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# The nematode *Caenorhabditis elegans* and diverse potential invertebrate vectors predominantly interact opportunistically

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Some small animals migrate with the help of other, more mobile animals (phoresy) to leave short-lived and resource-poor habitats. The nematode *Caenorhabditis elegans* lives in ephemeral habitats such as compost, but has also been found associated with various potential invertebrate vectors. Little research has been done to determine if *C. elegans* is directly attracted to these invertebrates. To determine whether *C. elegans* is attracted to compounds and volatile odorants of invertebrates, we conducted chemotaxis experiments with the isopods *Porcellio scaber*, *Oniscus asellus*, and *Armadillidium* sp. and with *Lithobius* sp. myriapods, *Drosophila melanogaster* fruit flies, and *Arion* sp. slugs as representatives of natural vectors. Because phoresy is an important escape strategy in nature, especially for dauer larvae of *C. elegans*, we examined the attraction of the natural *C. elegans* isolate MY2079 in addition to the laboratory-adapted strain N2 at the dauer and L4 stage. We found that DMSO washing solution of *Lithobius* sp. and the odor of live *D. melanogaster* attracted *C. elegans* N2 L4 larvae. Surprisingly, the natural isolate MY2079 was not attracted to any invertebrate during either the dauer or L4 life stages and both *C. elegans* strains were repelled by various compounds from *O. asellus*, *P. scaber*, *Armadillidium* sp., *Lithobius* sp., and *Arion* sp. feces. We hypothesize that this is due to defense chemicals released by the invertebrates. Although compounds from *Lithobius* sp. and *D. melanogaster* odorants were mildly attractive, the lack of attraction to most invertebrates suggests a predominantly opportunistic association between *C. elegans* and invertebrate vectors.

## KEYWORDS

*Caenorhabditis elegans*, chemotaxis, migration, invertebrates, *Arion* sp. slugs, *Drosophila melanogaster*, phoresy, isopods

## Introduction

For many small animals, the ability to travel longer distances is severely limited. However, migrating longer distances and thus settling in a new environment may offer advantages: Competition for food and mating partners, as well as pressure from predators, parasites, and pathogens, may be lower. In addition, mating outside of their own population can help organisms increase their genetic diversity (Lacy, 1987; Gustafson et al., 2017). Especially for animals in short-lived habitats, migration is essential to avoid limited resource availability and improve survival (Reid et al., 2018). To travel longer distances some animals therefore use other, more mobile animals as a means of transportation. One example are oribatid mites, which use a variety of hosts including beetles, birds, and mammals for migration (Dusbabek and Bukva, 1991; Krivolutsky

and Lebedeva, 2004; Knee et al., 2013). This type of temporary interaction for the purpose of dispersal is called phoresy (Farish and Axtell, 1971).

It is often unclear whether the association between small invertebrates and their vectors is purely incidental or whether the invertebrates actively search a vector. Odorants or chemicals released into the environment may play a crucial role in finding a host. Snails can track their mating partners using water- or air-borne pheromones, and mucus trails (Ng et al., 2013). A particular larval stage of the mite *Myianoetus muscarum* is attracted to a volatile substance produced by the pupa of the fly *Muscina stabulans*. The mite larvae gather at the anterior end of the pupa where the fly hatches and thus position themselves to be dispersed with the hatching fly (Greenberg and Carpenter, 1960). Entomopathogenic nematodes are attracted to volatile components of insect feces and odors emitted by live insects (Schmidt and All, 1979; Dillman et al., 2012).

The nematode *Caenorhabditis elegans* is often found in microbe-rich, short-lived habitats such as compost and other decomposing plant material (Schulenburg and Félix, 2017). In these habitats, *C. elegans* is frequently found in association with invertebrates, e.g., snails, slugs, isopods, and myriapods (Barrière and Félix, 2005; Caswell-Chen et al., 2005; Cutter, 2006; Kiontke and Sudhaus, 2006; Félix and Braendle, 2010; Félix and Duveau, 2012; Petersen et al., 2015a). These associations are assumed to be part of an escape strategy to migrate to new habitats if conditions deteriorate. Thus, migration using invertebrates allows *C. elegans* to travel comparatively long distances and establish populations in new locations.

It is known that *C. elegans* responds to chemical signals from food bacteria and pathogens (Pastan and Perlman, 1971; Ward, 1973; Andrew and Nicholas, 1976; Grewal and Wright, 1992; Schulenburg and Müller, 2004), but little research has been done to determine whether *C. elegans* can sense potential invertebrate vectors and move toward them (Dillman et al., 2012; Lee et al., 2017; Archer et al., 2020). This would be a prerequisite for *C. elegans*-initiated migration using invertebrates in response to food shortages or overpopulation. Previous studies suggest that *C. elegans* and the terrestrial isopod *Porcellio scaber* likely interact opportunistically (Archer et al., 2020). Information on the nature of interactions with other migration partners is scarce (Dillman et al., 2012; Lee et al., 2012, 2017).

The aim of the current study was to investigate if the interaction between *C. elegans* and invertebrates of different taxa representative of potential natural migration partners is based on *C. elegans* perception of a chemical signal from the invertebrates. We tested the chemotactic response of *C. elegans* toward different extracts of compounds and odorants from the isopod species *Porcellio scaber*, *Oniscus asellus*, and *Armadillidium* sp.; *Lithobius* sp. myriapods, the fruit fly *Drosophila melanogaster* and *Arion* sp. slugs. We included two *C. elegans* strains, the laboratory-adapted strain N2 and the natural strain MY2079, to account for the potentially higher importance of an escape strategy for free-living strains. To test whether attraction is stage-specific, we tested dauer larvae, which are often associated with invertebrates (Félix and Braendle, 2010), and fourth instar (L4) larvae. *Caenorhabditis elegans* N2 L4 larvae were attracted to *Lithobius* sp. myriapods washed in dimethyl sulfoxide (DMSO) and odor of live *D. melanogaster* while *P. scaber*, *O. asellus*, *Armadillidium* sp., and *Arion* sp. had no attractive effect. Surprisingly, the natural *C. elegans* strain MY2079 was not attracted to chemical compounds or odorants of any of the tested invertebrates. Both N2 and MY2079 were repelled by some chemical compounds or odorants. Based on these results, *C. elegans* appears to be attracted to some

compounds, but most of the associations between *C. elegans* and the invertebrates studied are likely opportunistic rather than directed.

## Materials and methods

### Nematodes, invertebrates, and bacterial strains

All experiments were performed with the standard laboratory *C. elegans* strain N2 and the natural *C. elegans* isolate MY2079. N2 was originally obtained from the *Caenorhabditis* Genetics Center (CGC) and MY2079 was isolated from compost in the Botanical Garden in Kiel, Germany (Petersen et al., 2015b). All worms were maintained on nematode growth medium (NGM) plates with *Escherichia coli* strain OP50 as food following standard procedures (Stiernagle, 2006). *Caenorhabditis elegans* strains were bleached and kept for approximately 48 h on OP50 at 20°C to obtain L4 larvae or for 2 to 3 weeks at 25°C to obtain dauer larvae. All worms were washed from the plates freshly for every experiment.

Isopods, myriapods, and slugs for the experiments were caught from nature in Kiel, Germany (Supplementary Figures 1A–H). The isopod species *Porcellio scaber*, *Oniscus asellus*, and *Armadillidium* sp. originated from a garden plot and were mostly found below old bricks. Myriapods and slugs originated from the same compost as the natural *C. elegans* strain MY2079. Myriapods were sampled below tree trunks or tree bark stored close to compost. Isopods and myriapods were either directly used in experiments or kept for up to 4 days in plastic boxes with wet tissue paper and thick tree branches and leaves from their natural habitat. The wet tissue paper was replaced daily. *Arion* sp. slugs were kept individually for one night in boxes containing plant material from their natural habitat to reduce adhering debris.

The *Drosophila melanogaster* laboratory strain w[1118] was grown on standard yeast/cornmeal/agar medium at 25°C until day 4–6 of adulthood as previously described (Rahn et al., 2013; Li et al., 2015). Flies were starved in a sterile, empty fly container for 16 h at 25°C prior to the experiment with a piece of wet paper to prevent desiccation.

### Invertebrate washes and extractions

Washes and extracts were prepared freshly for each replicate. One isopod, one *Lithobius* sp. or five *D. melanogaster* were transferred to a sterile 1.5-ml microcentrifuge tube. Subsequently, 180 µl sterile deionized water, DMSO or ethanol were added to isopods, 250 µl to *Lithobius* sp. and 120 µl to *D. melanogaster*. Washes were obtained from invertebrates washed in the solvent with the tube rotated on an orbital rotator for 30 min. Extracts were produced from washed invertebrates ground in the solvent using a sterile pestle.

Slug feces was collected from slugs kept for 1 day in a box and placed in a 1.5-ml microcentrifuge tube. After stirring, 1 mg feces was transferred to a new tube with 180 µl deionized water, DMSO, or ethanol, homogenized with a sterile pestle, briefly centrifuged and the liquid phase transferred to a new tube. All washes and extracts were used undiluted.

### Chemotaxis assays

We tested the chemotactic response of *C. elegans* to wash supernatants (washes) and invertebrates ground in deionized water,

DMSO, or ethanol (extracts). All experiments were performed on 6-cm NGM plates if not stated otherwise. A letter code was used to prevent any observer bias. Petri dishes were divided into quadrants as described previously to obtain an alternating pattern of test and control to avoid any bias that might result from the initial placement of the worms (Margie et al., 2013). A circle with a diameter of 1 cm marked the center of the plate (Supplementary Figure 1I). Sodium azide (0.5 M) diluted 1:3 in all test and control liquids was used as anesthetic. Immediately after preparation of the washes and extracts, 3  $\mu$ l were pipetted to opposing quadrants of the plate and 3  $\mu$ l of the corresponding solvent without extract or wash to the two remaining quadrants in-between. The general response of worms to chemical stimuli was tested using 3  $\mu$ l of isoamyl alcohol diluted to  $10^{-3}$  in ethanol (attractant) or undiluted 1-octanol (repellent) as the test compound. Plates were left for 2 h to allow all liquids to sink into the agar. Finally, 30–250 *C. elegans* L4 or dauer larvae were added to the center, a number that allows fast processing of the assay plates, is sufficient to limit the influence of individual worms and prevents overcrowding.

Slug mucus was stamped directly from the foot of a living slug onto one side of a 9-cm agar plate. The other side of the plate remained blank. 30–250 L4 or dauer larvae were pipetted to the center of the plate.

After 1 h, worms in the test and control quadrants or halves were scored. Worms remaining in the plate center were ignored and only replicates in which at least 10 worms left the center were considered. A choice index was calculated by subtracting the number of worms in the control quadrants (or halves) from the number of worms in test quadrants (or halves) and dividing the result by the total number of worms on the plate. A positive choice index indicates the choice of the test compound (attraction), a negative choice index indicates the choice of the control (repulsion) and a choice index around zero indicates equal choice of both.

## Chemotaxis gas assay

The assays were performed on unseeded 6-cm NGM plates. The center was marked with a circle of 1 cm in diameter and plates were divided into test and control halves each half with a drilled hole in the lid. One isopod, one *Lithobius* sp., or five *D. melanogaster* were placed in a sterile 1-ml pipette tip with a 3-mm opening. The tip was placed in the lid of one half and an identical tip without invertebrate in the other half. All tips were sealed at the top with a heat-sterilized 8-mm cigarette filter and a piece of heat-sterilized mesh in the tip prevented escape of *Lithobius* sp. and *D. melanogaster*. The gas exchange was not actively promoted but tested using 20  $\mu$ l of isoamyl alcohol diluted to  $10^{-3}$  in ethanol (attractant) or undiluted 1-octanol (repellent) on filter paper in a pipette tip as the test odorant. Additionally, the controls were used to test the general response of worms to air-borne stimuli.

30–250 *C. elegans* L4 or dauer larvae were added to the plate center. After 1 h at room temperature, the tips were removed and worms on each half were scored. A choice index was calculated as described before for the chemotaxis assays.

## Statistics

The experiments were performed as time-independent, biological replicates for each combination of nematode strain, nematode developmental stage, and invertebrate. Mean choice indices were

reported. One-sample Wilcoxon-Signed rank tests (Wilcoxon, 1945) with false discovery rate correction (Benjamini and Hochberg, 1995) for multiple testing were performed to assess differences between a choice index of zero and the corresponding choice index of the nematode strain, developmental stage, and solvent. All statistics were performed using R-Studio (version 1.4.1717) and are presented in Supplementary Tables 1–3 and Supplementary File 1. The raw data can be found in Supplementary Tables 4–7. Composite figures were created in Inkscape (version 1.1) and Affinity Photo (version 1.10.5).

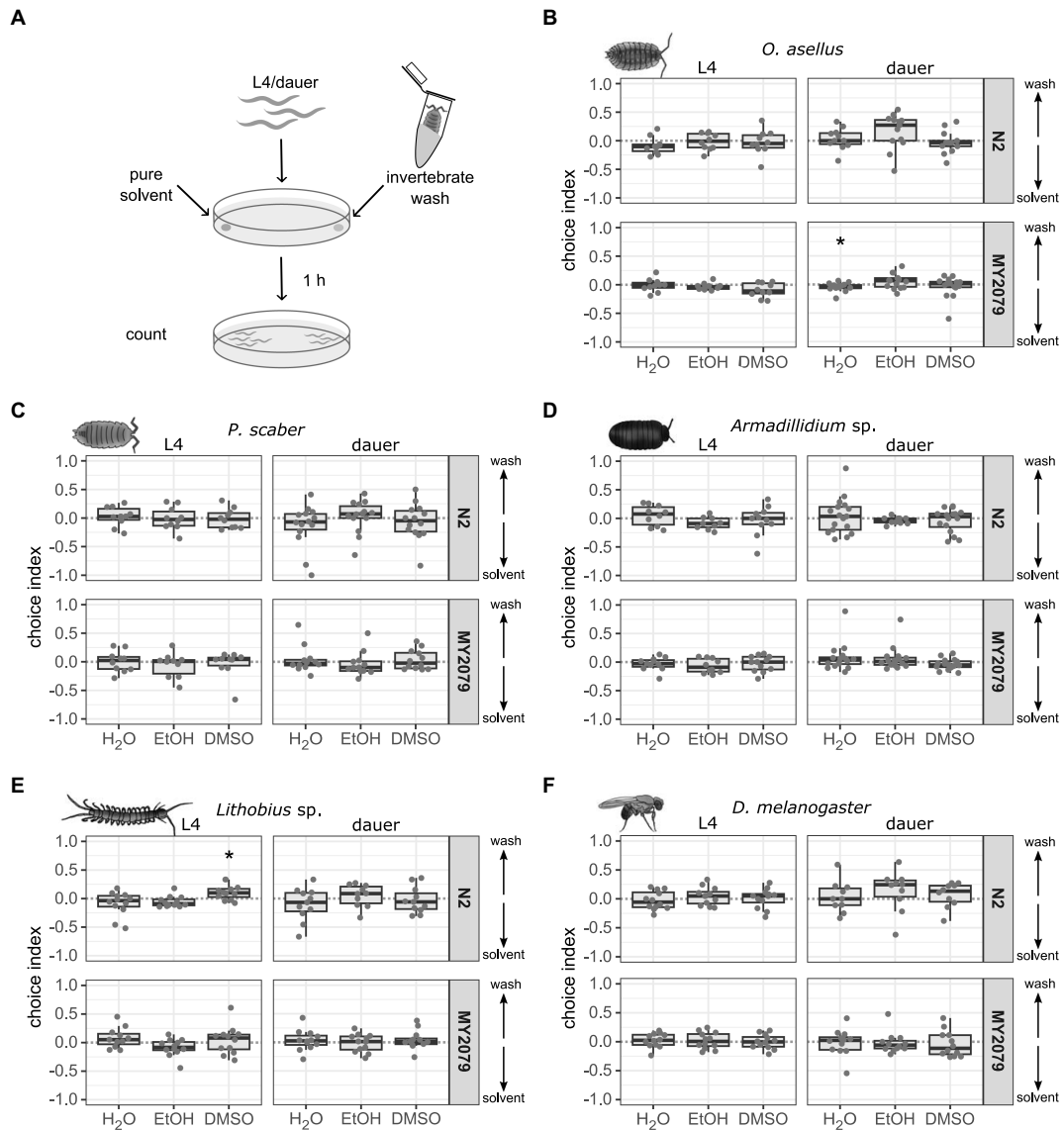
## Results

### *Caenorhabditis elegans* L4 and dauer larvae are not attracted to compounds washed from isopods or fruit flies, but N2 L4 larvae are attracted to compounds washed from myriapods

*Caenorhabditis elegans*' search for hosts used for migration may potentially be facilitated by chemical signals delivered by the host to the environment. The ability to emit chemical signals is known from various invertebrates, on which *C. elegans* has been repeatedly found. Snails are known to release substances with their mucus that enable other snails to follow their tracks (Ng et al., 2013). Isopods such as *Armadillidium vulgare* use chemical signals to attract mating partners (Beauché and Richard, 2013). These chemical signals used for intra-specific communication could guide *C. elegans* to suitable migration partners. To test whether invertebrate secretions are attractive to *C. elegans*, we used wash solutions of three isopod species, as well as of myriapods, and fruit flies as potential attractants in chemotaxis assays. We found that L4 *C. elegans* N2 were attracted to *Lithobius* sp. wash in DMSO ( $p=0.026$ ), but not to wash in deionized water and ethanol. In contrast, N2 dauer larvae and neither stage of MY2079 showed attractive or repulsive behavior toward *Lithobius* sp. wash (Figure 1, Supplementary Tables 1, 4). *Caenorhabditis elegans* MY2079 dauer larvae were repelled by *O. asellus* wash in deionized water ( $p=0.042$ ), while L4 MY2079 and both N2 stages showed no chemotactic response to *O. asellus* wash. Washes of *P. scaber*, *Armadillidium* sp., and *D. melanogaster* did neither attract nor repel any *C. elegans*. Both *C. elegans* strains were attracted to the control attractant isoamyl alcohol and repelled by the control repellent 1-octanol as L4 larvae (Supplementary Figure 2, Supplementary Tables 1, 4). However, there was a high variability in the response of dauer larvae to both the attraction and repellent controls.

### *Caenorhabditis elegans* L4 and dauer larvae are not attracted to compounds extracted from diverse ground invertebrates

Washing dissolves only the compounds that adhere to the outside of the invertebrates. The concentration of these washed compounds might be too small to trigger a chemotactic response in *C. elegans* due to dilution with the solvents. Other compounds could be located inside the invertebrates, for example, in ingested food or gut content. To extract potentially attractive compounds from the inside of invertebrates, whole *O. asellus*, *P. scaber*, *Armadillidium* sp., *Lithobius* sp., and *D. melanogaster* were first washed in deionized water, ethanol or DMSO and then ground with a sterile pestle (Figure 2A). *Caenorhabditis elegans* N2 and MY2079 were not attracted to any of the ground invertebrates in either as L4 or



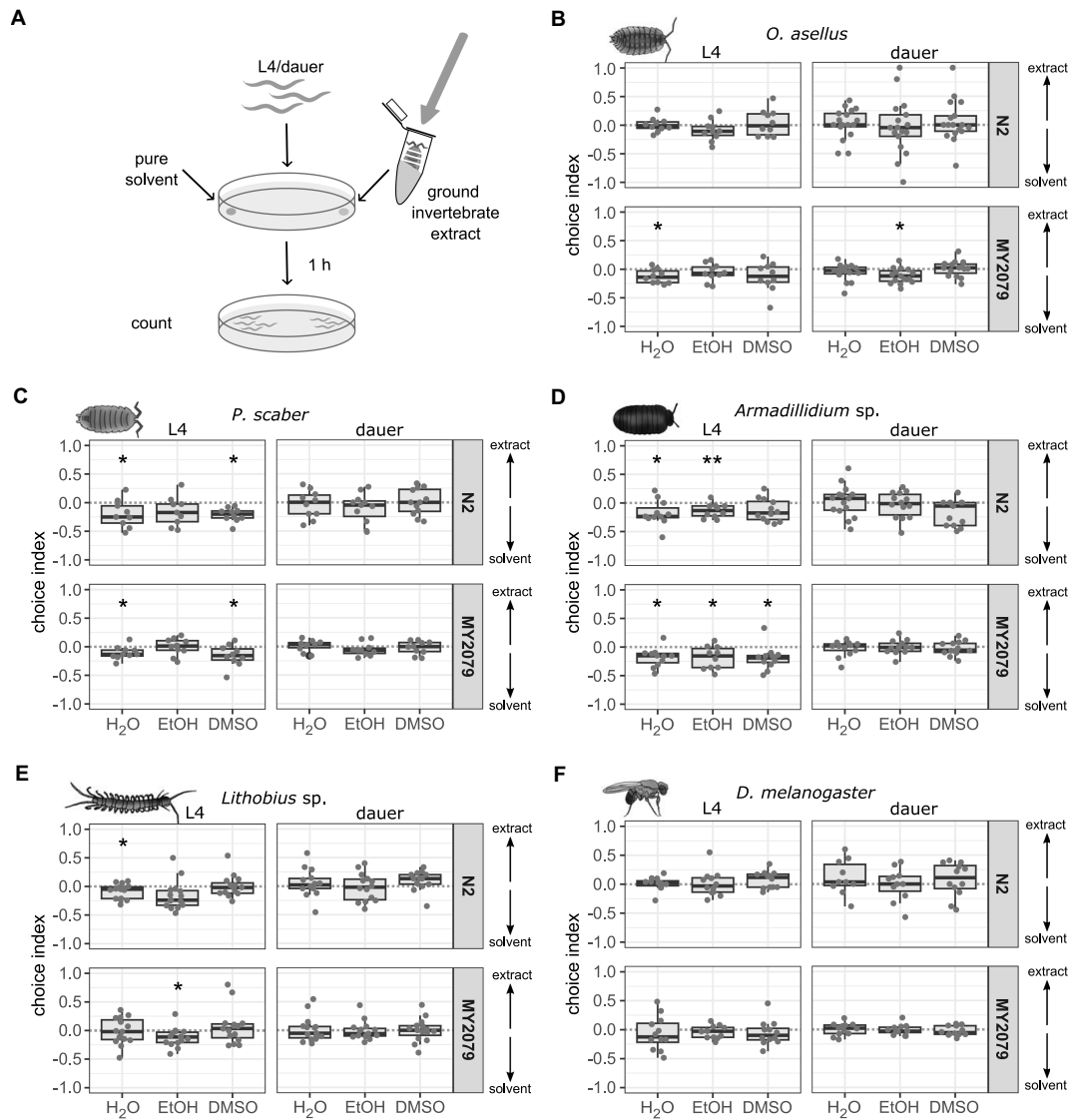
**FIGURE 1**  
*Caenorhabditis elegans* strains N2 and MY2079 do not respond to most invertebrate washes with three different solvents. **(A)** *Caenorhabditis elegans* strains N2 and MY2079 were grown on OP50 and used as L4 or dauer larvae to evaluate their chemotactic response toward washes of potential invertebrate migration partners in three different solvents. A positive choice index indicates attraction to the invertebrate wash from **(B)** *Oniscus asellus* ( $n = 10-17$ ), **(C)** *Porcellio scaber* ( $n = 10-16$ ), **(D)** *Armadillidium* sp. ( $n = 10-20$ ), **(E)** *Lithobius* sp. ( $n = 10-15$ ), and **(F)** *Drosophila melanogaster* ( $n = 11-12$ ). A negative choice index indicates repulsion from the invertebrate wash, a choice index of 0 indicates equal choice of both sides. For comparison of choice indices with 0, the Wilcoxon signed-rank test with false discovery rate correction for multiple testing was applied. Data are presented as boxplots with the median as a thick horizontal line, the interquartile range as box, the whiskers as vertical lines, and each replicate depicted by a dot. Significance is designated to \* $p < 0.05$ . From left to right each panel shows boxes representing deionized water (H<sub>2</sub>O), ethanol (EtOH), or dimethyl sulfoxide (DMSO) washes (with 30–250 worms per replicate).

dauer larvae (Figures 2B–F, Supplementary Tables 2, 5). In contrast, some invertebrate extracts had a repellent effect. L4 *C. elegans* N2 were repelled from *P. scaber* in deionized water ( $p = 0.044$ ) and DMSO ( $p = 0.004$ ), *Armadillidium* sp. in deionized water ( $p = 0.017$ ) and ethanol ( $p = 0.005$ ), and *Lithobius* sp. in deionized water ( $p = 0.005$ ). N2 dauer larvae were neither attracted nor repelled by any invertebrate. L4 *C. elegans* MY2079 were repelled by *O. asellus* in deionized water ( $p = 0.016$ ), *P. scaber* in deionized water ( $p = 0.018$ ) and DMSO ( $p = 0.033$ ), *Armadillidium* sp. in deionized water ( $p = 0.022$ ), ethanol ( $p = 0.047$ ), and DMSO ( $p = 0.024$ ), and *Lithobius* sp. in ethanol ( $p = 0.034$ ). MY2079 dauer larvae were repelled by *O. asellus* in ethanol ( $p = 0.014$ ). L4 larvae of *C. elegans* N2 and MY2079 were attracted to the control attractant isoamyl alcohol and

repelled by the control repellent 1-octanol (Supplementary Figure 3, Supplementary Tables 2, 5). However, dauer larvae of both *C. elegans* strains reacted variable to the attraction and repellent controls.

### *Caenorhabditis elegans* L4 and dauer larvae do not respond to *Arion* sp. mucus and are not attracted to *Arion* sp. feces

Slugs and snails have been repeatedly identified as vectors of *C. elegans* (Petersen et al., 2015a; Schulenburg and Félix, 2017). Since mucus plays a role in communication between snails and their



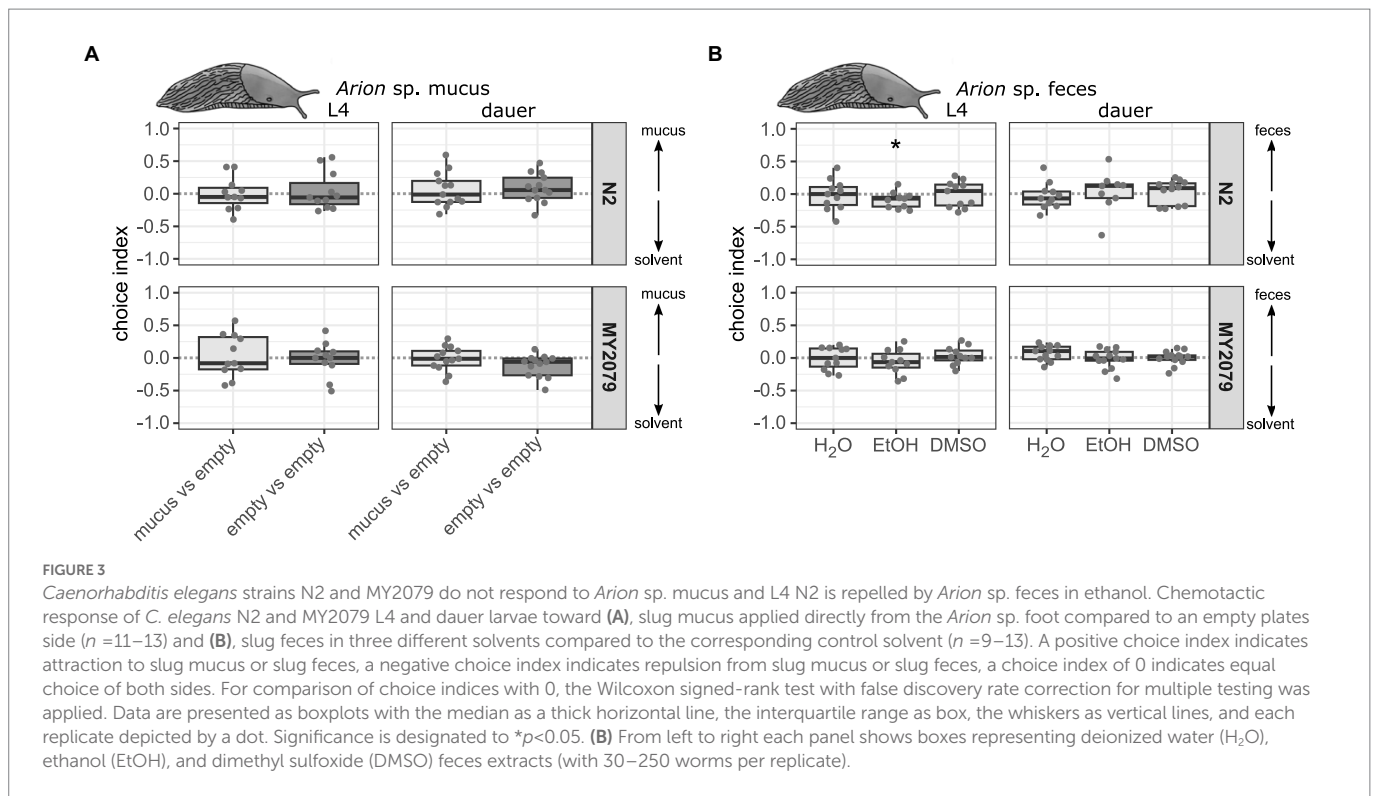
**FIGURE 2**  
*Caenorhabditis elegans* strains N2 and MY2079 are not attracted to extracts from ground invertebrates in three solvents. **(A)** *Caenorhabditis elegans* strains N2 and MY2079 were grown on OP50 and used as L4 or dauer larvae to evaluate their chemotactic response toward extracts obtained from invertebrates ground in three different solvents. A positive choice index indicates attraction to the extract from **(B)** *Oniscus asellus* ( $n = 9-17$ ), **(C)** *Porcellio scaber* ( $n = 9-14$ ), **(D)** *Armadillidium* sp. ( $n = 11-18$ ), **(E)** *Lithobius* sp. ( $n = 13-15$ ), or **(F)** *Drosophila melanogaster* ( $n = 11-12$ ). A negative choice index indicates repulsion from the invertebrate (solvent), a choice index of 0 indicates equal choice of both sides. For comparison of choice indices with 0, the Wilcoxon signed-rank test with false discovery rate correction for multiple testing was applied. Data are presented as boxplots with the median as a thick horizontal line, the interquartile range as box, the whiskers as vertical lines, and each replicate depicted by a dot. From left to right each panel shows boxes representing deionized water (H<sub>2</sub>O), ethanol (EtOH), and dimethyl sulfoxide (DMSO) extracts (with 30–250 worms per replicate). Significance is designated to the following scale: \*\* $p < 0.01$ , \* $p < 0.05$ .

conspecifics (Ng et al., 2013; Vong et al., 2019) and is therefore also a potential source of attraction for *C. elegans*, the attraction of mucus from the foot of *Arion* sp. was tested as potential attractant in the chemotaxis assay. We found that *Arion* sp. mucus did neither attract nor repel *C. elegans* (Figure 3A, Supplementary Tables 2, 6). *Caenorhabditis elegans* has often been isolated from the slug gut and both *C. elegans* and potential food bacteria can survive passage through the slug gut and be found in slug feces (Petersen et al., 2015a; Schulenburg and Félix, 2017; Pees et al., 2021). Therefore, we tested the chemotactic attraction towards slug feces next. Neither L4 nor dauer larvae of *C. elegans* N2 and MY2079 were attracted to *Arion* sp. feces (Figure 3B, Supplementary Tables 2, 6). L4 *C. elegans* N2 were repelled from feces

in ethanol ( $p = 0.034$ ). As seen before, both *C. elegans* strains were attracted to the control attractant isoamyl alcohol and repelled by the control repellent 1-octanol as L4 larvae (Supplementary Figure 3, Supplementary Tables 2, 6). However, the response of dauer larvae to the attraction and repellent controls was highly variable.

### *Caenorhabditis elegans* L4 and dauer larvae are not attracted to invertebrate odorants

Entomopathogenic nematodes are attracted to volatile components of insect feces and odors emitted by live insects (Schmidt and All, 1979;



Dillman et al., 2012). Carbon dioxide (CO<sub>2</sub>) has been identified as an essential host cue for entomopathogenic nematodes (Dillman et al., 2012). Therefore, gaseous components of odorants secreted by invertebrate hosts could play a role as attractants for *C. elegans*. We tested this hypothesis in simple gas chemotaxis assays using live isopods, myriapods, and fruit flies. L4 *C. elegans* N2 were attracted to *D. melanogaster* ( $p = 0.006$ ), but not to any other invertebrate odorant (Figure 4, Supplementary Tables 3, 7). N2 dauer larvae were not attracted to or repulsed by any invertebrate odorant. L4 and dauer larvae of *C. elegans* MY2079 were not attracted to or repulsed by any invertebrate odorant. Both *C. elegans* strains were attracted to odor of the control attractant isoamyl alcohol in as L4 larvae (Supplementary Figure 4, Supplementary Tables 3, 7). L4 larvae of N2 were repelled by the odor of the control repellent 1-octanol while *C. elegans* MY2079 L4 larvae showed variability in response to 1-octanol odor. Dauer larvae of both *C. elegans* strains showed a high variability in the response to both the attraction and repellent controls.

## Discussion

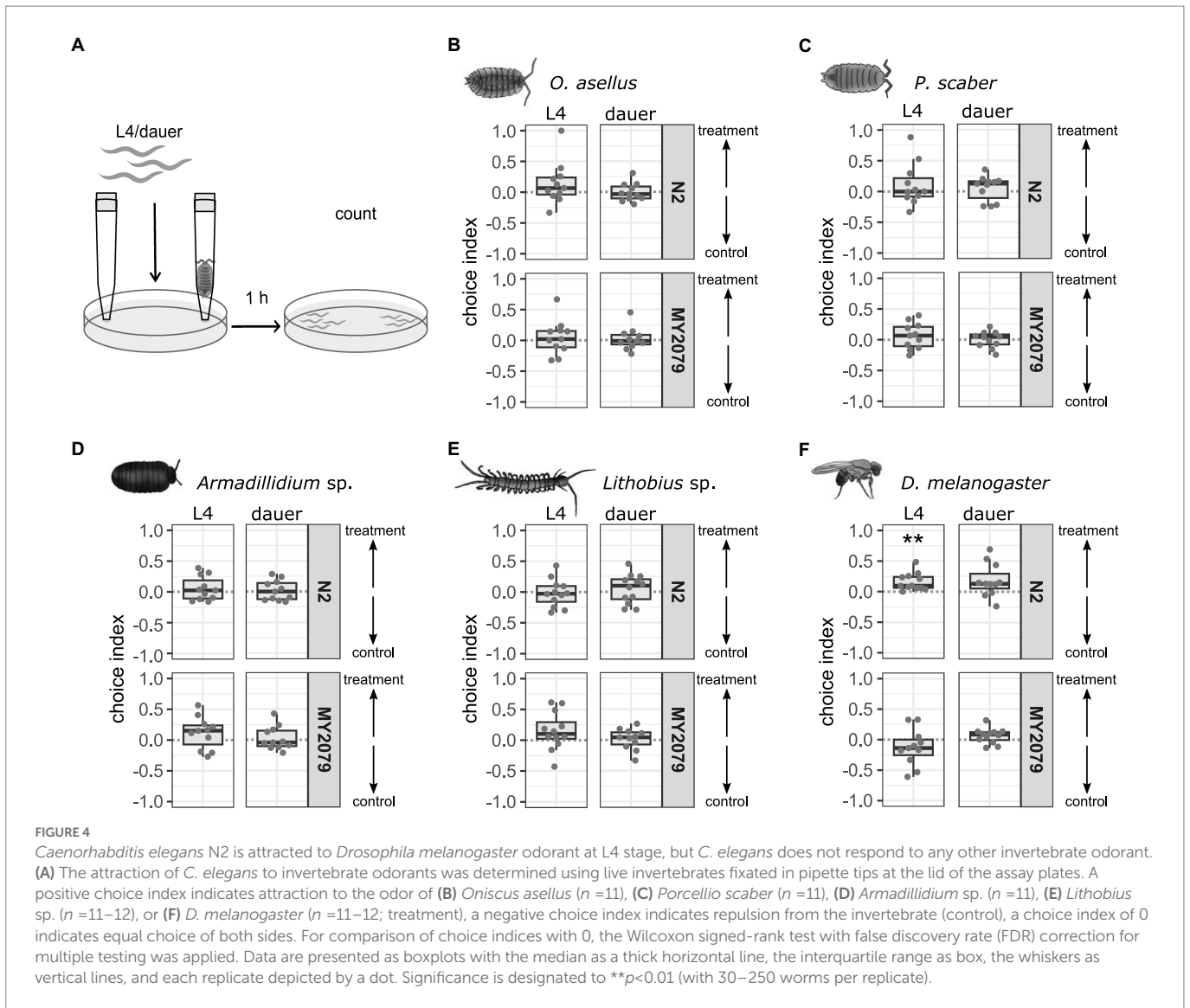
We studied the attraction of *C. elegans* to secreted compounds and volatile odorants of representatives of natural hosts. Our results indicate that *C. elegans* is not attracted to secreted compounds and volatile odorants of invertebrates, with the exception of *Lithobius* sp. wash and *D. melanogaster* odorants that were slightly attractive to N2 L4 larvae. The attraction was only observed towards *Lithobius* sp. DMSO wash and not towards ground animals or *Lithobius* sp. odorants indicating that (a) soluble attractive compounds are likely located at the body surface of *Lithobius* sp. and (b) the attracting compound is likely insoluble or slightly soluble in water and ethanol. DMSO is an efficient solvent, and some compounds may be more soluble in DMSO than in

water or ethanol (Clark et al., 2008; Alastruey-Izquierdo et al., 2012), hinting to a dose-dependent attraction towards compounds in *Lithobius* sp. wash. However, a dose-dependent attraction of *C. elegans* to the isopod *P. scaber* was not detected (Archer et al., 2020). *Drosophila* occurs in the natural habitat of *C. elegans* and can disperse nictating *C. elegans* dauer larvae, but not non-dauer stages, under laboratory conditions (Lee et al., 2012). In contrast, N2 L4 larvae but not dauer larvae were attracted to live *D. melanogaster* odorants in our experiments indicating that more than only nictation behavior may be involved in *C. elegans* dispersal.

No other extracted invertebrate compound or odorant attracted *C. elegans*. These results were unexpected. In particular, we expected that some invertebrates would have an attracting effect for the natural *C. elegans* isolate MY2079, as a change of location is likely important for survival in the wild. Although this natural strain has been shown to be generally capable of chemotaxis (Petersen et al., 2021), it was surprisingly not attracted to any invertebrate. Similarly, Archer et al. found no attraction of *C. elegans* N2 and a wild isolate to the isopod *P. scaber* (Archer et al., 2020). Overall, our results suggest that migration of *C. elegans* by invertebrates is opportunistic rather than *C. elegans* being attracted to invertebrates.

All isopods, myriapods, and slugs used in this study were collected in the wild. The altered environment of the laboratory may have resulted in a change in the chemical signals emitted and the subsequent chemoattraction of *C. elegans*. Furthermore, the sampling sites for isopods and myriapods were poor in decaying plant material and may have resulted in a microbe-poor environment. This could play a role if the chemical signals emitted by invertebrates are influenced by factors from the natural environment, e.g., microbial products could contribute to odorants, leading to microbially mediated olfactory communication (Carthey et al., 2018).

It has been reported that *C. elegans* dauer larvae respond neutral or repulsive to odorants of potential hosts such as mole crickets, earwigs,



flatheaded borers, pill bugs, and slugs, whereas parasitic nematodes from the genera *Heterorhabditis*, *Steinernema*, and *Oscheius* are attracted to some or all the mentioned hosts (Dillman et al., 2012). Similarly, extracts of ground invertebrates repeatedly had a repellent effect in our study. We hypothesize that this is due to the release of chemical defenses stored in the invertebrates or produced in response to the stressful washing procedure. Production, storage, and release of chemical defenses is known to occur in many invertebrates. Herbivorous insects such as the horseradish flea beetle selectively accumulate plant toxins in the hemolymph to defend themselves against predators (Yang et al., 2021). The secretions of chemical defense glands located along the body of some millipedes may consist of irritants, repellents, antifeedants, or even hydrogen cyanide gas, which is lethal to some other species (Eisner et al., 1978; Shear, 2015). Additionally, microbial symbionts may contribute to the production of repellent chemicals against predators, as hypothesized for the ladybird *Harmonia axyridis* (Schmidtberg et al., 2019).

Responses of dauer larvae to many chemical attractants are similar to responses of non-dauer stages (Ward, 1973; Cassada and Russell, 1975). However, sometimes dauer larvae do not respond to known attractants, such as sodium ions (Na<sup>+</sup>), and lack the rapid chemotactic response to food bacteria seen in well-fed larvae and adults (Albert and

Riddle, 1983). In our study dauer larvae were not attracted to any invertebrate and failed repeatedly to respond in particular to the control repellent 1-octanol, a lack of response not seen in Archer et al. (2020). This may be due to the fact that Archer et al. generated dauer larvae within 48h through exposure to dauer pheromone in liquid medium while we placed agar plates for 2–3 weeks at 25°C. Therefore, the dauer larvae in our study were older and thus exposed longer to the continuously secreted dauer pheromone, which may have affected olfactory plasticity (Yamada et al., 2010).

In summary, our results suggest that *C. elegans* appears to be attracted to some invertebrate compounds, but most of the associations between *C. elegans* and the invertebrates studied are likely opportunistic rather than directed. The future use of different dosages of the invertebrate extracts and washes, as well as the use of solvents with a different solubility spectrum, could help to further deepen the knowledge gained. It is possible that factors other than chemical signals play a role in attraction to a host. Diverse sensory neurons enable *C. elegans* to sense not only chemical cues, but also to respond to physical stimuli (Goodman and Sengupta, 2019). Thus, mechanical stimuli from host movement or thermal stimuli from host body temperature could contribute to host perception. Testing attraction in

response to different stimuli from potential hosts is an interesting future step.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

CP: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, visualization, writing—original draft, and writing—review and editing. AK: investigation. ML: resources. All authors read and approved of the final manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2023.1069056/full#supplementary-material>



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