Trends in Parasitology

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Review

Making sense of sensory behaviors in vector-borne helminths

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Migrations performed by helminths are impressive and diverse, and accumulating evidence shows that many are controlled by sophisticated sensory programs. The migrations of vector-borne helminths are particularly complex, requiring precise, stage-specific regulation. We review the contrasting states of knowledge on snail-borne schistosomes and mosquito-borne filarial nematodes. Rich observational data exist for the chemosensory behaviors of schistosomes, while the molecular sensory pathways in nematodes are well described. Recent investigations on the molecular mechanisms of sensation in schistosomes and filarial nematodes have revealed some features conserved within their respective phyla, but adaptations correlated with parasitism are pronounced. Technological developments are likely to extend these advances, and we forecast how these technologies may be applied.

Highlights

Sensory behaviors are used during parasite development and transmission.

The sensory behaviors of schistosomes are well documented, and new phenotyping platforms promise to reveal the molecular effectors behind these behaviors.

Sensory signal transduction pathways in parasitic nematodes have been established from free-living models, but mosquito-borne filarial nematodes have unique adaptations that enable sensory reception of host-derived cues.

Approaches for parasite control that exploit sensory behaviors could be implemented in hotspots, or regions recalcitrant to mass drug administration.

Recent technological advances in single-cell genomics, microfluidics, microscopy, and image processing enable more comprehensive profiling of sensory cells and tissues and the development of higher-throughput and more reproducible sensory assays.

Sensory behaviors are critical to the success of vector-borne helminths

The survival and reproductive success of many helminths hinge on their ability to actively seek out competent hosts, penetrate host tissues, and perform complex intra-host migrations. The extent to which these behaviors are guided by specific sensory modalities remains poorly understood, especially for vector-borne parasitic nematodes and flatworms. Deciphering sensory behaviors that are critical to the transmission and development of vector-borne helminths will lead to a better understanding of host–parasite interactions and may reveal new considerations for disease control.

Associating sensory processing with migratory behaviors in vector-borne helminths has been historically challenging. First, it is often unclear how well sensory behaviors observed in the laboratory reflect meaningful activity in nature. Survivorship bias in the mapping of parasite migrations can cause stochastic processes to be understood as precisely coordinated sensory events. For most helminths, we lack statistical resolution of how frequently parasites invade aberrant hosts or migrate in noncanonical ways within compatible hosts. Second, little is known about how the sensation of chemical, mechanical, thermal, geophysical, and photo stimuli interact to determine or refine locomotory outcomes. Third, sensory structures, receptors, and neural and molecular signaling pathways that transduce environmental cues in vector-borne helminths are poorly defined. Finally, it remains a matter of speculation how intraspecific and interspecific variations in parasite and host genetics affect parasite migration and therefore parasite–host compatibility and host range.

Headway is being made in these areas of investigation. We focus this review on recent progress in the understanding of sensory behaviors in mosquito-borne nematodes that cause human lymphatic filariasis and the snail-borne flatworms that cause human schistosomiasis. We use these systems to explore phylum- and species-specific aspects of sensory biology and to identify potential sensory behaviors and their molecular basis. We highlight unique facets of development

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within arthropod and molluscan intermediate hosts and contrasting states of knowledge about cues and molecular pathways that govern taxis in these important vector-borne helminths. While the literature describing taxis phenotypes in schistosomes runs deeper than in filarial nematodes, the signal transduction pathways that underlie nematode sensory biology are better understood. Lastly, we discuss how research in this arena will be enriched by recent technological advancements.

Sensation in human schistosomes

Schistosomiasis is a snail-borne **neglected tropical disease** (**NTD**) (see Glossary) that infects over 200 million people throughout much of the developing world [1]. Pathology results from egg-induced inflammation in the preferred locales of paired male and female worms – the mesenteric vasculature of the liver and intestines (*Schistosoma mansoni* and *S. japonicum*) and the urogenital system (*S. haematobium*). Schistosomes are transmitted by freshwater snail species belonging to genera including *Biomphalaria*, *Bulinus*, and *Oncomelania*. Schistosomes perform aquatic, intramolluscan, and intramammalian migrations, each of which is coordinated in response to external chemical cues.

Miracidia hatch from human host-excreted eggs upon exposure to light and water (Figure 1A). Miracidia are nonfeeding and have approximately 6–12 h to find a suitable snail host before depletion of their glycogen stores [2]. Miracidia initially disperse along straight trajectories, guided

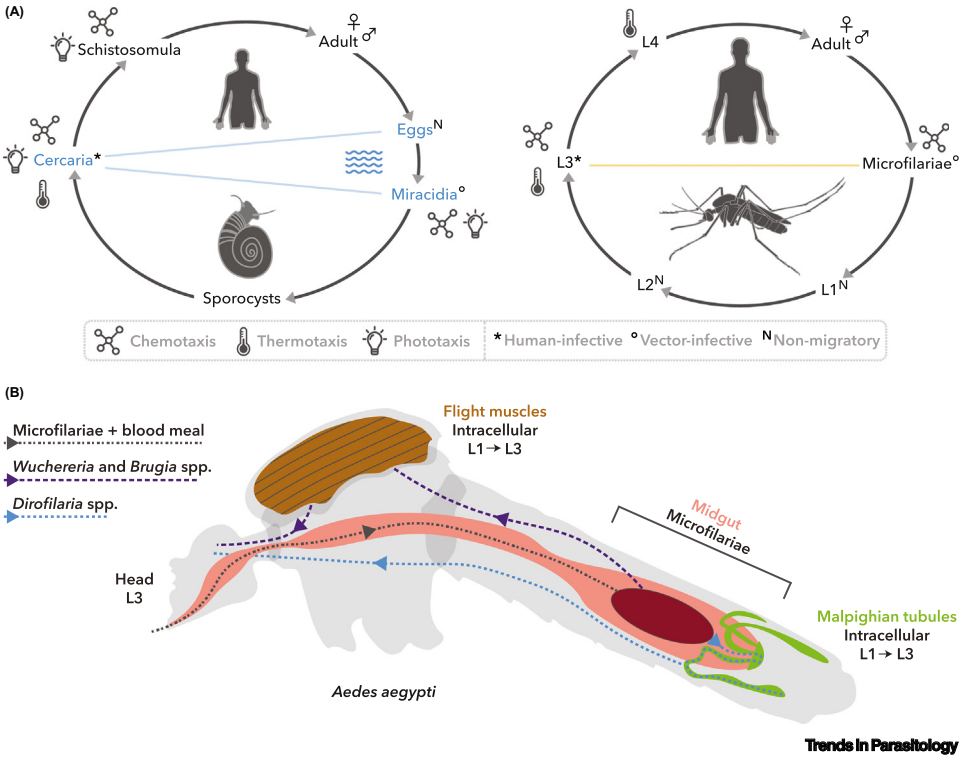

Figure 1. Vector-borne helminths exhibit stage-specific migratory patterns that are influenced by environmental signals. (A) The life cycles of human schistosomes (left) and mosquito-borne human filarial parasites (right) with nonmigratory stages annotated. Environmental signals known to be sensed by each stage are symbolized. (B) Different species of mosquito-borne filarial nematodes show divergent migrations within the same mosquito host (*Aedes aegypti*), suggesting that the nematodes rely on sensory-driven navigation for intra-host migration.

Glossary

Cercaria (*pl.* cercariae): the third larval stage of schistosomes; it swims in water and infects humans.
CRISPR/Cas9-mediated targeted mutagenesis: a technology that allows for the introduction of precise mutations in a genome.
Cyclic nucleotide-gated (CNG) channels: ion channels that are activated by cyclic nucleotides; they are often involved in the transduction of sensory signals.
ERK/MAPK: widely conserved kinases typically involved in intracellular signaling initiated by an extracellular cue.
FES: tyrosine kinase conserved throughout the animal kingdom.
G-protein-coupled receptors: a large superfamily of cell-surface receptors; they have seven transmembrane-domains, and some function as chemoreceptors in nematodes and other metazoa.
Gene drive: a technology that drives a selfish genetic element through a population; it can be used to replace or suppress populations of disease vectors.
Gonochoristic: refers to a system involving two sexes.
Guanylyl cyclase (GC): an enzyme that produces cyclic guanosine monophosphate, a second messenger that is often downstream of a cell-surface receptor's activation; some nematode GCs are direct receptors for sensory stimuli.
Malpighian tubules: the mosquito's functional analog of the kidney, involved in regulation of water and ions.
Microfilaria (*pl.* microfilariae): the mosquito-infectious, blood-dwelling stage of filarial nematodes.
Microfluidic devices: polydimethylsiloxane (PDMS) chips that precisely control the flow of fluid, used in a diverse range of bioengineering approaches.
Miracidium (*pl.* miracidia): the first larval stage of schistosomes; it swims in water and infects snails.
Neglected tropical disease (NTD): infectious diseases categorized by the WHO as 'neglected' due to a lack of economic incentive for research investment.
Opsin: a conserved type of GPCR that primarily senses photons.
PKC: a serine/threonine kinase often activated by calcium or diacylglycerol

by positive phototaxis, negative geotaxis, and chemical cues [3–5]. Miracidia respond to chemical cues that result in the accumulation of larvae near snail hosts or snail-conditioned water (SCW), though the movement patterns differ by species [6–10].

Fractionation of SCW identified macromolecules, including glycoconjugates and proteins, that stimulate these behaviors in miracidia of *S. mansoni* and other schistosomes [6,8]. Sensation of these macromolecules increases the rate of change of direction and the turnback response, and stimulates repeated investigation, attachment, and penetration of the host after contact [7]. Other simpler cues, such as salt ions, produce a similar behavior [11], but it is hypothesized that macromolecule recognition is the dominant stimulus for miracidial taxis [6]. Recently, multiple SCW-derived peptides were shown to recapitulate these chemosensory behaviors, although it is unclear if these are the same cues described in earlier studies [9,10].

S. mansoni miracidia penetrate snail hosts nearly indiscriminately along the exposed tissues [12]. After penetrating, miracidia shed their ciliated plates and begin to develop into mother **sporocysts** (mSps). The musculature of mSps degenerates within 48 h of penetration, limiting migratory capacity, but their daughter sporocysts (dSps) show directed migration through the loose connective tissue to the hepatopancreas [12]. Aberrant migrations do occur, resulting in degenerate dSps that yield few developing cercaria [12]. *S. mansoni* cercariae emerging from dSps can be found in several hemolymph-filled sinuses and veins, much less frequently in arteries and other ducts, and they preferentially emerge from the pseudobranch and mantle collar [12]. Thus, dSps and cercariae show intramolluscan migratory preferences that may involve sensory behaviors, but the experimental intractability of these stages has hindered study of migratory processes.

Cercariae shed from the snail tissue into fresh water, after which they use a complex mixture of strategies to find a mammalian host. Like miracidia, cercariae do not feed and rely on glycogen stores that last for 24–36 h [13]. *S. mansoni* cercariae will naturally alternate between an active phase involving tail-first upward swimming and a passive phase involving downward sinking with the tail slowing its speed [14]. A combination of this bimodal motility with thermotaxis, phototaxis, and geotaxis enables cercariae to maintain a preferred vertical position in the water column [15,16]. *S. mansoni* cercariae are stimulated to attach to a surface by physical cues associated with large mammals moving through water – namely, increased water flow and shadows – in addition to chemical cues. Fatty acids, L-arginine, peptides, ceramides, glucosylceramides, and phospholipids associated with host tissue all play a role in stimulating the complex behaviors that cercariae exhibit during host identification and penetration [16].

Cercariae shed their tails during penetration of the mammalian host and transform into juvenile **schistosomula**. *S. mansoni* schistosomula burrow through the epidermis and dermis by negative phototaxis (in stark contrast to the positive phototaxis of cercariae) and attraction to glucose, L-arginine, and peptides [17,18]. The vast majority of *S. mansoni* schistosomula enter the vasculature through dermal venules (although aberrant migration through lymphatics and lymph nodes does occur) and passively traverse through venous blood flow to the lungs, through the pulmonary veins into the systemic circulation, and finally via several possible routes to the hepatic portal system [19,20]. It seems that no directed taxis is needed once in the systemic circulation, and entrance into the hepatic portal system occurs by chance, as an individual schistosomulum can traverse the pulmonary–systemic circuit multiple times and will passively arrive at the liver over the range of several weeks postinfection [19,20].

Schistosomes are unique among the trematodes in that they are **gonochoristic**, and male and female adults must physically pair to produce offspring. Opposite-sex worms pair in hosts

downstream of a G-protein-coupled receptor.
Platyhelminth-specific, rhodopsin-like orphan family (PROF): a family of G-protein-coupled receptors that have unknown function but have been hypothesized to be involved in chemosensation.
Receptor tyrosine kinase: a single transmembrane domain receptor that has an intracellular kinase domain.
Schistosomulum (*pl.* schistosomula): the fourth larval stage of schistosomes; it resides in humans and migrates through blood.
Sporocyst: the second larval stage of schistosomes; it resides in snails, where mother sporocysts asexually reproduce to generate daughter sporocysts.
Transient receptor potential (TRP) channels: ion channels that are activated by diverse mechanisms; they are often involved in sensory detection.

experimentally infected with only three cercariae, emphasizing a remarkable ability to locate compatible mates [21]. Schistosomes can readily pair with other species, which has led to the proliferation of hybrid species in the wild, but some species show homospecific mate preference and heterospecific pairs will change partners to accommodate homospecific pairs [22,23]. Further, laterally bisected male carcasses can pair with viable females, stimulating oocyte maturation and embryogenesis [24,25]. Recent investigations have revealed the long-sought molecular controller of this male-dependent development – the dipeptide β-alanyl-tryptamine, produced by a nonribosomal peptide synthetase (NRPS) that is induced by the transcription factor GLI1 after pairing [26]. NRPS is synthesized in ciliated sensory neurons in the gynecophoral canal of paired male schistosomes, but the initial sensory stimulant, whether it be mechanical, chemical, or other, has yet to be uncovered [26]. Adult *S. mansoni* individuals exhibit 'chemo-orientation' and attraction toward each other or toward excretion/secretion products [27–32]. These observations suggest that pairing and development are regulated by both mechanical and chemical cues. Once paired, adult *S. mansoni* individuals migrate through the mesenteric circulation in a manner that appears to be guided by the males [21,33].

Sensation in filarial nematodes

Lymphatic filariasis (LF) is a mosquito-borne NTD that affects over 30 million people throughout 81 countries [1]. High worm burdens and chronic infection lead to physical disfigurement and disability resulting from the swelling and thickening of limbs (lymphedema) and scrotal tissues (hydrocele). These clinical manifestations result from dysfunction of the lymphatics, where adult worms reside. The etiological agents of LF in humans include the clade III nematodes *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. Although *B. malayi* accounts for less than 10% of global LF infections, it serves as the primary experimental model for human lymphatic filariasis, as the entire *B. malayi* life cycle can be maintained in the laboratory using jirds and susceptible substrains of *Aedes aegypti*.

Microfilariae of *Wuchereria* and *Brugia* spp. circulate in peripheral blood and are ingested by female mosquitoes during blood feeding (Figure 1A). In susceptible mosquito strains, these sheathed pre-L1 larvae passively move through the esophagus and arrive in the midgut. Within a few hours, microfilariae escape the blood and traverse the midgut epithelium to invade the hemocoel [34]. Although there are no studies of the sensory potential of *Brugia* spp. and *W. bancrofti* microfilariae, microfilariae of other filarial species have been shown to sense vector saliva [35]. It is possible that microfilariae move toward the midgut epithelium through sensing of epithelium-derived molecules or tactile cues that differentiate blood, midgut lumen, and the epithelial barrier. Indeed, sensory dysregulation of *Brugia* microfilariae resulted in fewer larvae establishing a successful mosquito infection [36]. The potential sensory crosstalk between microfilariae and mosquito microbiome constituents, in addition to endogenous host cues, has not been explored but presents an intriguing ecological question that could have ramifications for vector competence and host–parasite interactions [37].

The likelihood that midgut escape of *Wuchereria* and *Brugia* microfilariae is sensory driven is strengthened by contrasting their migration paths with that of the dog heartworm *Dirofilaria immitis* (Figure 1B). *D. immitis* microfilariae do not leave the mosquito alimentary canal, but instead migrate to the **Malpighian tubules**. Microfilariae of all three genera are approximately the same size but follow different migration routes within the same *Ae. aegypti* strain (Figure 1B) [38,39], strongly suggesting that the microfilariae actively migrate to distinct microenvironments. In addition, *D. immitis* moves against the flow of tubule contents and shows a preference for the distal tip, again suggesting that migration into the Malpighian tubules is driven by active taxis. Further, filaria larvae tend not to occupy all tubules, increasing the odds of mosquito survival and

perhaps indicative of a sensory preference (B. Christensen, personal communication). However, alternative explanations for the differences between migratory routes of microfilariae could involve differences in enzyme secretions that promote midgut penetration in *Brugia/Wuchereria* but not *Dirofilaria*, the fact that *Dirofilaria* microfilariae are unsheathed and possibly incapable of puncturing the midgut, or differences in the cuticular surface of the microfilariae that lead to unique biochemical or immunological interactions with the host [40].

In susceptible mosquitoes, microfiliariae ascend into the indirect flight muscles of the thorax, where *Brugia* spp. show a preference for the dorsal-longitudinal fibers and undergo intracellular development [41]. Confined within single muscle cells, larvae molt twice and grow to reach the human-infective L3 stage. L3s break out of the muscles and migrate to the lumen of the mouthparts. Migration through the body cavity to the head and proboscis begins roughly 8 days postinfection and represents the final journey within the mosquito [42]. Most L3s will accumulate in the head region, but larvae can still be found in other regions within the mosquito body cavity [42]. These large and motile L3s move throughout the hemocoel, and it is probable that their preference for the head is mediated by an anterior–posterior chemical gradient.

The mechanisms by which L3s exit from the head of the mosquito onto the skin of the mammalian host are poorly understood. Large numbers of larvae rapidly escape the mouthparts during the act of mosquito probing and blood feeding [43]. It is hypothesized that parasite release is mainly stimulated by the mechanical bending of the mosquito labium, with other factors such as temperature, humidity, and components of host blood playing a minor role [44]. Both *Brugia* and *Dirofilaria* L3s will emerge from infected mosquitoes when submersed in warm, nutrient-rich media [45], suggesting that mechanical bending of the labium is not necessary for proboscis escape. However, the relative importance of mechanical bending, thermal cues, and chemical cues during a blood meal are unknown. L3s that escape from the proboscis are dropped onto the skin in a small volume of hemolymph and must then actively penetrate host skin [46–48]. Both chemosensory and thermosensory cues appear to be important for the process of skin penetration. For example, exposure of *Brugia pahangi* L3s to mammalian serum results in chemotaxis toward the serum and skin-penetration behavior [49,50], and this phenotype is inhibited by chemical treatment or reverse genetics against putative sensory effectors [36]. In the case of *Brugia*, L3s exhibit a mixture of aversive and attractive responses to a panel of vector and skin-associated cues, and exposure to human skin cells increases larval motility [51–55]. Host body temperature also stimulates increased L3 motility in *B. pahangi*, *W. bancrofti*, and *D. immitis* as well as positive thermotaxis in *D. immitis* [36,46,56]. These results strongly suggest that orientation toward and penetration into skin are active sensory-driven processes. A plausible model for L3 sensation during penetration proposes sodium ions from blood as the primary attractant, supplemented by thermal cues and amino acids such as arginine [53].

After infection of the human host, larvae migrate to the lymphatics where they develop into sexually dimorphic adult worms over the course of months. A recent investigation of the route of infection of the filarial parasite *Litomosoides sigmodontis* in rodents carefully tracked the early migrations of larvae [57]. Unlike *Brugia* and *Wuchereria*, which remain in the lymphatics, *L. sigmodontis* enters the lymphatics and continues to the pleural cavity. *L. sigmodontis* larvae mechanically break the basement membrane of lymphatic collectors and use their tail to push off lymphatic walls and valves to achieve translational motion. Larvae travel in the same direction as fluid flow *in vitro*, respond positively to this flow, and progress through a microfluidic chamber in a way that cannot be explained by fluid flow alone. Interestingly, free-living nematodes are also able to invade and translate down the lymphatics, and the lymphatics are a known route for systemic dissemination for nonfilarial nematode parasites such as *Strongyloides stercoralis*

[57]. It is clear that larvae are active during this process, but unclear how much of that activity is coordinated by sensory cues.

A role for sensory processes in mating and copulation in filarial nematodes has not been investigated. Clade V nematodes synthesize and secrete ascarosides, which are involved in chemoreceptor-mediated mate attraction [58], but ascarosides have yet to be isolated in filarial nematodes, and they are notably absent from another clade III nematode (*Ascaris suum*) [59]. Sensory neurons expressing chemoreceptors and mechanoreceptors are present in the caudal regions near the sexual organs of some male nematodes, and some chemoreceptors are expressed in the posterior portion of *B. malayi* males [36,60]. Extracellular recordings from *B. pahangi* adults revealed that they show increased neural activity in the presence of fetal calf serum and glutathione, suggesting that adults are capable of responding to host chemosensory cues [61].

Sensory structures and molecular pathways

Descriptions of schistosome sensory behaviors have been aided by the ability to observe the aquatic stages in laboratory contexts that better reflect natural environments, while the host-restriction of the filarial nematode life cycle complicates such investigations. In contrast, much more is known about nematode sensory structures and pathways, largely owing to the extensive study of sensory biology in the model nematode *Caenorhabditis elegans*. While observational studies and information gleaned from model systems set a foundation, the molecular pathways and physical structures that control sensory behaviors in schistosomes and filarial nematodes are an open area of investigation.

Schistosomes

Schistosomes undergo significant morphological changes during development, and sensory structures are not well characterized. However, schistosomes contain many types of papillae that are spread throughout the body but often concentrated on the anterior portion. These structures contain nerve endings, cilia, and/or tegumental openings, and likely provide sensory function [62–66]. Recently, staining of the secreted protein SmK77 in cercariae was found to specifically demarcate the peripheral nervous system, which included bulbous sensory endings on the apical surface [67]. Similar markers of sensory structures in other stages have yet to be identified.

There are a few leads regarding the schistosome receptors and effectors involved in described sensory responses (Figure 2). Several kinases (**ERK/MAPK**, **FES**, and **PKC**) have been implicated in larval responses to different environmental stimuli, suggesting that **receptor tyrosine kinases** and **G-protein-coupled receptors** (**GPCRs**) may serve as upstream receptors [68,69]. SmHSP70 may be involved in the skin-attachment response of cercariae, and the second messenger cAMP is important in miracidia motility and chemokinesis [70,71].

Flatworms express a clade of GPCRs, the **platyhelminth-specific rhodopsin-like orphan family** (**PROF**), that show taxon-restriction, expansion relative to other GPCR families, and neuropeptide receptor-like features [72,73]. PROFs are structurally similar to the nematode chemosensory GPCR family *srw* [74,75], but whether this similarity reflects a conserved chemosensory function is unknown. The implication of L-arginine, peptides, and intracellular cAMP in miracidial and cercarial sensory behaviors point to peptide-sensing GPCRs as potential chemosensory pathway initiators [70]. Trematodes also express **opsin**-like GPCRs that are likely to be involved in the phototaxis of cercariae and miracidia [72,73].

Transient receptor potential (TRP) channels are another potential player in sensory behavior, as in other animal systems. TRP channels have been studied in schistosomes as putative drug targets and as a molecular target for the antischistosomal drug praziquantel [76–78]. Schistosomes

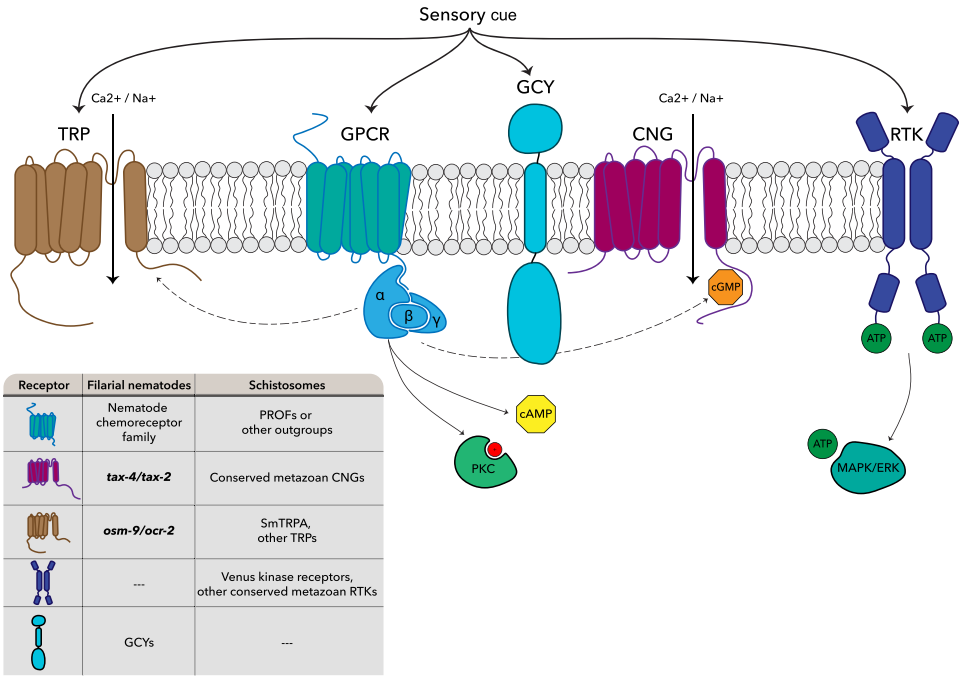

Figure 2. Molecular sensory signaling pathways in vector-borne helminths. The pathways in nematodes are better modeled, but only the CNGs and TRPs (bold and italic) have been definitely shown to function in sensation in filarial nematodes. The sensory effectors in schistosomes are hypothetical and require experimental validation. Abbreviations: CNG, cyclic nucleotide-gated; GCY, guanylyl cyclase Y; GPCR, G-protein-coupled receptor; MAPK/ERK, mitogen-activated protein kinase; PKC, extracellular signal-regulated kinases; PROF, platyhelminth-specific, rhodopsin-like orphan family; RTK, receptor tyrosine kinase; TRP, transient receptor potential.

have lost the TRPV family that is associated with sensation in nematodes and other metazoans, but it is possible that these functions have been co-opted by other TRP subfamilies. For instance, mammalian TRPV1 agonists have effects on SmTRPA and ShTRPA, which are TRPA1-like channels, and agonists including host-derived compounds significantly increased *in vitro* motility of schistosomula and adults and activated these channels when heterologously expressed [79,80]. In *Schmidtea mediterranea*, a free-living flatworm, *Smed*-TRPA1 functions in noxious heat and chemical avoidance, but it is unclear if this function is shared in schistosomes [81].

Filarial nematodes

The canonical nematode nervous system is substantially different from that of flatworms, and more is known about its function. Like all nematodes, filarial parasitic nematodes have sensory amphids [52]. These organs contain ciliated neurons with dendrites extended into pores, where they receive environmental cues. The neuroanatomical structure of amphids is incredibly conserved across nematode clades, with *Ascaris* (a clade III intestinal parasite related to filarial nematodes) containing positional homologs of most of the *C. elegans* amphid neurons [82]. Other somatosensory neurons are found along the body wall, and some nematodes, including *Brugia*, contain phasmids at the cephalic end. The phasmid neurons of *C. elegans* are polymodal, responding to temperature, chemicals, osmolarity, and touch.

The chemosensory transduction pathways of *C. elegans* are well-studied (Figure 2). Emerging evidence suggests that the primary components of these pathways – GPCRs, ion channels such as TRP channels and **cyclic nucleotide-gated (CNG) channels**, and **guanylyl cyclases**

(**GCs**) – are shared among diverse parasitic and free-living nematodes [83–85]. For instance, filarial nematodes share most of the key CNG channels and TRP channels such as TAX-2/TAX-4 and OSM-9/OCR-2 [36]. However, the chemoreceptive GPCRs are greatly diverged in parasites, including filaria, and parasites in general have fewer chemoreceptors than their free-living counterparts [36,86,87]. Indeed, there is a strong correlation between the number of chemoreceptive GPCRs in a nematode's genome and the amount of time it spends in a free-living, terrestrial state during its life cycle [36]. While *osm-9* and *tax-4* have been linked to chemosensory and thermosensory transduction in filarial nematodes, the chemoreceptive GPCRs have yet to be studied experimentally [36].

Other nematode sensory modalities (e.g., thermal and mechanical) often use the same or similar components as the chemosensory pathway, allowing integration of multiple stimuli [88]. For example, GCs, which can operate downstream of chemoreceptors, can receive thermal cues directly and synthesize cGMP to activate TAX-2/TAX-4 [85,89], and TRPs can be primary mechanosensory receptors [90,91].

Sensory-mediated migration as a unique target for parasite control

Current strategies for treatment and transmission control of both schistosomiasis and LF rely almost entirely on chemotherapeutics, primarily through mass drug administration (MDA). Vector control of mosquitoes and snails has been attempted in some regions and has been lauded as a key effort for elimination of both diseases, but success thus far has been sporadic [92,93]. Conversely, inhibiting or disorienting sensation has not been considered as an exploitable behavior. In fact, overlooking the ability of schistosomes to host seek has resulted in at times misguided efforts for parasite control through the introduction of decoy snails.

Some successful non-MDA strategies that focus on prevention may be instructive and provide a foundation for developing schemes targeting sensation. In veterinary medicine, canine heartworm is prevented by the regular administration of small molecules that inhibit the development of larval filaria after transmission from the mosquito to the definitive host [94]. Routine disbursement of the drugs active against early larvae ensures that disease is never established, and adult worms do not develop and reproduce. Dysregulation of early-infective processes that require sensation, such as larval invasion and migration to the blood/lymphatics, in a strategy analogous to the control of heartworm disease could be used. While such a strategy would not be feasible using MDA given the requirement of drug administration at precise developmental time points, it may be pertinent in hotspots that have proven to be recalcitrant to MDA, or as one part of a combination therapy.

Schistosomiasis hotspots may also be areas in which an alternative sensation-targeting strategy might prove to be effective. In these areas, snail population reduction via molluscicide treatment, habitat reduction, or **gene drives** has been encouraged by public investigators, and chemical-based snail control is a strong recommendation in the WHO's 2022 guidelines on control and elimination of human schistosomiasis [95–98]. A better understanding of the process by which miracidia seek and infect snails could lead to the discovery and development of compounds that block these processes. Alternatively, if transgenesis and gene drives in snails come to fruition, it may be possible to generate 'odorless' snails, imperceptible to miracidia. Indeed, studies suggest that a major attractive component of snail secretions is a glycoprotein, which could conceivably be knocked-out via genetic strategies [9,10].

Technological advancements will allow deeper understanding of sensation in vector-borne helminths

CRISPR/Cas9-mediated targeted mutagenesis has recently been applied to filarial nematodes and schistosomes to study nonsensory processes [99,100]. This approach has already

been used to study the molecular basis of chemosensation and thermosensation in the skin-penetrating gastrointestinal nematode *S. stercoralis* [101]. In the first of such studies, the role of the *S. stercoralis tax-4* gene in driving the host-seeking behaviors of infective larvae was investigated. Like *C. elegans tax-4*, *Ss-tax-4* is required for temperature- and odorant-driven host seeking [36,84,102,103]. Thus, sensory-driven host seeking in some parasitic nematodes occurs at least in part through the use of sensory pathways that are conserved in free-living nematodes [36,84,102,103]. It remains to be seen if new approaches for targeted mutagenesis can be leveraged in schistosomes and filarial nematodes to functionally characterize additional sensory effectors. These approaches would focus on life stages that have readily deployable *in vitro* sensory assays (L3s in filarial nematodes, aquatic stages in schistosomes), and would therefore require adaptation of current knock-out strategies to make mutations heritable (not yet demonstrated in schistosomes) or allow for propagation of the mutants through the vector (not yet performed in filarial nematodes).

Even amid exciting developments in genome editing of vector-borne helminths, transient gene knockdown strategies like RNA interference (RNAi) remain crucial and exhibit certain advantages over permanent gene knockout. RNAi has been used recently in larval stages of schistosomes and filariae to investigate sensory behaviors [36,69]. The transient nature of RNAi enables the study of genes that may be essential in other life stages and thus unamenable to permanent knockout. This limitation in genome editing is especially important to note for studies of sensory behaviors, which are integral to parasite success. Indeed, *S. stercoralis* infective larvae with a homozygous deletion of *tax-4* do not activate or exit developmental arrest upon exposure to host sensory cues [84]. In the case of *Strongyloides* species, mutations in genes such as *tax-4* that are essential for progression through the entire life cycle can be maintained in the laboratory by propagating the worms through mammalian hosts as heterozygotes and then mating heterozygous free-living adults to generate homozygous infective larvae [104]. However, this approach may not be feasible for helminths that lack a free-living generation. An inducible knockout approach like the auxin-inducible degron system in *C. elegans* could also circumnavigate this limitation and provide temporal control of protein expression, but such a system has not yet been adapted to parasitic helminths [105].

These functional genomic tools can also be used to knock out potential sensory stimulants emitted by the vectors. Genome editing and RNAi is routine in mosquitoes and continues to be further developed in mollusks. Since most putative chemical stimulants of parasite sensory behaviors are not proteins, this approach would require targeting biosynthetic pathway components rather than the sensory cue itself. However, *S. mansoni* miracidia are highly responsive to a snail peptide, and RNAi could be used to knock down its expression [9,10].

Microfluidic devices will also benefit the study of the sensory behaviors of both schistosomes and filarial parasites by increasing reproducibility and throughput. The only chemotaxis assay that exists for filarial nematodes is restricted to the L3 stage and relies on agarose plates that do not resemble the environments that infective larvae naturally encounter [50], and those used for aquatic stages of schistosomes are not easily scalable [9,106,107]. Microfluidic devices can be used to present aqueous environments and physical surfaces that can better scaffold parasite motility [57]. Designs can be extended to generate simple chambers to create stable gradients and measure parasite translational movement toward an attractant or away from a repellent [108]. Alternatively, devices can restrain the body of the parasite while allowing free swinging and choice of the head [109]. Chemosensory choice chambers could be readily adapted for schistosome miracidia and cercaria, which are small and motile in aquatic environments, but filarial larvae and larger adult stages will likely necessitate more complex approaches. The pairing

of high-content phenotypic assays with functional genomic tools such as CRISPR will enable the discovery of pathways that underlie the critical chemosensory behaviors of these important vector-borne parasites.

Most sensory assays rely on manual tallying of experimental subjects in regions of interest followed by the calculation of single metrics such as the chemotaxis index. Video recording of assay chambers with aqueous environments has been performed, but measurement of behaviors from these videos do not always provide readily interpretable quantitative outputs, and video platforms often capture only part of the chamber. While many trackers have been utilized for worms that readily crawl on an agarose plate (e.g., [103,110]), these have not been easily translated to either filarial nematodes or schistosomes. Advancements in imaging now allow for the unmagnified, high-resolution recordings of assay chambers in precisely controlled environments [111,112]. Paired with object tracking and machine learning, these platforms show great promise in capturing, annotating, and quantifying complex sensory behaviors.

Finally, new technologies will also enable the identification of sensory cells in vector-borne helminths. Recently, single-cell atlases of adult and juvenile *S. mansoni* have been generated, enabling the annotation of transcriptionally distinct clusters of cell types [113–115]. These data identified clusters of ciliated neurons in adult *S. mansoni* that display structural features consistent with sensory function. Probing neuronal clusters show that PROF receptors are mainly expressed in neurons, but none are enriched in ciliated neurons. In contrast, a TRP channel (*Smp_130890*) was highly enriched in ciliated neurons [116]. Interestingly, this TRPM protein is the protein most closely related to a target of praziquantel, $TRPM_{PZQ}$ [117]. Deeper analyses of these cells' transcriptomes will likely uncover additional putative chemoreceptors, and experimental selection for neuronal cells or integration with spatial information will greatly enrich these studies [118,119].

Outstanding questions

What are the molecular and neural pathways that mediate flatworm sensory behaviors?

To which host-derived cues do filarial nematodes respond in the natural course of infection and migration?

Can sensory assays used to study vector-borne helminths be developed to enable high-content behavioral assays that better reflect their natural environments?

Is sensation an exploitable process for parasite control and treatment?

Concluding remarks

Vector-borne helminths engage in sensory-driven behaviors at multiple stages of their life cycle, both inside and outside the host. Recent functional genomic advances across parasitic helminths have begun to illuminate the molecular mechanisms that drive these behaviors. Many questions remain about the underlying neural mechanisms and the potential for translating knowledge of these behaviors into real opportunities for parasite control (see Outstanding questions). Even if not directly exploited in control strategies, understanding the basic biology of vector-borne helminth sensation could better inform mathematical modeling efforts that explore infection dynamics and the potential results of altered treatment and control regimens. New technologies for probing the functions of genes, neurons, and circuits in parasitic helminths hold great promise for answering these questions in the coming years. Though sensory behaviors in the wild often occur in macroscopic spatial contexts, miniaturized assays could be developed that enable high-content approaches, providing a means by which sensation can be examined with greater throughput and resolution. A toolkit that includes high-resolution neural and behavioral analyses, experimental frameworks that accurately mimic *in vivo* sensory stimuli, and a panoramic perspective that places parasites in the context of their real-world hosts will cooperate to build a comprehensive model of these host–parasite interactions.

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Declaration of interests

The authors declare no competing interests.

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