Chemotactic and temperature-dependent responses of the Strongyloidoidea superfamily of nematodes

Alex Dulovic[1,2] (iD), Mat Norman[1,3], Dorothee Harbecke[1] and Adrian Streit[1] (iD)

[1]Max Planck Institute for Developmental Biology, Tübingen, Baden Württemberg, Germany; [2]NMI Natural and Medical Sciences Institute at the University of Tübingen, Tübingen, Baden Württemberg, Germany and [3]UBC Faculty of Medicine, Vancouver, British Columbia, Canada

Abstract

Host-seeking behaviour and how a parasite identifies the correct host to infect remains a poorly understood area of parasitology. What is currently known is that host sensation and seeking behaviour is formed from a complex mixture of chemo-, thermo- and mechanosensory behaviours, of which chemosensation is the best studied. Previous studies of olfaction in parasitic nematodes suggested that this behaviour appears to be more closely related to target host and infection mode than phylogeny. However, there has not yet been a study comparing the chemotactic and temperature-dependent behaviours of very closely related parasitic and non-parasitic nematodes. To this end, we examined the temperature-dependent and chemotactic responses of the Strongyloidoidea superfamily of nematodes. We found differences in temperature response between the different species and within infective larvae. Chemotactic responses were highly divergent, with different attraction profiles between all species studied. When examining direct stimulation with fur, we found that it was insufficient to cause an attractive response. Overall, our results support the notion that olfactory sensation is more closely related to lifestyle and host range than phylogeny, and that multiple cues are required to initiate host-seeking behaviour.

Introduction

Parasitic nematodes are of great agricultural, medical, veterinary and economic importance (Hotez *et al.*, 2008), yet despite this relatively little is known about how parasitic nematodes identify and infect their hosts. Growing evidence suggests that chemosensation and other senses such as thermosensation and mechanosensation play important roles in nematode parasite transmission and intra host migration (Dillman *et al.*, 2012; Tsubokawa *et al.*, 2017; Bryant *et al.*, 2018). Critical to this process is olfactory sensation, which is also important for and best studied in non-parasitic nematodes, whereby nematodes sense carbon dioxide or other volatile chemicals which attract them towards the correct host, invasion area or food source, or repel them from undesired targets (Bargmann, 2006; Hallem *et al.*, 2007; Hallem and Sternberg, 2008; Hart and Chao, 2010; Castelletto *et al.*, 2014; Hong, 2015).

The Strongyloidoidea superfamily (Hunt *et al.*, 2016) of nematodes contains the important human and veterinary parasitic *Strongyloides* nematodes (Schär *et al.*, 2013; Beknazarova *et al.*, 2016), the facultative parasitic *Parastrongyloides* nematodes (Kulkarni *et al.*, 2013) and the free-living *Rhabditophanes* nematodes (Dulovic *et al.*, 2020). The phylogeny of this superfamily can be seen in Fig. 1A. Members of the *Strongyloides* genus are of great research interest due to their life cycle and other biological features, like the availability of closely related culturable facultative and non-parasitic species (Viney and Lok, 2007; Streit, 2014, 2016; Streit *et al.*, 2016; Viney, 2017). Within *Strongyloides*, species often have a narrow range of possible host species which they are capable of infecting, yet the susceptible species do not need to be phylogenetically closely related (Eberhardt *et al.*, 2008; Jaleta *et al.*, 2017; Nutman, 2017). A general overview of the life cycles of all species used in this paper can be found at (Eberhardt *et al.*, 2007; Viney and Lok, 2007; Kulkarni *et al.*, 2013; Dulovic *et al.*, 2020) and Fig. 1B. Of note, is the fact that the parasitic generation of *Parastrongyloides trichosuri* (*P. trichosuri*) is dioecious (unlike in *Strongyloides* spp.) and it is therefore possible to generate a high, if not unlimited, number of consecutive free-living generations on agar plates (Grant *et al.*, 2006; Kulkarni *et al.*, 2013), whereas *Rhabditophanes diutinus* (*R. diutinus*) is free-living, reproduces asexually and can form dauer larvae as opposed to infective larvae (Dulovic *et al.*, 2020).

In a landmark paper (Castelletto *et al.*, 2014), Castelleto *et al.* performed a comparative analysis of the thermotaxic and chemotactic responses of various parasitic, entomopathogenic and free-living nematodes. This study compared closely related parasitic and entomopathogenic species with similar life cycles but different hosts and phylogenetically remote species either with similar life cycles and ecology or differing life cycles and ecology, finding that parasites with similar hosts respond similarly to odorants. Here, we decided to complement this extensive study with an analysis of phylogenetically close nematodes of the Strongyloidoidea superfamily, which have different life cycles and ecology. Previous research within this superfamily has found that urocanic acid is a strong chemoattractant in *Strongyloides stercoralis*
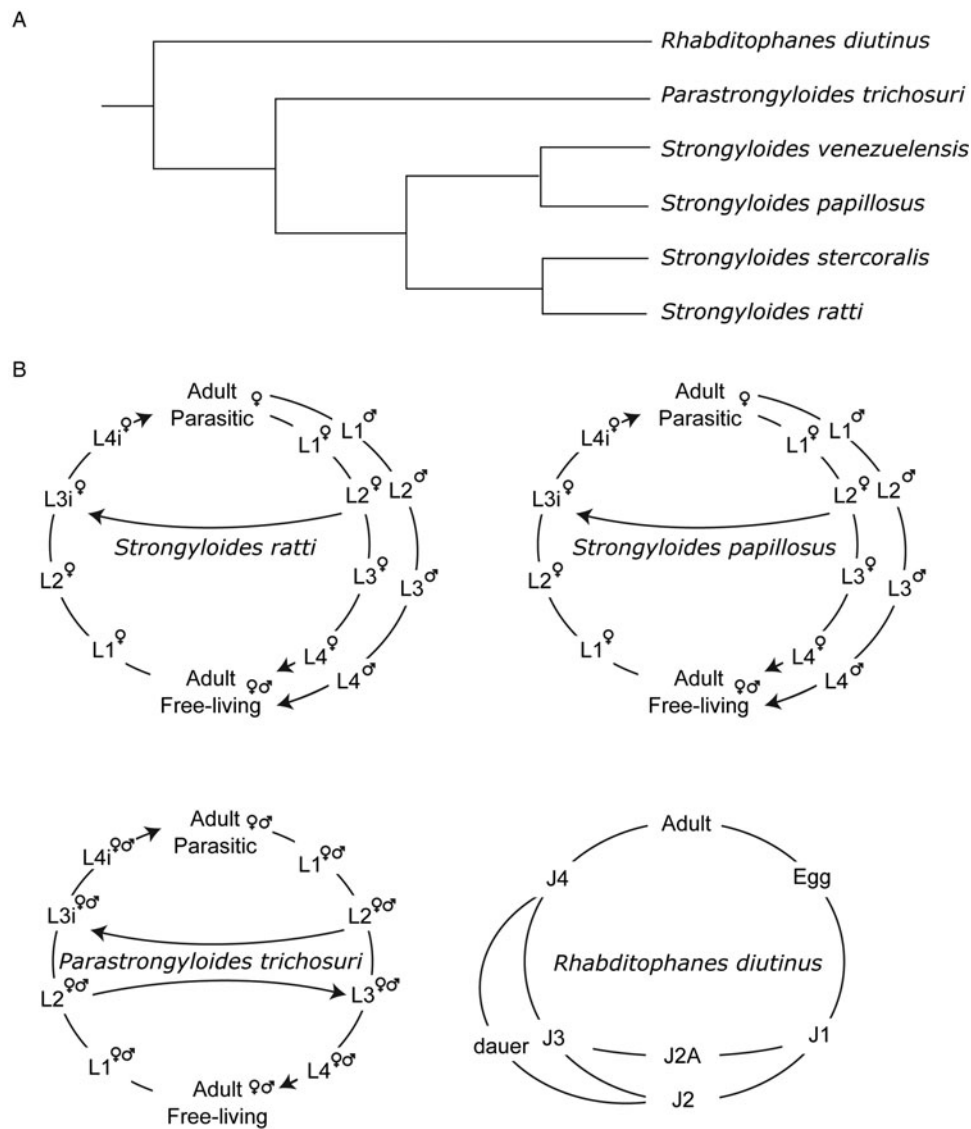
Fig. 1. Phylogeny and life cycles of the Strongyloidoidea. (A) Proposed phylogenetic relationship of the Strongyloidoidea based on whole genome sequence, according to Hunt *et al.* (2016). Only the topology is given, the branch lengths in the figure are arbitrary. (B) Life cycles of Strongyloidoidea. Life cycles of *S. ratti*, *S. papillosus*, *P. trichosuri* and *R. diutinus*. i indicates infective larvae.

(Safer *et al.*, 2007), while CO_2 was shown to be a repellent in contrast to many other parasitic nematodes (Castelletto *et al.*, 2014). Differences in olfactory behaviour between *S. stercoralis* and *S. ratti* have been reported (Safer *et al.*, 2007; Castelletto *et al.*, 2014), suggesting that host-seeking behaviour even between these two closely related species is rather different. Using the four species (*S. ratti*, *S.papillosus*, *P. trichosuri* and *R. diutinus*) of the Strongyloidoidea family that are in permanent culture in our lab, we set out to characterize temperature-dependent and chemotactic behaviours within this family, orienting our experimental approaches after (Castelletto *et al.*, 2014).

Materials and methods

Species and strains

Strongyloides ratti (strain ED321) was originally a gift from Mark Viney, University of Bristol, now University of Liverpool, in 2010 and has since been maintained in the laboratory in continuous culture. *Strongyloides papillosus* (strain LIN) was isolated from sheep at the University of Hohenheim Field Station on the Swabian Alp in 2006 (Eberhardt *et al.*, 2007) and has since

been maintained in the laboratory in continuous culture. *Parastrongyloides trichosuri* was originally a gift from Robin Hirst, Ag Research, New Zealand and Warwick Grant, Ag Research, New Zealand, now LaTrobe University, Australia in 2007 and has since been maintained in the laboratory and subjected to 12 rounds of inbreeding by selecting a single gravid female in each round, resulting in strain QA414. *Rhabditophanes diutinus* (strain KR3021, previously also called *Rhabditophanes* sp. KR3021) was originally isolated by Ann Rose in 1994 in British Columbia (Dulovic *et al.*, 2020) and was sent to us by Dee Denver, Oregon State University, in 2014 and has since been maintained within the laboratory in continuous culture. *Pristionchus pacificus* (strain PS312) was a gift from Ralf Sommer, MPI for Developmental Biology and has been maintained in the laboratory in continuous culture. *Caenorhabditis elegans* (strain N2) was originally obtained from the *Caenorhabditis* Genetic Center (CGC) and has since been maintained in the laboratory in continuous culture. All *Strongyloides*, *Parastrongyloides* and *Rhabditophanes* species and strains used in this paper are available upon request. *Pristionchus pacificus* (PS312) and *C. elegans* (N2) should be requested from Ralf Sommer and the CGC, respectively.

Maintenance of Strongyloides nematodes

Strongyloides ratti are maintained within rats at our in-house animal facility, as described in Dulovic *et al.* (2016). *Strongyloides papillosus* are maintained within rabbits at our in-house animal facility, as described in Eberhardt *et al.* (2007). Infective larvae for both species were collected by Baermann funnel from water surrounding minimum 7-day-old fecal cultures.

Maintenance of P. trichosuri, R. diutinus, P. pacificus and C. elegans

Parastrongyloides trichosuri, *R. diutinus*, *P. pacificus* and *C. elegans* were all maintained on NGM plates supplemented with a lawn of OP50 bacteria (Stiernagle, 2006). *Parastrongyloides trichosuri* were maintained at 20 °C, and the plates were further supplemented with a piece of autoclaved rabbit feces (Grant *et al.*, 2006). *Rhabditophanes diutinus* was maintained at 15 °C and *P. pacificus* and *C. elegans* at 20 °C. To obtain infective *P. trichosuri* larvae, plates were incubated for 2 weeks post-worms being added, then washed off and the worms concentrated by Baermann funnel for 1 h, following which the infective larvae obtained were used directly for experimentation. To obtain *R. diutinus* dauer larvae, *R. diutinus* was grown by liquid culture. Briefly, growing plates of *R. diutinus* were washed off into S-Medium (Stiernagle, 2006) without cholesterol, supplemented with 50 μg mL Streptomycin, 50 μg mL Nystatin and 1% w/v OP50 (from fresh overnight culture that had been pelleted by centrifugation). Cultures were then incubated at 17 °C and shaken at 130 rpm for 12 days. Following this, worms were isolated from the culture by centrifugation, washed once with ddH$_2$O, and then used directly for experimentation. To obtain *P. pacificus* and *C. elegans* dauer larvae, plates were incubated for 3 weeks post-worms being added and then washed off with ddH$_2$O and concentrated by Baermann funnel for 1 h.

Dispersal assay

Dispersal assays were performed similarly to those described in Bargmann (2006) and Castelletto *et al.* (2014) with minor modifications. Following a brief pilot study, HEPES agar was determined to allow the largest range of locomotion for all species of Strongyloidoidea examined. In total, 6 cm HEPES agar plates [per litre: 20 g (2%) agar and 3 g (0.05 M) NaCl autoclaved and then supplemented with 10 mL 1 M HEPES, 1 mL 1 M MgSO$_4$ and 1 mL 1 M CaCl$_2$] were marked with a 3.5 cm inner zone, with worms then spotted to the centre of this zone. Following 1 h incubation, the total amount in the inner zone and outer zone (everything outside the inner zone including the rim of the plate) was counted. Dispersal as a measurement was then calculated as follows:

$$\text{Dispersal Index (DI)} = \frac{\text{Worms outside of inner zone}}{\text{Total worms on plate}}$$

To determine the dispersal response in response to heat stress, the experiment was repeated using a range of temperatures from 4 to 37 °C. Experiments followed the same protocol as above, except the plates were incubated at the required temperature for 1 h. Plates were tested in batches of 10 for each temperature per species, with at least two different species being tested at once. The experiment was repeated to generate three biological replicates.

Chemotactic assay

Chemotactic assays were performed similarly to those described in Castelletto *et al.* (2014) with a few changes. Firstly, 6 cm

HEPES Agar (as above) plates were used, following a pilot study to determine the best agar for the experiments. Plates were marked with 1.5 cm circles which were extended to the boundary of the plate in a rectangle (as seen in S1 Fig.). The centre of these scoring zones was then spotted with 5 μL 10 mg mL^{-1} sodium azide to act as an anaesthetic, followed by 10 μL of either the chemical being tested or a control. Worms (a minimum of 100) were then spotted in the centre of the plate and incubated for 3 h at room temperature after which the number of worms in both scoring zones was counted. A selection of odorants used within (Castelletto *et al.*, 2014) were used to allow direct comparison of results, in addition to urea. Odorants were chosen from this paper to comprise a variety of chemical classes and contain a mixture of strong attraction/repulsion or neutral profiles. All chemicals used were undiluted with the exceptions of urea (1 M in water), methyl palmitate (0.05 g in 2.5 mL paraffin oil) and palmitic acid (0.5 g in 10 mL ethanol). Water was used as a control, with the exceptions of ammonia (ethanol), methyl palmitate (paraffin oil) and palmitic acid (ethanol). When odorants were spotted on the lid, small squares (8 mm × 8 mm) of filter paper were stuck to the lid above the scoring zones using double-sided tape, onto which the odorants were spotted. The Chemotactic Index (CI) was calculated as in Castelletto *et al.* (2014) as follows:

$$\text{Chemotaxic Index (CI)} = \frac{(\text{\# Worms in Test Scoring Zone}) - (\text{\# Worms in Control Scoring Zone})}{(\text{\# Worms in Test Scoring Zone}) + (\text{\# Worms in Control Scoring Zone})}$$

As a result, a positive CI score indicates attraction while a negative CI score indicates repulsion. No minimum value was used for each scoring zone, however at least 10 worms in total had to move into the scoring zone(s) for the plate to be considered valid.

Fur assay

For fur assays, rat fur and mice fur from our in-house animal facilities were collected. Assays were performed as above with the chemotactic assay with either rat fur and mice fur, or rat fur and no fur placed in opposite scoring zones. Following this, *S. ratti* infective larvae were spotted in the centre and incubated for 1 h at room temperature. The amount of fur (if necessary) used was sufficient to cover the entire scoring zone. The number in both scoring zones and the CI calculated as above.

Fecal assay

HEPES agar plates were again marked as in the chemotaxis assay. Rat and rabbit feces were collected using the same procedure described earlier for the maintenance of *S. ratti* and *S. papillosus*, except uninfected rats and rabbits were used. Rat feces was either used freshly (i.e. on the day it was collected) or was stored for a maximum of 2 days at 19 °C prior to being used. Rabbit feces was autoclaved and stored long term at 4 °C. Both feces were warmed to RT for a minimum of 1 h prior to use. A piece of rat or rabbit feces (depending on species tested) was placed in one scoring zone and the other scoring zone left empty. Either adults or infective larvae were spotted in the middle of the plates and then incubated for 1 h at room temperature, after which the worms in both scoring zones were counted. To search for worms in the fecal scoring zone, small amounts of OP50 bacteria in
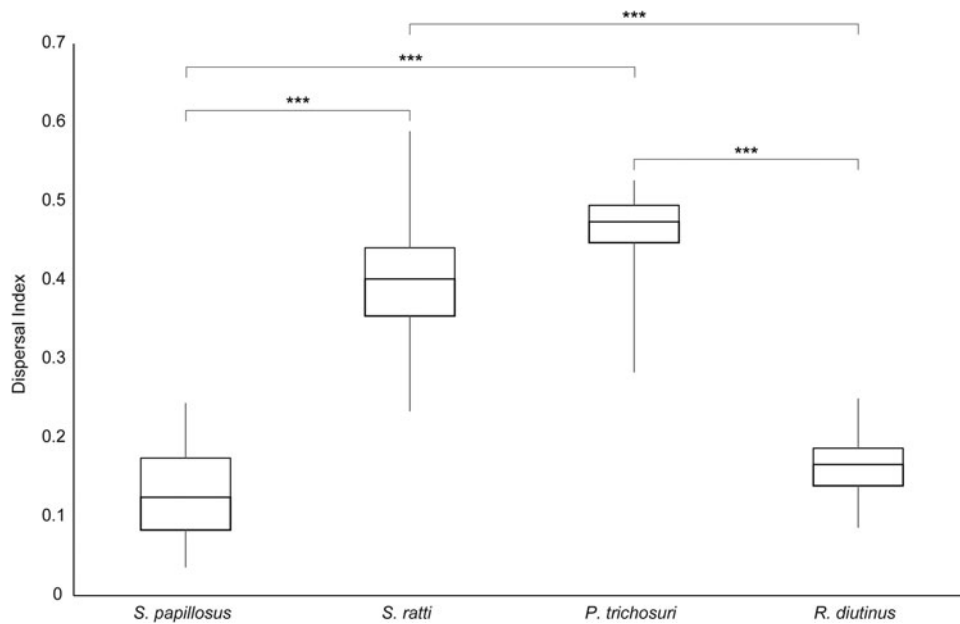
Fig. 2. Dispersal of Strongyloidoidea at room temperature – Strongyloidoidea have different dispersal profiles at room temperature (22 °C). Boxplots of dispersal on plates of *S. papillosus*, *S. ratti*, *P. trichosuri* and *R. diutinus*. For each species, plates were tested in batches of 10, with three biological replicates. Box and whisker plot shown here is produced from all 30 data points (per species), with the maximum and minimum values shown by the whiskers, and the box representing the interquartile range with the median indicated. Kruskal–Wallis H was performed to determine statistical significance between the samples. P values *<0.05, **<0.01, ***<0.001.

solution were added and the feces broken up. Fecal attraction was determined using the same calculation as for CI.

Statistical analysis and figure generation

Appropriate statistical analysis was performed using Excel or R, with figures generated using Excel and Inkscape. The exact statistical test used is stated in the figure legends where necessary. Significance was determined by P value of <0.05.

Results

Following the example of Castelletto *et al.* (2014), we first compared the locomotion activity of infective larvae/dauer larvae of the different species on agar plates in the absence of any experimental olfactory stimuli (see Materials and methods 'Dispersal assay'). We observed substantial differences in the dispersal at room temperature among the four species of Strongyloidoidea tested (Fig. 2). While *P. trichosuri* [mean Dispersal Index (DI) = 0.47] moved slightly more than *S. ratti*, *S. papillosus* (mean DI = 0.13) and *R. diutinus* (mean DI = 0.17) moved only minimally. Our results for *S. ratti* (mean DI = 0.40), the only species included in this study and in Castelletto *et al.* (2014), are very similar to the *S. ratti* results of Castelletto *et al.* (2014), confirming that our results are comparable to the results of this publication.

To assay for the effect of temperature on locomotion, and to exclude that the results presented above just reflected different temperature preferences, we examined motility at a range of temperatures from 4 to 37 °C (Fig. 3). For comparison, we also included dauer larvae of the two well-studied, phylogenetically distant, free-living nematodes *C. elegans* and *P. pacificus* in the analysis. Consistent with the results in Fig. 2, *S. ratti* and *P. trichosuri* showed higher locomotory activity than *S. papillosus* and *R. diutinus* over the intermediate temperature range (10–22 °C). Interestingly, iL3s of the two *Strongyloides* species responded differently to the more extreme temperatures than did the other species tested. All three types of dauer larvae showed peak dispersal at intermediate temperatures and reduced activity at lower

temperatures and at high temperature (37 °C). In contrast, *S. ratti* and *S. papillosus* showed a significant (P values <0.001) increase in dispersal at 4 °C and at 37 °C

To profile the olfactory response of the Strongyloidoidea, infective/dauer larvae were subjected to a variety of odorants known to be emitted by skin, sweat and microbiota on the skin (Fig. 4A). We tested a subset of the odorants used in Castelletto *et al.* (2014) plus urea. As seen by other authors (Dillman *et al.*, 2012; Castelletto *et al.*, 2014), we found that all species had a unique odorant response profile. Even the two species of the same genus, *S. ratti* and *S. papillosus* showed clearly different olfactory response profiles (Fig. 4B), similar to what had been observed in previous research for *S. ratti* and *S. stercoralis* (Castelletto *et al.*, 2014). *Rhabditophanes diutinus* had a significant olfactory response (attraction or repulsion) to more and was indifferent to fewer odorants, compared to all of the parasitic species [10 of 13 odorant cues triggered a statistically significant response (Fig. 4C) as opposed to 3–5 of 13 in the parasitic species (Fig. 4B, D)]. Of those odorants tested, only one odorant (acetic acid) triggered a statistically significant response in all four species, acting as an attractant in *S. ratti* (CI = 0.31) and *P. trichosuri* (CI = 0.19) and as a repellent to *S. papillosus* (CI = −0.64) and *R. diutinus* (CI = −1.00). To confirm that this olfactory preference was olfactory and not hygroscopic or gustatory, some odorants were also tested by using spotted filter paper on the plate lid, such that they could not soak into and spread through the agar. We found the response was near identical for all odorants tested (S2 Fig), confirming that the responses we saw are olfactory and not hygroscopic or gustatory, in agreement with what was found by Castelletto *et al.* (2014). To test whether *Strongyloides* spp. would actively seek out fur of its host species, we examined *S. ratti*'s response to rat fur (its natural host) and mouse fur (mice are barely susceptible hosts for *S. ratti*), such that infections are very short lived and of very low productivity if they are established at all (Gemmill *et al.*, 2000). We found that *S. ratti* has no significant preference for host fur over non-host fur (CI = 0.36 ± 0.40, P value 0.329, S3 Fig). As we noticed the infective larvae were barely crawling towards the fur samples, we then determined
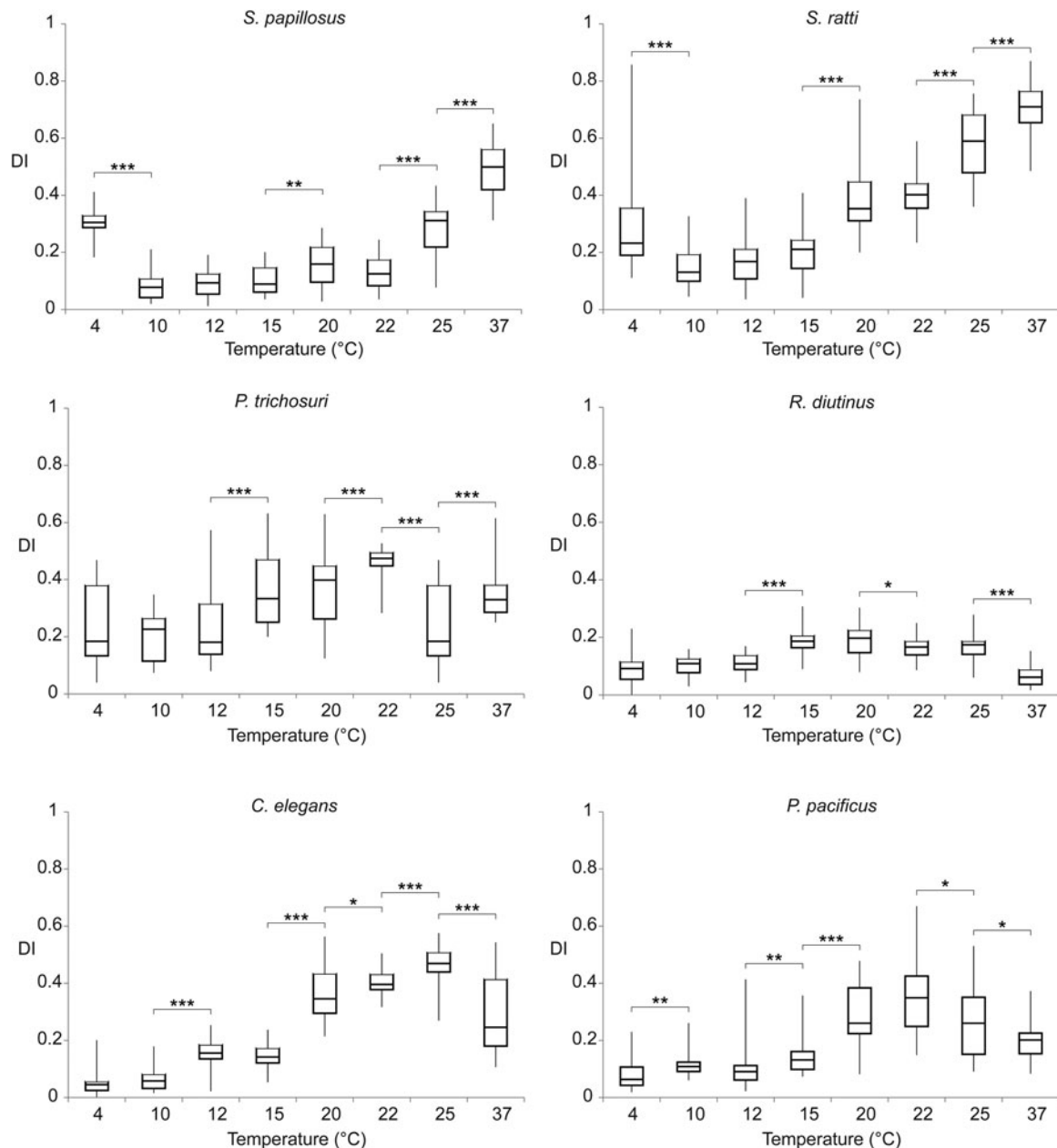
Fig. 3. Strongyloidoidea exhibit differing responses to thermal stress –temperature-dependent motility response of *S. papillosus*, *S. ratti*, *P. trichosuri* and *R. diutinus* infective/dauer larvae at a range of different temperatures between 4 and 37 °C. As a comparison, the temperature-dependent motility response of *P. pacificus* and *C. elegans* are included. For each temperature and species, 10 plates were tested, with all temperatures being tested at the same time. This experiment was repeated to generate three biological replicates. Data shown here in the box and whisker plots comprise the interquartile range with the median indicated, and the maximum and minimum shown from all 30 data points. To determine statistical significance, Mann–Whitney U (two-way) was performed. *P* values *<0.05, **<0.01, ***<0.001. Significance between neighbouring temperatures is shown only when significant.

whether they were attracted to fur at all. We found that *S. ratti* is in fact repulsed from fur (CI = −0.63 ± 0.17, *P* value <0.0001, S3 Fig).

Finally, as it is known that *Strongyloides* infective larvae actively migrate out of feces in search of a new host (Viney and Lok, 2015) while free-living adults tend to stay in the feces. To determine if this is reflected in the chemotactic behaviour of these different stages, we performed chemotaxis assays with adults and infective larvae of *S. ratti* and *S. papillosus* using feces of the host they had been raised in (rat for *S. ratti*, rabbit for *S. papillosus*) as attractants. As Castelletto *et al.* (2014) had found for *S. stercoralis* and *S. ratti*, we found that adults were attracted towards feces (*S. papillosus* CI = 0.61, *S. ratti* CI = 0.59) (Fig. 5) while infective larvae were indifferent (*S. papillosus* CI = −0.04, *S. ratti* CI = −0.13).

Discussion

Similarly to other authors (Castelletto *et al.*, 2014), we identified differences in chemotactic behaviours among closely-related species. These differences within a single genus appear to confirm what other authors previously proposed, in that host-seeking behaviour may have evolved independently even in closely related species in order to accommodate host behaviour and ecology (Dillman *et al.*, 2012; Castelletto *et al.*, 2014).

When examining the effect of temperature on motility, we observed small differences in temperature-dependent motility profiles between the two *Strongyloides* species, with *S. ratti* showing higher motility at all examined temperatures. Interestingly both species showed increased motility at 4 °C compared with more moderate temperatures (10–15 °C). This could be due to 4 °C being close to the lower limit of the tolerable temperatures
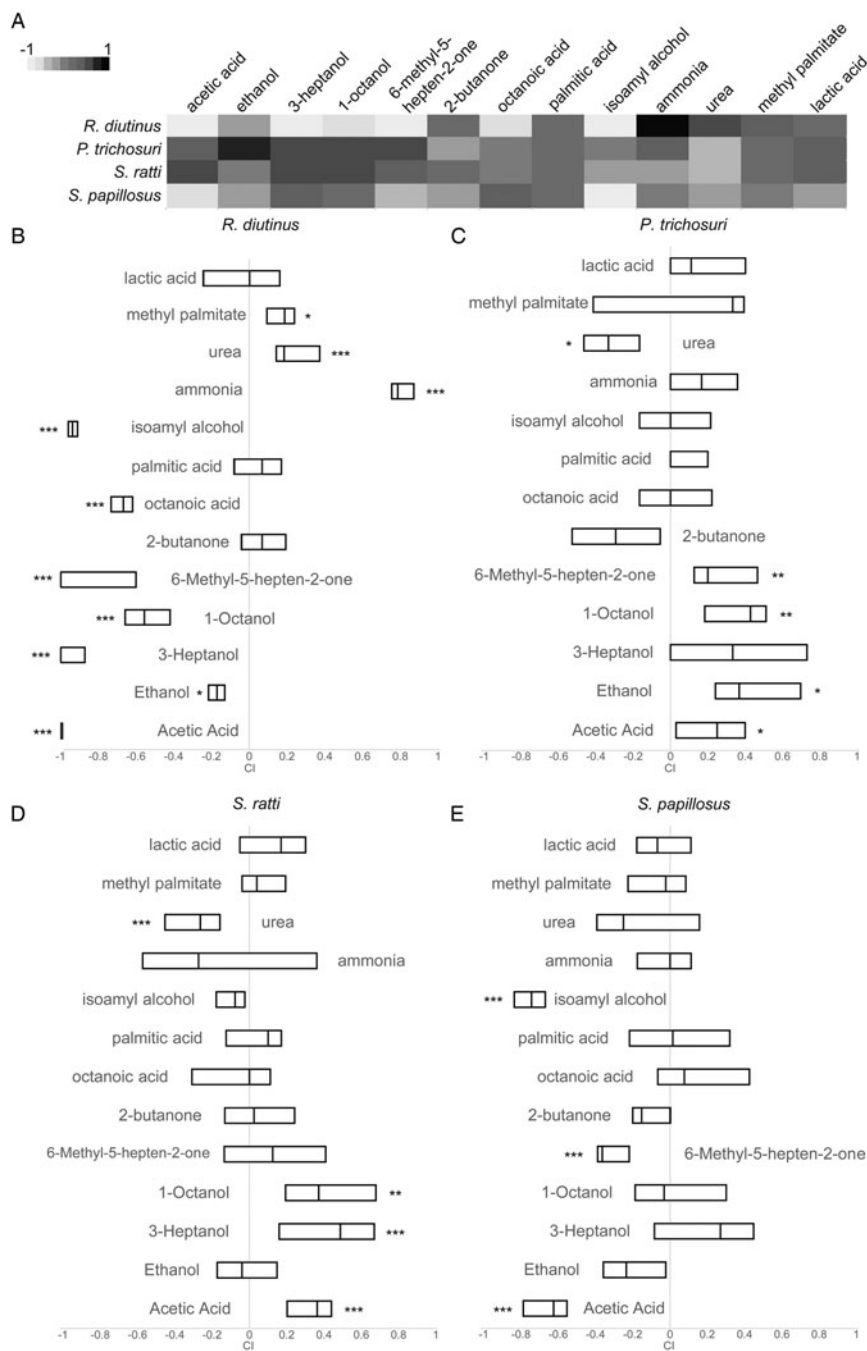
Fig. 4. Olfactory response profiles of the Strongyloidoidea – olfactory response of all four species in response to various volatile odorants is shown as a heatmap (A), with white indicating a strong repulsion and black strong attraction. Olfactory response of *R. diutinus* (B), *P. trichosuri* (C), *S. ratti* (D) and *S. papillosus* (E) to odorant cues. *P value <0.05, **P value <0.01, ***P value <0.001, if not significant than no star is shown. Two-way ANOVAs between odorant and control for all odorants and species tested. Each odorant was tested in batches of five, with at least three biological replicates. Boxplots show interquartile range of response, with mean response indicated by the middle bar.

for these two species, leading to them to try to escape and find a new host. Indeed, for *S. stercoralis*, several authors have noticed that refrigeration is detrimental for the development and the survival of infective larvae (Sultana *et al.*, 2013; Zhou *et al.*, 2019). In contrast to the two *Strongyloides* species, *P. trichosuri* showed neither a clear increase nor a decrease towards the ends of the temperature range tested. This may mean that the infective larvae of this facultative parasite are less temperature responsive than the two *Strongyloides* spp. tested. However, it is also possible that for *P. trichosuri*, the range of tolerable temperatures is wider than for the others and the temperatures tested are all within the comfort zone of this species. Further, we cannot exclude that the low responsiveness to temperature of the *P. trichosuri* iL3s has arisen in the laboratory. Our culture of *P. trichosuri* has been maintained exclusively through the free-living cycle for years such that features of developmental stages specific for the parasitic life cycle might have been lost. Further investigation into the temperature response of *P. trichosuri* is required. The

minimal (by comparison) locomotory activity that we saw in *R. diutinus* is unlikely to be due to its dauer lifestyle (both *C. elegans* and *P. pacificus* moved more during the experiment), but instead may be a species trait.

Similar to other authors (Castelletto *et al.*, 2014), the species-specific olfactory response we see is consistent with the proposal that olfactory preference is more closely associated with host species than with taxonomic position, with different chemotactic profiles among members of even the same nematode genus. *Strongyloides ratti* actually appeared to differ more from *S. papillosus* than from the more distantly related *P. trichosuri* since all four odorants leading to a significant response in *S. ratti* also triggered a significant response in the same direction in *P. trichosuri*. Of the odorants used in this assay, only a single one, acetic acid, produced a significant response for all species tested and this response did not go in the same direction in all four species. Acetic acid is known to be emitted from the skin (Bernier *et al.*, 2000; Gallagher *et al.*, 2008) and feet (Ara *et al.*, 2006) which
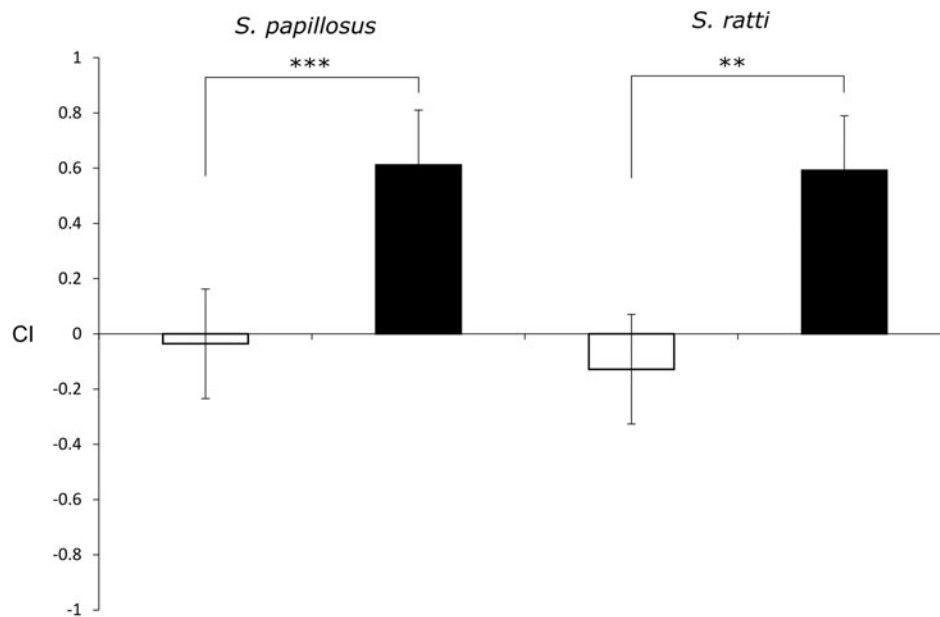
Fig. 5. Response of *Strongyloides* larvae to feces – only *Strongyloides* adults (black) are attracted towards their host feces, infective larvae (white) are indifferent to it. Two-way ANOVA with Bonferroni Post Test performed to determine significance between life stage and species. *P* value ***<0.001, **<0.01. No significant difference found between the two species. Bars indicate mean response towards feces with errors bars indicating the standard deviation. Each response variable (i.e. host feces for *S. ratti*) was tested in batches of five, with two biological replicates.

may explain why *S. ratti* and *P. trichosuri* are attracted to it as it is their normal entry site for infecting a new host (Grant *et al.*, 2006; Viney and Lok, 2007). It is unclear however why it would have an opposite response in *S. papillosus*. Interestingly *R. diutinus*, the only non-parasitic species included in our study, responded to more odorants than any of the parasitic species. If this profile is typical for being a dauer rather than an infective larva or is a *R. diutinus*-specific feature remains to be determined. The lack of response to fur in *S. ratti* while interesting, is not surprising. It suggests that fur is not a dominant carrier of attractive cues and that a combination of multiple cues (i.e. temperature, odorants) is required to trigger host-seeking behaviour within *S. ratti*. The lack of attraction towards rat fur in *S. ratti* could also be partially explained by an inability to penetrate the skin at areas with heavy fur and therefore an adaptive response to increase the efficiency of host-seeking behaviours. These olfactory results, combined with the thermosensory response, indicate that fur alone is not sufficient to generate a response, and that multiple cues from the skin and body temperature are necessary to engage host-seeking behaviour.

It should also be kept in mind that a particular reaction is not necessarily triggered by a single cue but by the combination of all stimuli present. In their natural environments, which are rather different from the laboratory conditions, the species tested might respond differently to the same cues, because they are present in different combinations. Nevertheless, taken together, our results support the notion that the sensing of and responding patterns to cues that are important for host-seeking are predominantly the result of independent evolution within the different species in response to the different lifestyles and hosts and only to a minor extent they are the result of the phylogenetic history of the species in question.

References

Ara K, Hama M, Akiba S, Koike K, Okisaka K, Hagura T, Kamiya T and Tomita F (2006) Foot odor due to microbial metabolism and its control. *Canadian Journal of Microbiology* **52**, 357–364.

Bargmann CI (2006) Chemosensation in *C. elegans*. *WormBook*, 1–29. doi: 10.1895/wormbook.1.123.1

Beknazarova M, Whiley H and Ross K (2016) Strongyloidiasis: a disease of socioeconomic disadvantage. *International Journal of Environmental Research and Public Health* **13**, 517. doi: 10.3390/ijerph13050517

Bernier UR, Kline DL, Barnard DR, Schreck CE and Yost RA (2000) Analysis of human skin emanations by gas chromatography/mass spectrometry. 2. Identification of volatile compounds that are candidate

attractants for the yellow fever mosquito (*Aedes aegypti*). *Analytical Chemistry* **72**, 747–756.

Bryant AS, Ruiz F, Gang SS, Castelletto ML, Lopez JB and Hallem EA (2018). A critical role for thermosensation in host seeking by skin-penetrating Nematodes. *Current Biology* **28**, 2338–2347.e2336.

Castelletto ML, Gang SS, Okubo RP, Tselikova AA, Nolan TJ, Platzer EG, Lok JB and Hallem EA (2014) Diverse host-seeking behaviors of skin-penetrating Nematodes. *PLoS Pathogens* **10**, e1004305.

Dillman AR, Guillermin ML, Lee JH, Kim B, Sternberg PW and Hallem EA (2012) Olfaction shapes host-parasite interactions in parasitic nematodes. *Proceedings of the National Academy of Sciences of the USA* **109**, E2324–E2333.

Dulovic A, Puller V and Streit A (2016). Optimizing culture conditions for free-living stages of the nematode parasite *Strongyloides ratti*. *Experimental Parasitology*, **168**, 25–30.

Dulovic A, Renahan T, Röseler W, Rödelsperger C, Rose AM and Streit A (2020) *Rhabditophanes diutinus* a parthenogenetic clade IV nematode with dauer larvae. *PLoS Pathogens* **16**, e1009113.

Eberhardt AG, Mayer WE and Streit A (2007) The free-living generation of the nematode *Strongyloides papillosus* undergoes sexual reproduction. *International Journal for Parasitology* **37**, 989–1000.

Eberhardt AG, Mayer WE, Bonfoh B and Streit A (2008) The Strongyloides (Nematoda) of sheep and the predominant Strongyloides of cattle form at least two different, genetically isolated populations. *Veterinary Parasitology* **157**, 89–99.

Gallagher M, Wysocki CJ, Leyden JJ, Spielman AI, Sun X and Preti G (2008) Analyses of volatile organic compounds from human skin. *British Journal of Dermatology* **159**, 780–791.

Gemmill AW, Viney ME and Read AF (2000) The evolutionary ecology of host-specificity: experimental studies with *Strongyloides ratti*. *Parasitology* **120**, 429–437.

Grant WN, Stasiuk S, Newton-Howes J, Ralston M, Bisset SA, Heath DD and Shoemaker CB (2006) *Parastrongyloides trichosuri*, a nematode parasite of mammals that is uniquely suited to genetic analysis. *International Journal for Parasitology* **36**, 453–466.

Hallem EA and Sternberg PW (2008) Acute carbon dioxide avoidance in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* **105**, 8038–8043.

Hallem EA, Rengarajan M, Ciche TA and Sternberg PW (2007) Nematodes, bacteria, and flies: a tripartite model for nematode parasitism. *Current Biology* **17**, 898–904.

Hart AC and Chao MY (2010) From odors to behaviours in *Caenorhabditis elegans*. In Menini A (ed.), *The Neurobiology of Olfaction*. Boca Raton, FL: CRC Press/Taylor & Francis.

Hong RL (2015) *Pristionchus pacificus* olfaction. In Sommer RJ (ed.), *Pristionchus pacificus – A Nematode Model for Comparative and Evolutionary Biology*, Vol. **11**. Brill, pp. 331–352.

Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ and Jacobson J (2008) Helminth infections: the great neglected tropical diseases. *The Journal of Clinical Investigation* **118**, 1311–1321.

Hunt VL, Tsai IJ, Coghlan A, Reid AJ, Holroyd N, Foth BJ, Tracey A, Cotton JA, Stanley EJ, Beasley H, Bennett HM, Brooks K, Harsha B, Kajitani R, Kulkarni A, Harbecke D, Nagayasu E, Nichol S, Ogura Y, Quail MA, Randle N, Xia D, Brattig NW, Soblik H, Ribeiro DM, Sanchez-Flores A, Hayashi T, Itoh T, Denver DR, Grant W, Stoltzfus JD, Lok JB, Murayama H, Wastling J, Streit A, Kikuchi T, Viney M and Berriman M (2016) The genomic basis of parasitism in the *Strongyloides* clade of nematodes. *Nature Genetics* **48**, 299–307.

Jaleta TG, Zhou S, Bemm FM, Schär F, Khieu V, Muth S, Odermatt P, Lok JB and Streit A (2017) Different but overlapping populations of *Strongyloides stercoralis* in dogs and humans – dogs as a possible source for zoonotic strongyloidiasis. *PLoS Neglected Tropical Diseases* **11**, e0005752.

Kulkarni A, Dyka A, Nemetschke L, Grant WN and Streit A (2013) *Parastrongyloides trichosuri* suggests that XX/XO sex determination is ancestral in Strongyloididae (Nematoda). *Parasitology* **140**, 1822–1830.

Nutman TB (2017) Human infection with *Strongyloides stercoralis* and other related *Strongyloides* species. *Parasitology* **144**, 263–273.

Safer D, Brenes M, Dunipace S and Schad G (2007) Urocanic acid is a major chemoattractant for the skin-penetrating parasitic nematode *Strongyloides stercoralis*. *Proceedings of the National Academy of Sciences* **104**, 1627–1630.

Schär F, Trostdorf U, Giardina F, Khieu V, Muth S, Marti H, Vounatsou P and Odermatt P (2013) *Strongyloides stercoralis*: global distribution and risk factors. *PLoS Neglected Tropical Diseases* **7**, e2288.

Stiernagle, T. (2006). Maintenance of *C. elegans*. WormBook, ed. The *C. elegans* Research Community, Wormbook, doi/10.1895/wormbook.1.101.1. Available at http://www.wormbook.org.

Streit A (2014) How to become a parasite without sex chromosomes: a hypothesis for the evolution of *Strongyloides* spp. and related nematodes. *Parasitology* **141**, 1244–1254.

Streit A (2016) Genetics: modes of reproduction and genetic analysis. *Parasitology* **144** (Special Issue 3: Strongyloides), 1–11. doi: 10.1017/s0031182016000342

Streit A, Wang J, Kang Y and Davis RE (2016) Gene silencing and sex determination by programmed DNA elimination in parasitic nematodes. *Current Opinion in Microbiology* **32**, 120–127.

Sultana Y, Jeoffreys N, Watts MR, Gilbert GL and Lee R (2013) Real-time polymerase chain reaction for detection of *Strongyloides stercoralis* in stool. *American Journal of Tropical Medicine and Hygiene* **88**, 1048–1051.

Tsubokawa D, Hatta T, Kikuchi T, Maeda H, Mikami F, Alim MA, Maruyama H and Tsuji N (2017) Venestatin, a Ca(++)-binding protein from the parasitic nematode *Strongyloides venezuelensis*, is involved in the larval migration process. *International Journal for Parasitology* **47**, 501–509.

Viney M (2017) How can we understand the genomic basis of nematode parasitism? *Trends in Parasitology* **33**, 444–452.

Viney ME and Lok JB (2007) *Strongyloides* spp. WormBook, ed. The *C. elegans* Research Community, Wormbook, doi:10.1895/wormbook.1.141.1. Available at http://www.wormbook.org.

Viney ME and Lok JB (2015) The biology of *Strongyloides* spp. *WormBook*, 1–17. doi: 10.1895/wormbook.1.141.2

Zhou S, Harbecke D and Streit A (2019) From the faeces to the genome: a guideline for the isolation and preservation of *Strongyloides stercoralis* in the field for genetic and genomic analysis of individual worms. *Parasites & Vectors* **12**, 496.