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Evaluation of host-seeking behavior in a diverse group of nematode species

A thesis submitted in partial satisfaction of the requirements for the degree of Master of Science in Microbiology, Immunology, & Molecular Genetics

By

Keely Ellen Chaisson

2013
ABSTRACT OF THE THESIS

Evaluation of host-seeking behavior in a diverse group of nematode species

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Keely Ellen Chaisson

Master of Science in Microbiology, Immunology, & Molecular Genetics

University of California, Los Angeles, 2013

Professor Elissa Hallem, Chair

One of the behaviors of parasitic nematodes that is essential for successful parasitism is host seeking, a complex behavior requiring nematodes to integrate sensory cues to find suitable hosts in which to complete their life cycles. Olfaction is a critical component of this response; many nematode parasites use carbon dioxide and other host-produced volatiles to locate their hosts. I investigated the odor responses of four species of skin-penetrating mammalian-parasitic nematodes: the rat parasites *Strongyloides ratti* and *Nippostrongylus brasiliensis*, the possum parasite *Parastrongyloides trichosuri*, and the human parasite *Strongyloides stercoralis*. For comparison, I also profiled responses of the insect parasites *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, as well as those of the free-living nematode *Caenorhabditis elegans*. The mammalian parasites were found to show significant response to some components of human/mammalian odors, though the matter of attraction to live hosts remains unresolved. Likewise, clustering analysis grouping species by odor responses was inconclusive.
The thesis of Keely Ellen Chaisson is approved.

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University of California Los Angeles
2013
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Chemosensory behaviors of parasites

INTRODUCTION

Many multicellular parasites seek out hosts by following trails of host-emitted chemicals. Host seeking is a characteristic of endoparasites such as parasitic worms as well as of ectoparasites such as mosquitoes and ticks. For host location, many of these parasites use CO$_2$, a respiration byproduct, in combination with host-specific chemicals. Recent work has begun to elucidate the behavioral responses of parasites to CO$_2$ and other host chemicals, and to unravel the mechanisms of these responses.

Chemosensory behavior of parasitic helminths

Parasitic helminths comprise a large group of worms from several different phyla, including roundworms in the phylum Nematoda and flatworms in the phylum Platyhelminthes. Some parasitize humans, whereas others parasitize non-human animals or plants. Human-parasitic species cause extensive morbidity, mortality, and economic loss worldwide, and are responsible for some of the most common neglected tropical diseases$^1$. Parasitic helminths utilize several different strategies for host infection: (i) reliance on passive host ingestion, (ii) transmission by intermediate vectors such as mosquitoes and flies, and (iii) engagement in active host-seeking and host-invasion behaviors. This latter group consists of some of the most devastating human-parasitic worms, including the skin-penetrating intestinal nematodes Strongyloides stercoralis, Ancylostoma duodenale, and Necator americanus, as well as blood flukes in the genus Schistosoma. For parasitic worms that actively seek out hosts, chemosensation is a crucial sensory modality, allowing the parasites to recognize appropriate hosts by detecting host-emitted chemicals.

The most well-studied of the helminths are the nematodes, which comprise a large and highly diverse phylum that includes both free-living species such as the model nematode
Caenorhabditis elegans and parasitic species. Human-parasitic nematodes infect over a quarter of the world population, and nematode parasites of livestock and plants cause economic and agricultural losses of billions of dollars each year\textsuperscript{1,2}. The infective stage of many parasitic nematodes is a developmentally-arrested larval stage called the infective juvenile (IJ) that actively seeks out hosts in which to complete its lifecycle. The group of nematodes that host seek includes insect parasites, plant parasites, and skin-penetrating mammalian parasites.

The entomopathogenic nematodes (EPNs) comprise a guild of lethal parasites of insects that are of interest both as models for human parasitic nematodes and as biocontrol agents for a wide variety of insect pests and disease vectors. EPN IJs infect insect hosts either by entering through a natural body opening or by penetrating directly through the insect cuticle. EPNs rapidly kill their host, develop and reproduce inside the host cadaver until resources are depleted, and then exit as IJs to search for new hosts. EPNs vary greatly in their host ranges; for example, *Steinernema carpocapsae* is a generalist that can infect over 250 different insect species under laboratory conditions, whereas *Steinernema scapterisci* is an orthopteran specialist whose only known natural host is the mole cricket\textsuperscript{3}. EPN IJs are strongly attracted to live hosts, as well as to CO\textsubscript{2} and a wide variety of other host-derived volatiles\textsuperscript{4-6}. Attraction to live insect hosts is greatly diminished in the absence of CO\textsubscript{2}, indicating that CO\textsubscript{2} is an essential host-seeking cue for these parasites\textsuperscript{5,6}.

In addition to displaying chemotaxis toward hosts, some EPNs in the genus *Steinernema* exhibit jumping, a highly specialized host-seeking behavior in which the IJ springs into the air and onto passing hosts\textsuperscript{7}. *Steinernema* IJs jump in response to complex odor blends emitted by live hosts, as well as to CO\textsubscript{2} and many individual host-derived odorants\textsuperscript{5,6}. A comparison of the odor response profiles of *Heterorhabditis bacteriophora* and *S. carpocapsae* IJs with *C. elegans*
dauer larvae, which are analogous life stages, revealed that the parasitic species cluster together despite their phylogenetic distance\(^5\), suggesting an important role for chemosensation in the evolution of host range among EPNs.

In addition to responding to host-derived chemicals, EPNs respond to several other ecologically relevant chemical cues. For example, EPNs are attracted by odorants produced by insect-damaged plants as an indirect defense against predation\(^8\)\(^-\)\(^10\). However, some of the plant volatiles that attract EPNs also attract plant-parasitic nematodes, suggesting an ecological cost to this plant defense mechanism\(^11\). EPNs also respond to ascarosides, a large family of small-molecule pheromones that regulate reproductive diapause as well as mating, aggregation, and chemotaxis behaviors in nematodes\(^12\)\(^-\)\(^16\).

Skin-penetrating mammalian-parasitic nematodes such as the human threadworm *Strongyloides stercoralis* and the human hookworms *N. americanus* and *Ancylostoma caninum* also engage in host-seeking and host-invasion behaviors. These nematodes are passed out of an infected host as eggs or newly hatched larvae in infested feces, develop into infective larvae in the feces, and then migrate into the environment in search of new hosts. Infection of new hosts occurs by skin penetration, primarily through the skin of the feet\(^17\). Many mammalian-parasitic nematodes have highly specific host ranges. For example, *Strongyloides fuelleborni kellyi* is an exclusive parasite of humans, and *Strongyloides ratti* is a natural parasite of rats but not of other rodents\(^17\),\(^18\). Chemosensation is thought to be an important component of host specificity: *S. stercoralis* is attracted to mammalian skin extract\(^19\) and the closely related rat parasite *S. ratti* is attracted to some components of mammalian sera\(^20\). For *S. stercoralis*, a major component of skin attraction is the odorant urocanic acid, which is found in the skin of many mammals and is a potent attractant even at low concentrations\(^19\). Both *S. stercoralis* and *S. ratti* also navigate
through sodium chloride gradients\textsuperscript{21,22}. Similarly to the \textit{Strongyloides} species, the dog hookworm \textit{A. caninum} is attracted to hydrophilic extracts of dog skin\textsuperscript{23}. CO\textsubscript{2} causes activation of \textit{A. caninum} and \textit{S. stercoralis}, and stimulates nictation (tail-standing) behavior in \textit{A. caninum}\textsuperscript{23,24}. In contrast to \textit{A. caninum} and the \textit{Strongyloides} species, chemoattraction to human skin has not been demonstrated for the human hookworms \textit{N. americanus} and \textit{A. duodenale}\textsuperscript{25}. However, these species do display activation (increased locomotion) and penetration behaviors in response to chemosensory cues present in skin extract\textsuperscript{26}. The fact that human hookworms have not been shown to display long-range host attraction is consistent with their foraging strategy: the infective larvae are thought to spend most of their time motionless, waiting for passing hosts\textsuperscript{24,26}. By contrast, \textit{Strongyloides} species spend most of their foraging time actively searching for hosts\textsuperscript{24}. This difference in foraging strategy is similar to the cruiser/ambusher distinction among EPNs: some EPNs are cruisers that actively undergo chemotaxis through the soil searching for hosts, whereas others are ambushers that are thought to remain relatively stationary and primarily infect passing hosts\textsuperscript{27}. However, ambushing EPNs are in fact capable of chemotaxis toward a wide variety of insect-derived odorants as well as live insects\textsuperscript{5,6}, although they display less robust host-attraction than cruising EPNs\textsuperscript{6}. This suggests that ambushing EPNs exhibit long-range host seeking in response to appropriate chemosensory cues. The chemotaxis behavior of human hookworms has not been as thoroughly examined, and whether they are also capable of long-range host attraction is not yet clear.

Plant-parasitic nematodes use CO\textsubscript{2} in combination with soluble and volatile host-specific chemicals to locate the roots of host plants\textsuperscript{8,11,28}. In the case of the root-knot nematodes \textit{Meloidogyne incognita} and \textit{Meloidogyne graminicola}, infective larvae are attracted to host plants, but show little or no attraction to non-host plants\textsuperscript{28}. Moreover, when given a choice between a
short route and a long route to reach a host plant, nematodes preferentially choose the short route; however, nematodes often travel the long route to reach a non-host plant\textsuperscript{28}. Thus, plant-parasitic nematodes respond differently to chemical blends produced by hosts versus non-hosts.

Several other nematode parasites also display chemosensory behaviors. One example is the gastropod-parasitic nematode \textit{Phasmarhabditis hermaphrodita}. \textit{P. hermaphrodita} shows attraction to mucosal extracts from slugs, indicating that it uses chemosensory cues to locate slug hosts\textsuperscript{29-31}. However, attraction of \textit{P. hermaphrodita} to potential hosts does not correlate with reproductive success inside the host, suggesting that other factors play an important role in host selection\textsuperscript{30}. By contrast, the response of \textit{S. carpocapsae} to host volatiles following exposure to host cuticle is positively correlated with both host mortality and parasite reproduction inside the host, suggesting that at least some EPNs use chemosensory cues for host selection\textsuperscript{32}. Thus, although \textit{P. hermaphrodita} and \textit{S. carpocapsae} have apparently similar lifestyles – both are lethal parasites of small invertebrates that engage in host seeking – the two species appear to rely on different mechanisms for appropriate host selection.

Insight into the cellular and molecular mechanisms underlying chemosensation in parasitic nematodes has come largely from studies of the free-living model nematode \textit{C. elegans}. Despite profound differences in body size and lifestyle among nematodes, sensory neural anatomy and function are highly conserved across species\textsuperscript{33}. Thus, many of the neural circuits and signaling pathways that operate in \textit{C. elegans} are likely to operate similarly in parasitic nematodes. \textit{C. elegans} shows directional chemotaxis in response to volatile chemicals such as alcohols, esters, and aldehydes; soluble chemicals such as salts and pheromones; and gases such as oxygen (\(\text{O}_2\)) and \(\text{CO}_2\)\textsuperscript{34}. Olfactory and gustatory responses are mediated primarily by neurons located in the main chemosensory organs of \textit{C. elegans}, the paired amphid sensilla\textsuperscript{34}, whereas
responses to O\textsubscript{2} and CO\textsubscript{2} are mediated by a distributed network of head and tail neurons\textsuperscript{35-39}. Chemoreceptors in \textit{C. elegans} are encoded by large families of G protein-coupled receptor (GPCR) and guanylate cyclase (GC) genes\textsuperscript{34}. Specific receptors have been identified for a few chemicals, including O\textsubscript{2}\textsuperscript{35,40}, ascarosides\textsuperscript{41,42}, and the odorant 2,3-butanedione\textsuperscript{43}. The CO\textsubscript{2} receptor has not yet been identified, although the receptor guanylate cyclase GCY-9 is required for the CO\textsubscript{2} response and may be a receptor for CO\textsubscript{2} or a CO\textsubscript{2} metabolite\textsuperscript{39,44}.

In parasitic nematodes, chemosensory neuron function has been investigated in only a few cases. As in \textit{C. elegans}, the amphidial ASE and ASH sensory neurons of \textit{S. stercoralis} mediate chemoattraction and chemorepulsion to soluble chemicals, respectively\textsuperscript{21,45}. In addition, the BAG sensory neurons mediate CO\textsubscript{2} repulsion by \textit{C. elegans} adults, and CO\textsubscript{2} attraction by \textit{C. elegans} dauers and EPN IJs, demonstrating that the neural basis of CO\textsubscript{2} response is at least partly conserved across species regardless of whether CO\textsubscript{2} is a repulsive or attractive cue\textsuperscript{5,37}. The molecular basis of chemoreception in parasitic nematodes has not yet been investigated. However, recent breakthroughs in the development of techniques for genetic transformation of \textit{S. stercoralis} and \textit{S. ratti}\textsuperscript{46,47} should enable new avenues of research into the molecular mechanisms that underlie chemosensation in these parasites.

**The varied use of carbon dioxide as a host cue across parasitic species**

A consistent theme across species that engage in host seeking is the reliance on a combination of general and specific host sensory cues. General cues typically include CO\textsubscript{2} as well as non-chemosensory stimuli such as heat, whereas specific host cues are often a unique blend of host-derived odorants. The requirement for general versus specific cues varies greatly for different parasites, different parasite–host combinations, and different host-seeking behaviors.
For example, the universal cue CO$_2$ is sufficient to elicit host-seeking behaviors from EPNs$^{5,6}$. However, removing CO$_2$ from the host odor blend abolishes attraction to some hosts but not others, indicating that the requirement for general versus specific host cues is host dependent$^{5,6}$. Parasites also differ in whether they rely on CO$_2$ for long-range versus short-range host-seeking behaviors. For example, in the case of the mosquito Culex quinquefasciatus, CO$_2$ is more important for long-range host-seeking behavior, whereas host-specific odorants are more important for short-range landing behavior$^{48}$. Similarly, S. carpocapsae requires CO$_2$ for long-range chemotaxis but not short-range jumping to waxworm odor$^{5}$. By contrast, CO$_2$ is a short-range activation cue for human hookworms in combination with warmth and/or moisture$^{25}$. Thus, although general host cues such as CO$_2$ are used by many parasites, the ways in which they are used for host seeking are often species-specific.

**Investigating host-seeking behaviors in mammalian parasitic nematodes**

The goal of the work described in this thesis was to investigate odor-driven host-seeking behavior in skin-penetrating mammalian-parasitic nematodes. I used three species of skin-penetrating mammalian-parasitic nematodes as models systems: the rat parasites *Strongyloides ratti* and *Nippostrongylus brasiliensis* as well as the possum parasite *Parastrongyloides trichosuri*. In addition, *Strongyloides stercoralis* was later tested in identical assays by a lab-mate thanks to cooperation from a collaborating lab. These parasites were chosen based on their relative ease of maintenance in the laboratory and their close similarity to soil-transmitted human parasites. In addition, *Strongyloides* species are amenable to genetic transformation$^{46}$. I examined the behavioral responses of these nematodes to a large and chemically diverse set of odorants to identify odorants that attract and repel mammalian-parasitic nematodes. I also examined the
responses to live hosts and host-derived odorants. This work is the first broad investigation of olfactory behavior in nematode parasites of mammals.
RESULTS

Responses of mammalian-parasitic nematodes to CO₂

Specificity of host-seeking behavior by many parasites is thought to result from integration of responses to carbon dioxide (CO₂) and host-specific odors. CO₂ is known to be an important host cue for several parasitic nematodes. I characterized the behavioral responses of *S. ratti* and *N. brasiliensis* to CO₂ concentrations ranging from 0.2% to 15% using a CO₂ gradient assay. In contrast to EPN IJs and *C. elegans* dauers, the mammalian-parasitic nematodes were found to be repelled by carbon dioxide (Figure 1). This result held up across concentrations and species—all mammalian parasitic nematodes tested thus far have been found to be repelled by carbon dioxide. This indicates that, unlike what is found in insect-parasitic nematodes, carbon dioxide alone is not a host cue for mammalian-parasitic nematodes. It is possible carbon dioxide simply plays no role in the host seeking of these nematodes. Alternatively, carbon dioxide may turn out to be an attractive cue but only in combination with other key indicators of host presence, such as other odors, movement, and heat.

Responses to general odorants

I used chemotaxis assays in which the IJs are allowed to disperse on plates in a chemical gradient to characterize the odor response profiles of IJs to a diverse panel of fifty-six volatile organic compounds, all of which are ecologically relevant odorants that soil-dwelling IJs are likely to encounter in nature. These responses were compared to previous data where the responses to this same panel were examined in the free-living nematode *C. elegans* as well as two entomopathogenic nematodes, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. The response profiles of the mammalian parasites to the general odors appeared different from
those of the insect parasites (Figure 2a); however, given the small panel it is difficult to determine the relevance of this difference. When grouped based on their odor response profiles, the species tested clustered seemingly randomly, reflecting neither host range nor phylogeny. In addition, it should be noted that most of the responses to the general odor panel found in the mammalian parasites were not very robust—that is, the responses disappeared quickly when the odor was diluted rather than at full strength (Figure 3). One odor, 2,3-butanedione could be argued to be an exception, as some response lingered even after a hundred-fold dilution. Notably, this odor, though originally included in the ‘standard’ panel, is known to be associated with mammalian odor.

**Responses to discrete ‘mammalian’ compounds**

After characterizing responses to a broad panel of compounds, I moved forward by characterizing the responses to additional candidate attractants that were identified from work investigating volatile odorants given off by mammalian skin and secretions\(^\text{50-55}\). Several attractive odorants were identified, including three that are known to be strongly attractive to mosquitoes, and one that has been previously identified as an attractant for the human-parasitic nematode *S. stercoralis*. This suggested that some compounds may serve as common attractants for parasites of mammals, and therefore mammalian compounds that are attractive for other parasites are useful starting points for investigation.

Out of the odorants I tested, several were found to be significantly attractive to one or more of the mammalian-parasitic nematodes (Figure 2). Impressively, *S. stercoralis* showed a significant positive response to seven out of the twelve odorants it has been tested with, some of
those being specifically identified as odorants present in human sweat (3-methyl-1-butanol, 2-methyl-1-butanol, 6-methyl-5-hepten-2-one, 7-octenoic acid)\(^49,56\).

**Responses to live hosts and host-derived odorants**

There is a great deal of evidence for host-seeking behavior in parasitic worms\(^5,23,57\). Notably, *S. stercoralis*, which is closely related to *S. ratti*, was recently shown to be attracted to the host-derived odorant urocanic acid. However, relatively little has been done to investigate the specific role of olfaction in attraction to live hosts. Of particular interest is whether olfaction alone can be used by these parasites to distinguish between similar hosts that vary in their suitability for infection.

I designed an experiment to investigate this behavior, largely adapted from a protocol developed to study attraction to live hosts in scabies mites\(^58\). As in our assay for chemotaxis towards gases, air flowed through tubes into opposite sides of the lid of a chemotaxis assay plate. One of these tubes flowed from a small tube containing a live host animal, and the other from an empty control tube. Initial experiments investigated response to the natural host for *S. ratti* and *N. brasiliensis*, the rat. These experiments were performed both with and without running the host air through soda lime-filled tubing to remove CO\(_2\), in order to allow us to determine whether CO\(_2\) plays a significant role in the response to live hosts.

Unfortunately, despite endless troubleshooting of the experimental apparatus (Figure 6), this experiment provided us with little useful information. *N. brasiliensis* shows a slight attraction to rats when soda lime is used to remove carbon dioxide (Figure 7a), but a C.I. of ~0.3 is quite low given that we were presenting the worms with their natural hosts. Even worse, *S. ratti* failed to show anything but a negative response to rat odor under all conditions (Figure 7b).
In parallel with the live-host project, I was interested in investigating attraction to other host-derived materials, such as one might find in a natural rat’s nest. Early in our experimentation, we were able to obtain male and female urine samples from three different common lab strains of rat and test them in our standard chemotaxis assay (Figure 5). Later, when I was failing to obtain clear responses to live rats, we began developing a number of different protocols to test the parasites against the odor of rat bedding, or of cotton t-shirts given in place of bedding (as wood shavings have natural odor), but none of these methods even gave consistent results (data not shown). Another member of the lab also tried presenting the parasites with rat feces, but this was also not found to be attractive (data not shown).
DISCUSSION

Mammalian parasite response to diverse panel of odors

The work detailed here was able to assess the attractiveness of a large number of compounds to *S. ratti* and *N. brasiliensis*, as well as a smaller but still significant number to *P. trichosuri*, *S. stercoralis*, *H. bacteriophora*, *S. carpocapsae*, and *C. elegans* (Hawaii strain). A number of compounds known to be human-derived odorants were shown to be attractive in particular to *S. stercoralis*, a human parasite. As such, some of these compounds may be useful for development of traps, especially as many of these odorants are also attractive to mosquitoes, and as such traps containing them may serve a dual purpose. A large amount of data has been generated that will continue to be used to compare odor-evoked behavior across parasite species.

That said, our sights for this project aimed much higher. In previous work, responses to many more compounds were characterized for several insect parasites, and this data was used to show that odor preferences between species that shared hosts were closer together than odor preferences of closely related species that lacked a shared host/lifestyle. Such a clustering did not arise from my work as described here, but it is possible that the completed odorant lists are just too small. *P. trichosuri* proved difficult to culture and as such worms were scraped together to even get as many animals as we managed to test, and *S. stercoralis* testing was only possible during a brief trip to a collaborating lab, as the Hallem lab has yet to begin culturing its own *S. stercoralis*. In addition, it has now been shown that the previously-generated insect parasite data may need to be repeated under more carefully controlled conditions, as we have now found that the odorant preferences of insect-parasitic nematodes can vary significantly with age and/or cultivation temperature (unpublished data). As such, while we may draw conclusions about
individual odors found attractive for all but the insect parasites, the aggregate data remains preliminary.

**Mammalian parasite response to host-derived odorants**

Initially, it seemed a simple proposition: that rat parasitic nematodes should be attractive to rats. The equivalent had already been proven in the case of several insect-parasitic nematodes, and these are *skin-penetrating nematodes*—surely, they must find their host somehow. Even the protocol to show this was roughly outlined—the insects had been put in syringes that slowly emptied their air through tubes running to the chemotaxis plate. All that remained, seemingly, was to scale up—bigger animals, bigger tubes, but the same principles.

Unfortunately, this was not the case. Before we even came to an experimental apparatus design that we were happy with, we went through two complete redesigns. But technical difficulties aside, there simply didn’t seem to be *anything* we could do to find consistent evidence of our nematodes’ attraction to their host. Urine didn’t work, and neither did bedding or feces. Several potential scenarios could explain these frustrating findings. First, it is possible that we simply have not tried the right experiment yet, and with a proper design of our experimental setup, we will solve our problems entirely. We have learned that the mammalian-parasitic nematodes are incredibly sensitive to vibration and air flow rates—it is entirely possible that our setup simply allowed so much variation in one or both of these that any response to hosts was masked. Second, it is possible that the mammalian parasitic nematodes we have tested actually don’t use host odor to actively seek their hosts. There are two ways that this could be explained—one, it is possible that the parasites use other cues such as heat for long-range host seeking, and only use chemical cues at short ranges, or to trigger penetration. This is exactly how
Schistosoma japonicum cercariae function, using very general cues to find a thing that might be a host, and then only triggering penetration behavior if the appropriate cues are found on the host’s skin\textsuperscript{67-69}. Alternatively, it is possible that one or more of our strains has lost the ability to host seek after dozens of generations of serial passage under conditions where, thanks to an experimenter’s needle and syringe, host seeking is unnecessary.

This second idea, that there is no host-seeking behavior in our mammalian parasitic nematodes, seems unlikely to be true, at least for all strains. In particular, Strongyloides stercoralis is very active in response to many mammalian odor cues. However, all possibilities must be considered, as the “live hosts” part of this project has so far seen over a year of work and nothing but disappointing results.

Conclusions

In conclusion, the work described here was the first to attempt a thorough investigation of the odor-based host-seeking behavior of mammalian-parasitic nematodes. A number of interesting findings were made, including the fact that in many cases mammalian parasites responded to odors previously identified as attractive for other human parasites. However, due to a number of unforeseen challenges, the work remains incomplete and the dataset cannot be evaluated as a whole.

Future directions

An important direction for future research will be to investigate further the extent to which chemosensory behavior contributes to host selection and the evolution of host specificity. Appropriate host selection is crucial for endoparasites: many species have narrow host-ranges,
and infection of a non-host or dead-end host is often fatal. For a few parasites, attraction to host-derived chemicals has been shown to correlate with host suitability. For example, *S. carpocapsae* is more attracted to hosts that support higher levels of mortality and parasite reproduction\(^{32}\). In the case of the human parasitic trematode, *Schistosoma haematobium*, the miracidia are more attracted to hosts than sympatric non-hosts but are unable to discriminate hosts from allopatric non-hosts, suggesting that their chemosensory preferences have evolved to facilitate host selection\(^{70}\). However, for both species, long-range chemosensory behavior is not sufficient to ensure appropriate host selection: *S. carpocapsae* is also attracted to non-host isopods\(^{6}\), and the preference of *S. haematobium* miracidia for hosts over sympatric non-hosts is not absolute\(^{70}\). This suggests either that these parasites sometimes attempt to infect poor hosts, that host selection is further refined by host recognition and penetration behaviors, or both. Further work will be necessary to distinguish between these possibilities, and to investigate the relationship between host attraction and host suitability in other parasites.

Recent progress in understanding the behavioral responses to host odor cues and the mechanisms that underlie these responses has already led to the design of more specific and effective parasite control strategies. In the case of *An. gambiae*, the discovery that human skin microbiota play an important role in host seeking\(^{49}\) led to the finding that the addition of some skin microbiota-derived odorants enhance the effectiveness of existing traps\(^{66,71}\), and the large-scale decoding of the AgOR repertoire\(^{72}\) led to the finding that some of the odorants that strongly activate AgORs can increase trap effectiveness\(^{73}\). In addition, a better understanding of insect odorant receptor function led to the finding that the broad spectrum insect repellent DEET directly modulates the odorant receptor complex\(^{74}\), paving the way for future studies aimed at designing alternative insect repellents. The odor-driven behaviors of human endoparasites are
much less well understood, but the possibility of developing chemically-baited traps or repellents for these parasites based on knowledge of the function and organization of their chemosensory systems is an exciting avenue for future research. Finally, a comparison of odorants that are attractants for different parasites reveals that many of the chemosensory cues that elicit host-seeking behaviors are conserved across phyla\textsuperscript{75}, raising the possibility that traps could be designed that target multiple phylogenetically diverse species of parasites. Multicellular parasites are a major cause of disease worldwide, and a better understanding of how they respond to host chemical cues at the molecular and cellular level should greatly facilitate the development of more effective control strategies.
METHODS

General chemotaxis assay

Chemotaxis plates were set out on the bench to dry for 1-2 days prior to the experiment. Worms to be used were obtained from fecal-charcoal cultures using a Baermann apparatus\(^5\) on the same day as assays were to be performed. Chemotaxis plates were marked by placing each of them into the upside-down lid of the guide lid, and marking a short vertical line on the plate that corresponds to the short vertical line on the edge of the lid. This was used to orient the plates. 2 microliters of 5% sodium azide was applied to each of the odorant points. Liquid was allowed to dry for approximately one minute. Then 5 microliters of odorant was applied to one side of the plate, and 5 microliters paraffin oil was applied (or appropriate diluent for solid odors) as a control to on the other side of the plate. Then approximately 3 microliters of worm pellet was applied to the center of the plate using a pipet. More worms were added if the initial number placed was insufficient, taking care to not exceed 10 microliters of liquid. The lid was then placed on the plate, and the plate then placed in a cardboard box, on a vibration-reducing table. Plates were left undisturbed for three hours, after which the number of worms in each of the odor circles was counted. The chemotaxis index was then computed as follows:

\[
\text{C.I.} = \frac{\text{(# worms in odor circle)} - \text{(# worms in control circle)}}{\text{(total worms in both circles)}}
\]

Carbon dioxide response assays

Plates and worms were prepared as for ordinary chemotaxis assays. Six gas-tight syringes were filled with gas from the appropriate tanks, three from the air control and three from the tanks containing air with the percentage of carbon dioxide to be tested. The syringe pump apparatus was assembled and tubes running to each aerotaxis plate lid were connected
appropriately, such that each lid had a tube containing air running into the hole on one side, and a tube containing the test air on the opposite side. The pump rate was set to 10 ml/min and the pump was allowed to run for 1 minute before the pump rate was changed to 0.5ml/min for the duration of the assay. Worms were then applied to the plates as described above and in previous work, and then plates were aligned under the aerotaxis lids on the vibration-reducing table. Assays ran for one hour. Scoring was completed as for chemotaxis assays.

Mammalian-parasitic worm culturing methods

*Nippostronglyus brasiliensis*: On day zero of each infection, two healthy adult rats (cagemates) were injected with approximately 3000 infective juveniles (IJs) in 200-300 microliters of sterile PBS. A 20.5 gauge needle was used to fill the syringe and a 25.5 gauge needle was used for subcutaneous injection. On day six, the rats were placed in a cage containing wire racks over wet techboard in the evening. Approximately 12 hours later, rat feces was collected and rats were placed back in an ordinary cage with bedding. The collection was repeated identically on days 7-11. Collected feces were divided between two 10 cm Petri dishes containing a damp circle of filter paper. A thin layer of bone char charcoal was then added to the plate, followed by sufficient vermiculite to cover both charcoal and feces completely. Plates were stored in a humidified chamber in a 22-degree incubator. Six to seven days following feces collection, infective larvae are visible nictating on vermiculite and IJs can be recovered using a Baermann apparatus.

*Strongyloides ratti*: On day zero of each infection, two healthy adult rats (cagemates) were injected with approximately 500-1000 infective juveniles (IJs) in 200-300 microliters of
sterile PBS. A 20.5 gauge needle was used to fill the syringe and a 25.5 gauge needle was used for subcutaneous injection. On day six, the rats were placed in a cage containing wire racks over wet techboard in the evening. Approximately 12-hours later, rat feces were collected and rats were placed back in an ordinary cage with bedding. The collection was repeated identically on days 7-21. Collected feces was dampened and mixed with an amount of bone char charcoal approximately equal to the volume of feces. A large popsicle stick was used to combine the mixture and break feces into smaller pieces. Sufficient vermiculite to absorb any standing water was added to the feces-charcoal mixture, creating a paste. This mixture was then divided between 2 or 3 10 cm Petri dishes lined with damp filter paper, such that the plates were full but the mixture did not press against the lid. Plates were stored in a humidified chamber in a 25 degree incubator. Five to six days following feces collection, second-generation infective larvae are visible in culture under a scope and IJs can be recovered using a Baermann apparatus\textsuperscript{59}.

**Insect-parasitic worm culturing**

Five waxworms were placed on filter paper in a small petri dish, then inoculated with water containing approximately 100 IJs/waxworm. During the infection, worms were intermittently monitored to check the progress of infection and any black worms (indicating bacterial infection) were discarded. After 7-10 days, the dead and dry waxworms were placed in a White trap\textsuperscript{60}—that is, they were placed on a piece of filter paper on top of a small Petri dish lid which was placed inside a larger Petri dish, which contained just enough water to cover the bottom of the dish. IJs emerge gradually and then can be collected from the water in the White trap. Following this, worms were washed three times in water and then stored at 15 degrees C in a 50 ml tissue culture flask.
Live animal attraction experiments

Animal(s) to be tested were placed on top of a sheet of techboard in a plastic tube of sufficient size that the animal was able to turn around freely. For all animals excluding the guinea pig, a 4 inch diameter tube was used. For the guinea pig, a 5- or 6 inch diameter tube was used. The tubes ranged in length from 4 to 12 inches, with the smallest length that accommodated the animal(s) being chosen in each instance. Matching tubes containing techboard but no animal were used as controls. Each tube was sealed tightly at each end with plastic caps. Air flowing from an aquarium pump at approximately 0.6 LPM as measured by a flow meter was pumped into each tube through a 0.5 inch hole on one side of the tube. On the opposite side of the tube, an equally sized air outlet was connected to tubing. This tubing flowed to a second flow meter that restricted airflow to 6.22 ml/min. Following the second flow meter, a y-connector split the airflow into two streams, each to be provided to one side of a chemotaxis plate. Each assay plate had control airflow on one side and animal airflow on the other. 1-hour chemotaxis assays were performed as previously described. When required, 6-inches of 0.25 inch diameter tubing containing soda lime was placed in-line between the tube containing the animal and the second flow meter. A diagram of the setup can be seen in Figure 6.

Creation of ‘mammalian’ odor compound list

The literature was searched to the best of my ability, using Google scholar and Pubmed, for (preferably recent) papers in which a) human parasite attraction to a discrete, identifiable compound was demonstrated or b) odor compounds of humans or rats were identified using GC-MS. From these papers, a list of twenty compounds that appeared in at least two sources
and could be obtained from Sigma-Aldrich was generated, with preference given to odors that appeared in multiple sources. The list was later reduced after several compounds were found to be toxic to the nematodes under assay conditions.
Figure 1: Nematode responses to carbon dioxide. a) Nematode response to 10% carbon dioxide delivered at .5 ml/min was measured in a one-hour chemotaxis assay. Featured species are the insect parasites *S. carpocapsae* and *H. bacteriophora*, free-living nematode *C. elegans* Hawaii strain, the rat parasites *S. ratti* and *N. brasiliensis*, and the possum parasite *P. trichosuri*. n = 6-11. b) Nematode responses to a range of carbon dioxide concentrations. Experimental set up and species assessed were the same as in a. n = 6-18
Figure 2: Odor response panels of diverse set of nematode species a) Profile of responses to panel of odorants commonly found in nature, assessed by chemotaxis assays. Featured species are the insect parasites S. carpocapsae and H. bacteriophora, free-living nematode C. elegans Hawaii strain, the rat parasites S. ratti and N. brasiliensis, the possum parasite P. trichosuri, and the human parasite S. stercoralis. n = 6-33 for each combination of odorant and nematode species. b) Odor response profiles of all previously mentioned species except S. stercoralis to a panel of odors known to be associated with mammalian hosts. n = 6-20. c) Subset of data from b, with the addition of data for S. stercoralis.
Figure 3: Response of mammalian parasites to dilutions of key odors. a-f are responses of *N. brasiliensis*, g-n are responses of *S. ratti*.
Figure 4: Cluster analysis of species based on odor preferences. Cluster analysis for A included only ‘standard panel’ odors, and no mammalian odors. Cluster analysis for B included all odors for which data was collected for all species. Clustering performed using Ward’s method in PAST.
Figure 5: Rat-parasitic nematodes’ responses to rat urine. N = 6 for all data points. Rat strains collected from were three common lab strains. Urine was frozen from collection until use.
Figure 6: Experiment set-up for testing response to live hosts. Figure is shown with inline soda lime columns (orange) to remove carbon dioxide from air stream. Gray boxes represent 1 LPM flow meters to control air flow to animal tube, blue boxes represent 70mm flow meters to control flow to chemotaxis plates.
Figure 7: Response of rat-parasitic nematodes to live hosts. A) *N. brasiliensis* response to odor of live rats, male and female assayed separately. N = 6 or more for all data. B) *S. ratti* response to only live male rats. N = 4 or more for all data.
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