37°C (Figure 4B–D) [26]. This observation suggests that heat stimulates local search and thereby increases the likelihood of host attachment. In contrast, *Ha. contortus* does not migrate to a heat source and does not increase its crawling speed in response to heat (Figure 4B) [26, 55]. *Ha. contortus* instead migrates to its cultivation temperature, a behavioral strategy resembling that of *C. elegans* [55]. Thus, passively ingested IJs may not use heat as a host-seeking cue. However, more quantitative studies will be necessary to better understand how mammalian-parasitic nematodes move within temperature gradients.

Responses of mammalian-parasitic nematodes to other sensory cues

Mammalian-parasitic nematodes respond to a number of additional sensory cues that may contribute to host seeking or promote host contact in the environment. *An. caninum, An. duodenale,* and *Ne. americanus* IJs respond to vibration and humidity changes by actively crawling [30, 34, 35]. *An. duodenale* and *Ne. americanus* IJs are also activated by light, but only *Ne. americanus* migrates toward light [30, 35]. Mechanical stimulation increases crawling speed in *Str. ratti* IJs [26]. How these responses to vibration, humidity, light, and mechanical stimulation contribute to host-seeking behaviors for skin-penetrating nematodes remains to be elucidated. Finally, *Ha. contortus* IJs exhibit migration toward light (phototaxis) and moisture (hygrotaxis) [39, 40]. Phototaxis may allow *Ha. contortus* IJs to move vertically on herbage during the day when grazing animals are more active. Hygrotaxis may serve as a protection mechanism, allowing IJs to migrate down herbage and into the relatively damp soil when surface conditions are unfavorable.

Host seeking by plant-parasitic nematodes

Some plant-parasitic nematodes (PPNs) also engage in host seeking, although their host-seeking behaviors remain poorly understood. For example, PPNs such as the potato cyst nematode *Globodera pallida* and root-knot nematodes in the genus *Meloidogyne* are attracted to plant roots [56–58]. Ethylene signaling in the plant modulates root attractiveness to PPNs, although whether ethylene signaling directly regulates the production of specific PPN attractants has not yet been determined [59]. *Meloidogyne* species are attracted to low pH, consistent with the fact that growing roots create a low pH environment [58]. CO_2

attracts a number of PPN species, including *Meloidogyne incognita* and *Rotylenchulus reniformis* [13, 60]. However, at least in the case of *Meloidogyne* species, the observed attraction to CO_2 may be primarily a response to low pH rather than molecular CO_2 [58]. PPNs are also attracted to some of the same root volatiles emitted by insect-damaged plants that attract EPNs [61]. Thus, the emission of specific volatiles by plants in response to insect damage comes at a potential ecological cost.

The neural basis of host-seeking behavior

The neural basis of host-seeking behavior in parasitic nematodes is poorly understood, due to the technical difficulty of working with these organisms and a disconnect between the fields of parasitology and neurobiology. However, the neural basis of sensory behavior is well-studied in the model free-living nematode *C. elegans,* and neural anatomy and function are often conserved across free-living and parasitic nematode species [48, 62, 63]. In addition, *C. elegans* has an developmentally arrested, long-lived alternative life stage called the dauer larva, which is developmentally analogous to the IJ stage of parasitic nematodes [64]. *C. elegans* dauers form when environmental conditions are unfavorable and engage in phoresy, using insects and other invertebrates for transport to more favorable environmental niches [65, 66]. A number of dauer behaviors, including nictation, are shared with parasitic IJs [66]. Thus, knowledge of the neural basis of behavior in *C. elegans*, particularly dauer behavior, can be leveraged to better understand the neural basis of host seeking in parasitic nematodes.

The neural basis of sensory behaviors in C. elegans

C. elegans responds robustly to sensory stimuli, including a wide variety of volatile compounds [67, 68]; water-soluble compounds such as cations, anions, nucleotides, and amino acids [68]; pheromones [69]; the gases O_2 and CO_2 [70]; and temperature [71]. The responses to most chemicals and temperature are mediated primarily by head sensory neurons that extend processes toward the tip of the anterior of the worm [68]. Odorants and pheromones are detected by large families of seven-transmembrane domain G protein-coupled receptors (GPCRs) [68, 72], while the gustatory response requires receptor guanylate cyclases (rGCs) [73]. CO_2 detection is mediated in part by the rGC GCY-9 [74–76]; however, GCY-9-independent mechanisms of CO_2 detection appear to operate in some sensory neurons but have not yet been characterized [77, 78]. O_2 detection is mediated by soluble guanylate cyclases and globins [79–81]. Thermosensation involves rGCs and TRP channels [82].

Dauer-specific nictation behavior in *C. elegans* is mediated by a set of six cholinergic sensory neurons in the head called IL2 neurons [66]. The processes of the IL2 neurons undergo extensive remodeling during dauer development, and the furan homolog *kpc-1* is required for both dauer-specific IL2 remodeling and nictation behavior [83]. Whether IL2 neurons and/or a *kpc-1* homolog are required for host seeking by parasitic nematodes has not yet been investigated.

The neural basis of chemosensation in parasitic nematodes

A number of sensory neurons that contribute to host seeking have been identified in parasitic nematodes based on analogy with *C. elegans* neurons (Figure 5A). For example, CO_2 chemotaxis in *C. elegans* was shown to require a pair of head sensory neurons called the BAG neurons, which directly sense molecular CO_2 [75, 76, 84, 85]. Subsequently, BAG neurons were shown to mediate CO_2 chemotaxis in the EPNs *Ste. carpocapsae* and *He. bacteriophora*, and CO_2-evoked jumping in *Ste. carpocapsae* (Figure 5B) [10]. Thus, the neural basis of CO_2 responsiveness is at least partly conserved across free-living and parasitic nematode species. Similarly, the ASE and ASH neurons mediate responses to gustatory cues in both *C. elegans* and the skin-penetrating human parasite *Str. stercoralis* [44]. The olfactory sensory neurons that mediate responses to host-specific odorants have not yet been identified in parasitic nematodes.

The neural basis of thermosensation in parasitic nematodes

In *C. elegans,* the primary thermosensory neurons are the AFD neurons, although the AWC and ASI chemosensory neurons are also thermosensory and contribute to thermotaxis [82]. The AFD neurons have a finger-like dendritic structure that results in an increased surface area in the amphid chemosensory organs, which is thought to be important for temperature sensing [82]. The positional analogs of the AFD neurons in the dog hookworm *Ancylostoma caninum* and the passively ingested ruminant parasite *Ha. contortus* also have a finger-like dendritic structure and are also required for thermotaxis [53, 55]. Moreover, the RIA interneurons, which function downstream of AFD to mediate thermosensation in *C. elegans* [82], were also found to be required for thermosensation in *Ha. contortus* [55]. Thus, as is the case for chemosensation, the neural basis of thermosensation is at least partly conserved across free-living and parasitic species.

Temperature sensing in *Str. stercoralis* may be somewhat different because *Str. stercoralis* does not have a pair of neurons with a finger-like dendritic structure [86]. Instead, *Str. stercoralis* has a pair of neurons, called the ALD neurons, with a lamellar dendritic structure that also results in a large surface area in the amphids [86]. Ablation of the ALD neurons in *Str. stercoralis* disrupted thermotaxis, demonstrating that the ALD neurons are required for thermosensory behavior (Figure 5C) [54]. The anatomical position of the ALD neurons appears to most closely resemble that of the *C. elegans* AWC neurons [54]. However, whether ALD neurons are functionally more analogous to the *C. elegans* AWC or AFD neurons remains unclear.

Unanswered questions regarding neural circuit function in parasitic nematodes

The studies described above demonstrate that sensory neuron function is often conserved across free-living and parasitic species. However, the extent to which functional conservation across species exists at the interneuron level remains unknown. With the exception of the RIA interneurons mentioned above, interneuron function has not yet been explored in parasitic nematodes. The fact that sensory neuron function is often conserved across species, yet sensory microcircuits support species-specific behaviors, suggests that significant differences in interneuron function exist across species. While the connections between neurons have been almost completely mapped for *C. elegans* [87, 88], connectome

data is not yet available for parasitic nematodes. Thus, whether positionally analogous interneurons participate in the same microcircuits across species but have different functional properties, or whether positionally analogous interneurons participate in different microcircuits across species, is not yet clear.

The molecular basis of host seeking by parasitic nematodes also remains to be investigated. Some insights into possible molecular mechanisms of host seeking have come from studies of the necromenic nematode *Pristionchus pacificus*, which uses beetles for transport to new environmental niches and feeds off beetle cadavers [89]. Closely related *Pristionchus* species show species-specific responses to insect pheromones and plant volatiles [89]. Natural variation in the response to insect pheromone across *P. pacificus* strains is associated with the cGMP-dependent protein kinase gene *egl-4*, suggesting a role for cGMP signaling in the host-seeking behavior of *P. pacificus* [90]. Future studies will be necessary to determine whether cGMP signaling also regulates host seeking in parasitic nematodes.

Conclusions and future directions

In summary, parasitic nematodes use multiple sensory modalities to find and infect hosts, including olfaction, gustation, thermosensation, and hygrosensation. Moreover, all parasitic nematode infective larvae that have so far been examined respond robustly to sensory cues, suggesting that parasitic nematodes rely on these to maximize their chances of a successful infection regardless of their host range, host-seeking strategy, or infection route. Although we are still at the early stages of understanding the neural basis of host seeking, a more detailed understanding of the molecular and cellular basis of this crucial behavior is likely to emerge over the next few years. Mechanistic studies of neural circuit function in parasitic nematodes are now feasible due to the large-scale sequencing of parasitic nematode genomes [91] and the development of new methods for genetic transformation of parasitic worms [92]. In addition, targeted gene disruption has recently been achieved in multiple free-living nematodes using the CRISPR-Cas9 system [93–95], and this system is likely to be applicable to parasitic nematodes. These exciting developments pave the way for in-depth molecular, cellular, and circuit-level analyses of the host-seeking behaviors of parasitic nematodes. A better understanding of neural circuit function in parasitic nematodes will provide important insights into how parasites target their hosts, and more generally, how the nervous systems of parasites evolve to mediate parasitic behaviors such as host seeking and host invasion. In addition, a more mechanistic understanding of host seeking may enable the development of new strategies for preventing harmful nematode infections of animals and plants, and for enhancing the efficacy of beneficial nematodes as biocontrol agents.

Acknowledgments

We thank Taylor Brown, Michelle Castelletto, and Joon Ha Lee for insightful comments on the manuscript. This work was funded by the Whitcome Predoctoral Training Program, UCLA Molecular Biology Institute; Ruth L. Kirschstein National Research Service Award AI007323; and a National Science Foundation East Asia and Pacific Summer Institute Fellowship (Award #1414655) to S.S.G.; and a MacArthur Fellowship, McKnight Scholar Award, Searle Scholar Award, Rita Allen Foundation Fellowship, and NIH New Innovator Award (1DP2DC014596-01) to E.A.H.

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Summary

Parasitic nematodes use a diverse array of host-emitted sensory cues to find and infect their hosts.

Highlights

- Parasitic worms use sensory cues to find and infect hosts
- Host seeking is a complex behavior that involves multiple sensory modalities
- Parasitic worms have specialized olfactory systems that support host finding
- Sensory neural function is often conserved across free-living and parasitic worms
- Mechanisms of host seeking are being elucidated based on knowledge of *C. elegans*

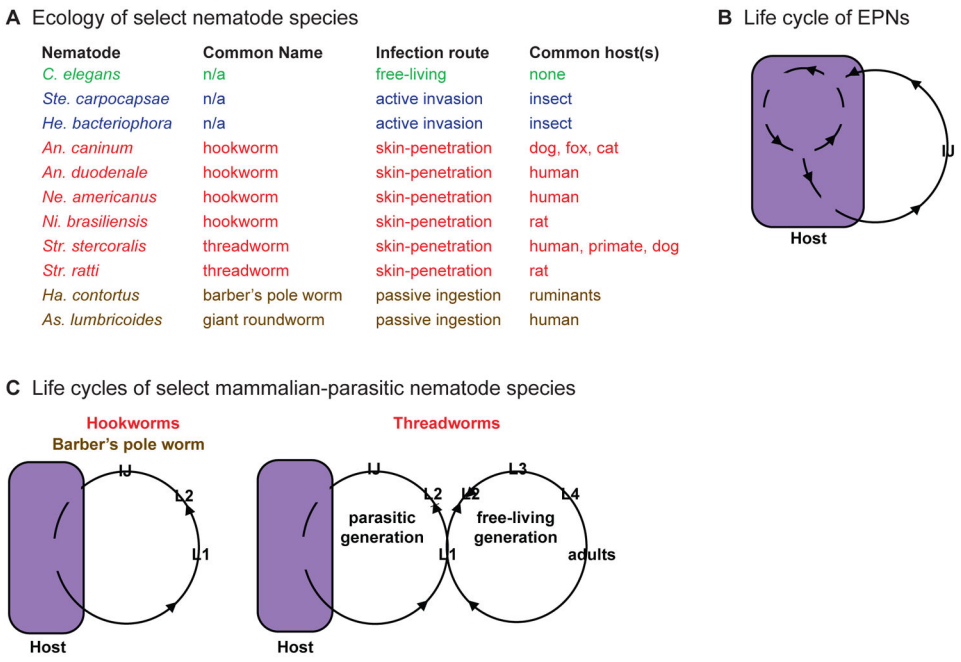

Figure 1. Ecology and life cycles of parasitic nematodes

A. Ecology of select nematode species, with common hosts indicated [28, 29, 31, 96–98]. Green = free-living, blue = insect-parasitic, red = skin-penetrating, brown = passively ingested. **B.** The life cycle of EPNs. EPN infective juveniles (IJs) infect by invasion through orifices or by penetration of the cuticle. The IJs and their associated bacteria rapidly kill the host. The nematodes develop and reproduce within the host cadaver for a number of generations, where they feed on bacteria in the insect body and the cadaver tissue. Once resources in the cadaver are exhausted, IJs emerge and search for a new host to infect [96]. **C.** Life cycles of mammalian-parasitic nematodes. Hookworms infect by skin penetration or orally. Inside the host, hookworms develop into adults that reproduce in the small intestine. Eggs are excreted with the host's feces and develop into IJs. IJs leave the feces in search of new hosts. Hookworms must infect a new host every generation [99]. Threadworms of the *Strongyloides* genus have a similar life cycle but can develop through a limited number of free-living generations outside the host. Some of the eggs excreted with the host's feces develop into IJs, while others develop into free-living adults that mate outside the host [28]. The life cycle outside the host of the barber's pole worm *Ha. contortus* is similar to that of hookworms, except that the IJs enter the host only by passive ingestion [97]. For **B**–**C**, L1–L4 are the first through fourth larval stages. For some parasitic nematode species IJs are alternatively described as infective third-stage larvae (L3i). Figures are adapted from Castelletto *et al.,* 2014 [26].

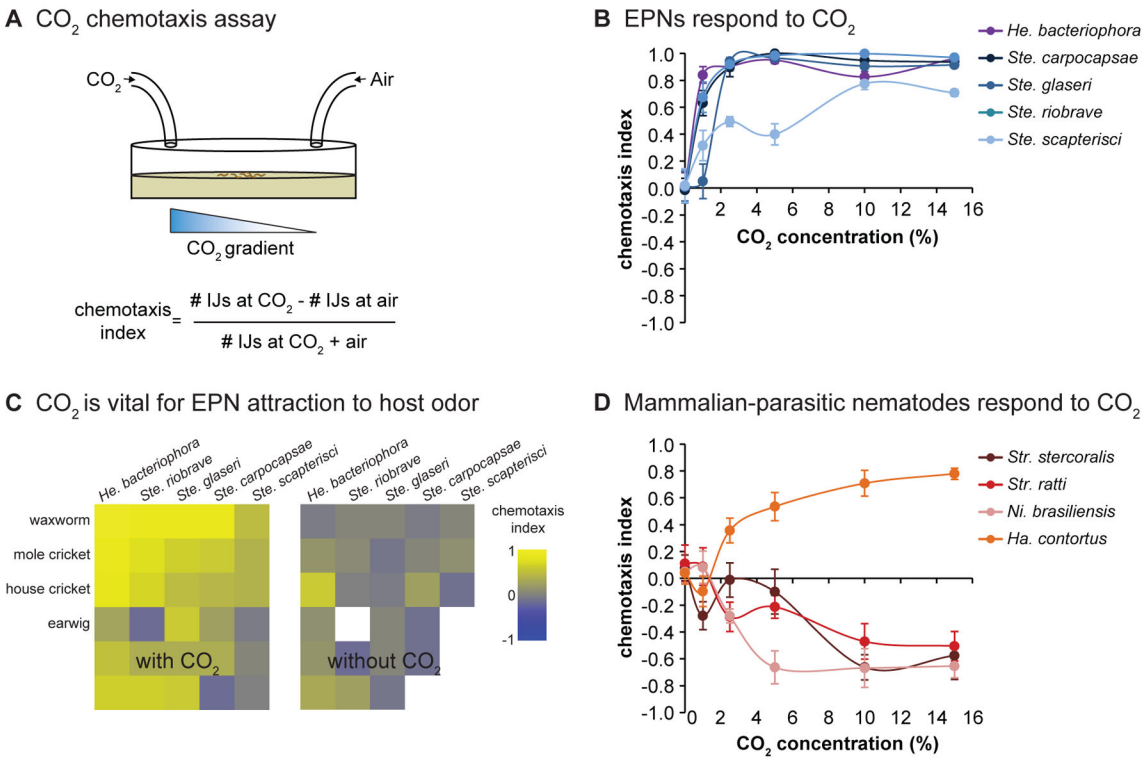

Figure 2. Responses of different parasitic nematode species to CO_2

A. A CO_2 chemotaxis assay. CO_2 is pumped into one side of a plate, and an air control is pumped into the other side. IJs are placed in the center of the plate and allowed to migrate in the CO_2 gradient for 1 hour. The number of IJs underneath the CO_2 inlet and the number underneath the air inlet are then counted, and a chemotaxis index (CI) is calculated according to the formula indicated. The chemotaxis index ranges from +1 to −1, with positive values indicating attraction to CO_2 and negative values indicating repulsion from CO_2. **B.** Responses of EPNs to CO_2 in a chemotaxis assay. All EPN species tested are attracted to CO_2 across concentrations. Data are from Dillman *et al.*, 2012 [11]. **C.** CO_2 is required for normal attraction of EPNs to insect odor blends. Left, EPN responses to host odor blends in a chemotaxis assay. Right, EPN responses to host odor blends with CO_2 chemically removed. Attraction of EPNs to insect odor is reduced or eliminated in the absence of CO_2. Responses are shown as a heatmap; yellow indicates attraction and blue indicates repulsion. White boxes in the heatmap indicate EPN-host combinations that were not tested with CO_2 removed because they were not attractive with CO_2 present. Reproduced from Dillman *et al.,* 2012 [11]. **D.** Responses of mammalian-parasitic nematodes to CO_2 in a chemotaxis assay. The skin-penetrating nematodes *Str. stercoralis, Str. ratti,* and *Ni. brasiliensis* are repelled by CO_2, while the passively ingested nematode *Ha. contortus* is attracted to CO_2. Data are from Castelletto *et al.,* 2014 [26].

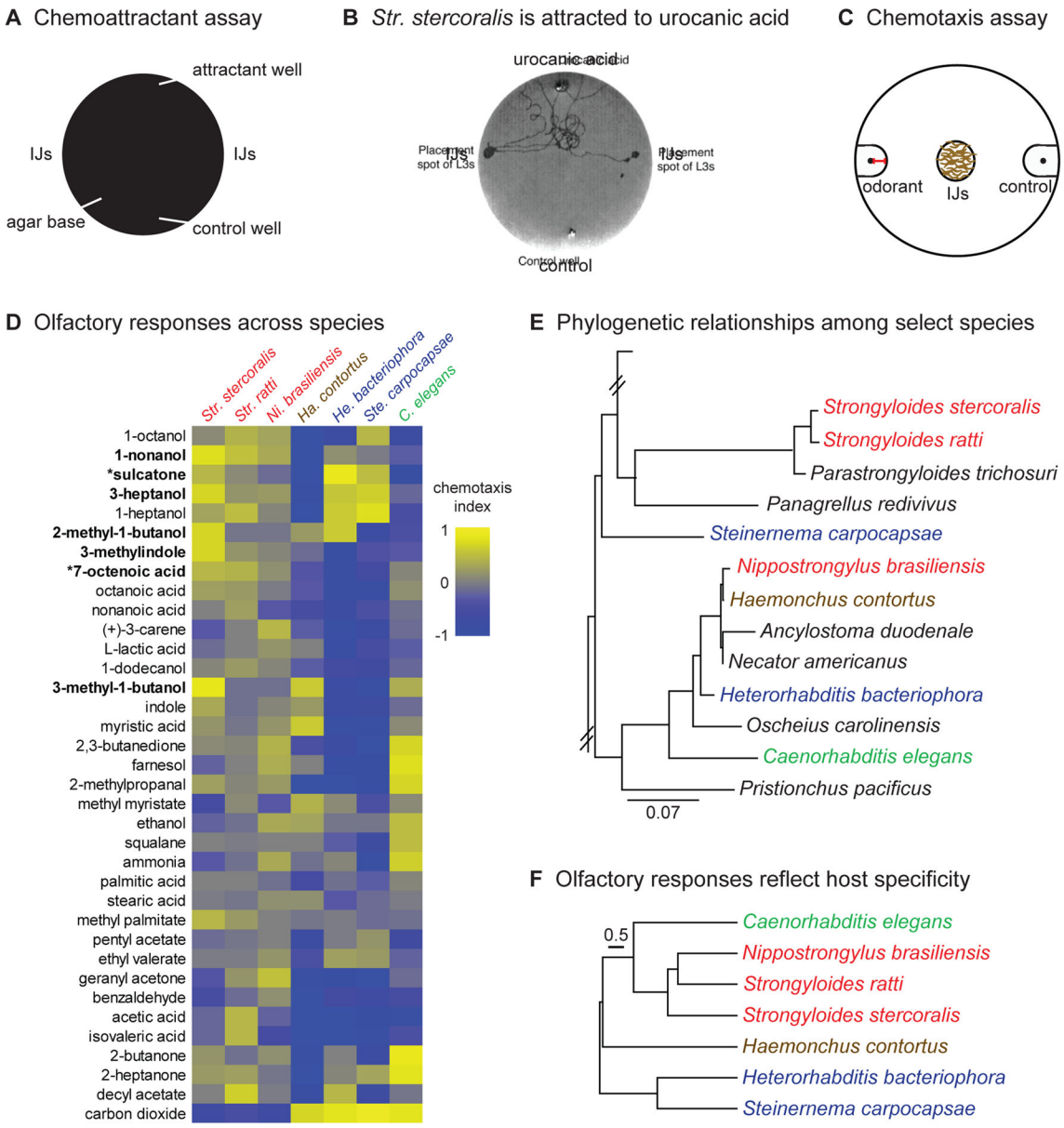

Figure 3. Responses of mammalian-parasitic nematodes to volatile host cues

A. A chemoattractant assay for *Str. stercoralis* IJs. IJs are placed on each side of the plate and allowed to migrate in the chemical gradient for 28 min. **B.** *Str. stercoralis* IJs are attracted to urocanic acid. For **A**–**B**, data are reproduced from Safer *et al.*, 2007 [42]. **C.** A chemotaxis assay for IJs. Odorant is placed on one side of the plate and control is placed on the other (black dots). IJs are placed in the center of the plate and allowed to migrate in the odorant gradient for 3 hours. The number of IJs in each scoring region (extended circles around the black dots) is then counted and a chemotaxis index (CI) is calculated as: CI = (# IJs at odorant − # IJs at control)/(# IJs at odorant + control). The chemotaxis index ranges from +1 to −1, with positive values indicating attraction to the odorant and negative values indicating repulsion from the odorant. Red scale bar = 1 cm. **D.** Olfactory responses across species. CI values are color-coded as shown to the right of the heatmap. For nematode

species included, red = skin-penetrating; brown = passively ingested; blue = insect-parasitic; green = free-living. Odorants in bold are known attractants for anthropophilic mosquito species. Odorants denoted by an asterisk are abundant in humans relative to other mammals. **E.** Phylogenetic relationships among select nematode species, based on Castelletto *et al.,* 2014 [26] and Dillman *et al.,* 2012 [11]. Species tested for olfactory behavior are color-coded. **F.** Behavioral dendrogram constructed from odorant responses in **D**. Olfactory responses reflect preferred host rather than genetic relatedness. For **C**–**F**, data are from Castelletto *et al.*, 2014 [26].

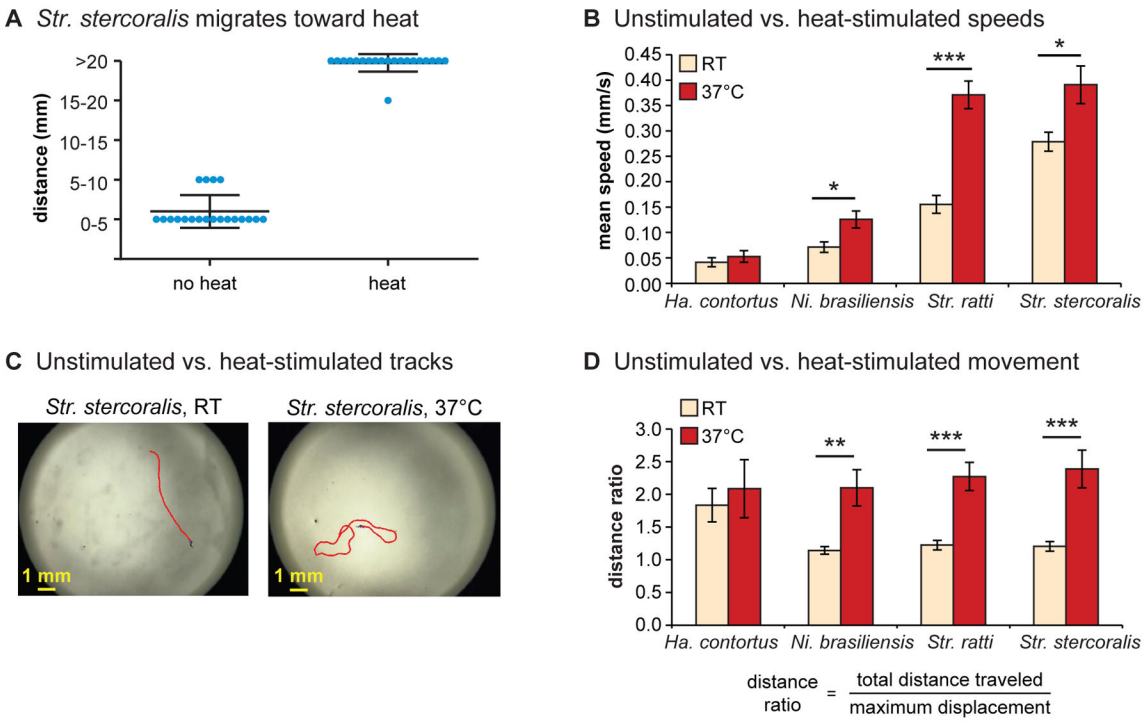

Figure 4. Skin-penetrating nematodes respond to thermosensory cues

A. *Str. stercoralis* IJs migrate toward heat. In the absence of a thermal gradient, *Str. stercoralis* IJs do not migrate far from their placement point ("no heat" condition). When the IJs are placed at 26°C in a thermal gradient ranging from 22°C to 43°C, they migrate toward the heated end ("heat" condition). Blue dots = migration distances of individual IJs from their initial placement point during a 1 minute assay; center bars = mean migration distances; upper and lower bars = standard deviations. Dot-plot data are reproduced from Lopez *et al.*, 2000 [54] with permission. **B.** Unstimulated vs. heat-stimulated speeds of mammalian-parasitic IJs. IJs of skin-penetrating nematode species exposed to an acute 37°C stimulus increase their crawling speeds. **C.** Representative tracks of *Str. stercoralis* from 20 s recordings at room temperature versus 37°C. **D.** Movement patterns at room temperature versus 37°C. Distance ratios were calculated according to the formula shown; a greater distance ratio indicates a more curved trajectory. Skin-penetrating nematodes exposed to an acute 37°C stimulus show curved crawling trajectories, indicative of local-search behavior. For **B–D,** data are from Castelletto *et al.*, 2014 [26].

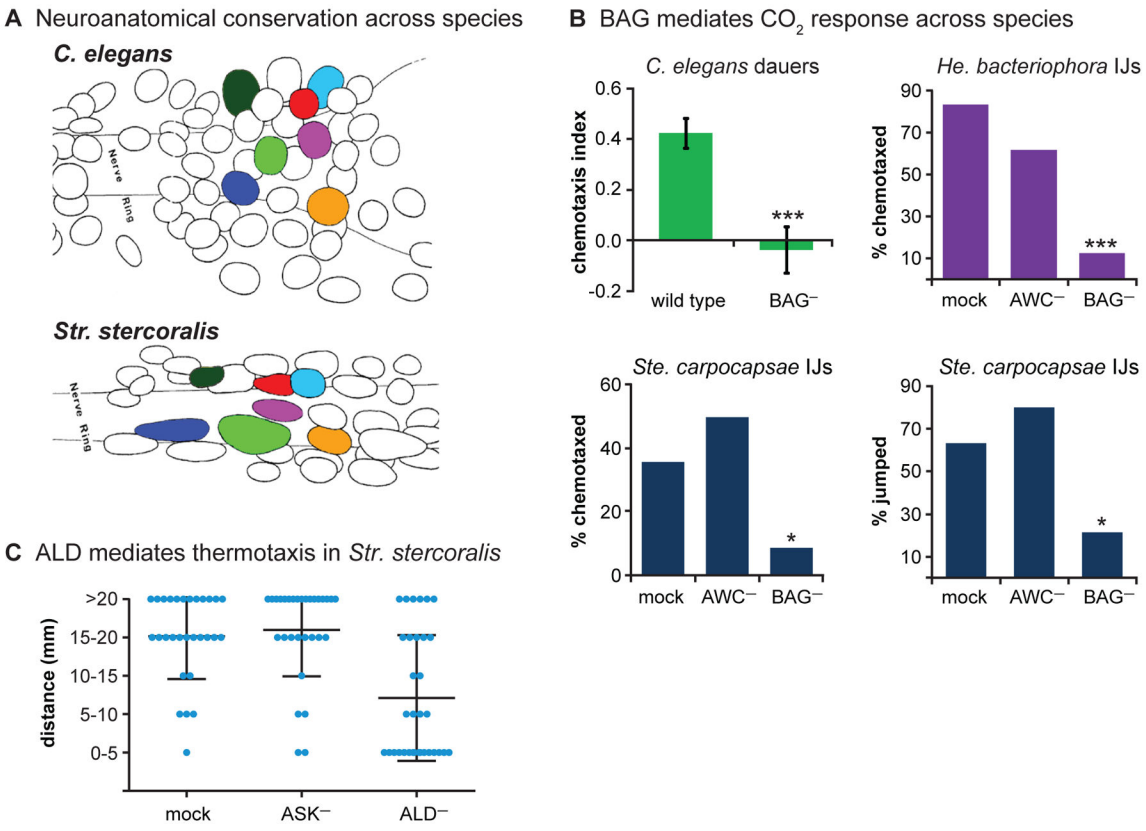

Figure 5. Sensory neuron function is often conserved across free-living and parasitic nematodes

A. Schematics of neurons in the head regions of *C. elegans* and *Str. stercoralis*. Color-coding indicates a few of the analogous sensory neurons. Schematics are reproduced from Ashton *et al.*, 1995 [86] with permission. **B.** BAG neurons mediate CO_2 response across species. Wild-type *C. elegans* dauer larvae are attracted to CO_2, but animals containing a genetic ablation of the BAG neurons (BAG^-) do not respond to CO_2. Reproduced from Hallem *et al.,* 2011 [10]. Wild-type *He. bacteriophora* and *Ste. carpocapsae* IJs, and IJs in which the AWC chemosensory neurons have been laser-ablated (AWC^-), are attracted to CO_2. However, IJs in which the BAG neurons have been laser-ablated (BAG^-) no longer respond to CO_2. BAG ablation also eliminates CO_2-evoked jumping by *Ste. carpocapsae* IJs (lower right graph). Reproduced from Hallem *et al.*, 2011 [10]. **C.** ALD neurons mediate thermotaxis in *Str. stercoralis*. Wild-type *Str. stercoralis* IJs, and IJs in which the ASK chemosensory neurons have been laser-ablated (ASK^-), migrate toward heat when placed at 26°C in a thermal gradient ranging from 22°C to 43°C. However, IJs in which the ALD neurons have been laser-ablated (ALD^-) do not migrate toward heat. Blue dots = migration distances of individual IJs from their initial placement point during a 1 minute assay; center bars = mean migration distances; upper and lower bars = standard deviations. Dot-plot data are reproduced from Lopez *et al.*, 2000 [54] with permission.