


Caenorhabditis elegans nematodes are not attracted to the terrestrial isopod Porcellio scaber   
 --Manuscript Draft--

<b>Manuscript Number:</b>	PONE-D-20-06744
<b>Article Type:</b>	Research Article
<b>Full Title:</b>	Caenorhabditis elegans nematodes are not attracted to the terrestrial isopod Porcellio scaber
<b>Short Title:</b>	C. elegans and P. scaber likely interact opportunistically
<b>Corresponding Author:</b>	Erik C Andersen, Ph.D. Northwestern University Evanston, IL UNITED STATES
<b>Keywords:</b>	C. elegans; P. scaber; Chemotaxis; Phoresy
<b>Abstract:</b>	Phoresy is a behavior in which an organism, the phoront, travels from one location to another by 'hitching a ride' on the body of a host as it disperses. Some phoronts are generalists, taking advantage of any available host. Others are specialists and travel only when specific hosts are located using chemical cues to identify and move (chemotax) toward the preferred host. Free-living nematodes, like Caenorhabditis elegans, are often found in natural environments that contain terrestrial isopods and other invertebrates. Additionally, the C. elegans wild strain PB306 was isolated associated with the isopod Porcellio scaber. However, it is currently unclear if C. elegans is a phoront of terrestrial isopods, and if so, whether it is a specialist, generalist, or developmental stage-specific combination of both strategies. Because the relevant chemical stimuli might be secreted compounds or volatile odorants, we used different types of chemotaxis assays across diverse extractions of compounds or odorants to test whether C. elegans is attracted to P. scaber. We show that two different strains – the wild isolate PB306 and the laboratory-adapted strain N2 – are not attracted to P. scaber during either the dauer or adult life stages. Our results indicate that C. elegans was not attracted to chemical compounds or volatile odorants from P. scaber, providing valuable empirical evidence to suggest that any associations between these two species are likely opportunistic rather than specific phoresy.
<b>Order of Authors:</b>	Heather Archer Selina Deiparine Erik C Andersen, Ph.D.
<b>Additional Information:</b>	
<b>Question</b>	<b>Response</b>
<b>Financial Disclosure</b>  Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the <a href="#">submission guidelines</a> for detailed requirements. View published research articles from <a href="#">PLOS ONE</a> for specific examples.  This statement is required for submission and <b>will appear in the published article</b> if the submission is accepted. Please make sure it is accurate.	This work was funded by an NSF CAREER Award (1751035) to E.C.A. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Unfunded studies**

Enter: *The author(s) received no specific funding for this work.*

**Funded studies**

Enter a statement with the following details:

- Initials of the authors who received each award
- Grant numbers awarded to each author
- The full name of each funder
- URL of each funder website
- Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript?
- **NO** - Include this sentence at the end of your statement: *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*
- **YES** - Specify the role(s) played.

\* typeset

**Competing Interests**

Use the instructions below to enter a competing interest statement for this submission. On behalf of all authors, disclose any [competing interests](#) that could be perceived to bias this work—acknowledging all financial support and any other relevant financial or non-financial competing interests.

This statement **will appear in the published article** if the submission is accepted. Please make sure it is accurate. View published research articles from [PLOS ONE](#) for specific examples.

The authors have declared that no competing interests exist.

**NO authors have competing interests**

Enter: *The authors have declared that no competing interests exist.*

**Authors with competing interests**

Enter competing interest details beginning with this statement:

*I have read the journal's policy and the authors of this manuscript have the following competing interests: [insert competing interests here]*

\* typeset

**Ethics Statement**

N/A

Enter an ethics statement for this submission. This statement is required if the study involved:

- Human participants
- Human specimens or tissue
- Vertebrate animals or cephalopods
- Vertebrate embryos or tissues
- Field research

Write "N/A" if the submission does not require an ethics statement.

General guidance is provided below. Consult the [submission guidelines](#) for detailed instructions. **Make sure that all information entered here is included in the Methods section of the manuscript.**

**Format for specific study types**

**Human Subject Research (involving human participants and/or tissue)**

- Give the name of the institutional review board or ethics committee that approved the study
- Include the approval number and/or a statement indicating approval of this research
- Indicate the form of consent obtained (written/oral) or the reason that consent was not obtained (e.g. the data were analyzed anonymously)

**Animal Research (involving vertebrate animals, embryos or tissues)**

- Provide the name of the Institutional Animal Care and Use Committee (IACUC) or other relevant ethics board that reviewed the study protocol, and indicate whether they approved this research or granted a formal waiver of ethical approval
- Include an approval number if one was obtained
- If the study involved *non-human primates*, add *additional details* about animal welfare and steps taken to ameliorate suffering
- If anesthesia, euthanasia, or any kind of animal sacrifice is part of the study, include briefly which substances and/or methods were applied

**Field Research**

Include the following details if this study involves the collection of plant, animal, or other materials from a natural setting:

- Field permit number
- Name of the institution or relevant body that granted permission

**Data Availability**

Authors are required to make all data underlying the findings described fully available, without restriction, and from the time of publication. PLOS allows rare exceptions to address legal and ethical concerns. See the [PLOS Data Policy](#) and [FAQ](#) for detailed information.

Yes - all data are fully available without restriction

A Data Availability Statement describing where the data can be found is required at submission. Your answers to this question constitute the Data Availability Statement and **will be published in the article**, if accepted.

**Important:** Stating 'data available on request from the author' is not sufficient. If your data are only available upon request, select 'No' for the first question and explain your exceptional situation in the text box.

Do the authors confirm that all data underlying the findings described in their manuscript are fully available without restriction?

**Describe where the data may be found in full sentences. If you are copying our sample text, replace any instances of XXX with the appropriate details.**

- If the data are **held or will be held in a public repository**, include URLs, accession numbers or DOIs. If this information will only be available after acceptance, indicate this by ticking the box below. For example: *All XXX files are available from the XXX database (accession number(s) XXX, XXX).*
- If the data are all contained **within the manuscript and/or Supporting Information files**, enter the following: *All relevant data are within the manuscript and its Supporting Information files.*
- If neither of these applies but you are able to provide **details of access elsewhere**, with or without limitations, please do so. For example:

*Data cannot be shared publicly because of [XXX]. Data are available from the XXX Institutional Data Access / Ethics Committee (contact via XXX) for researchers who meet the criteria for access to confidential data.*

*The data underlying the results presented in the study are available from (include the name of the third party*

All relevant data are within the manuscript and its Supporting Information files.

*and contact information or URL).*

- This text is appropriate if the data are owned by a third party and authors do not have permission to share the data.

\* typeset

Additional data availability information:

***Caenorhabditis elegans* nematodes are not attracted to the terrestrial isopod *Porcellio scaber***

Heather Archer, Selina Deiparine, and Erik C. Andersen\*

Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, USA

\*Corresponding author:

Erik C. Andersen

Department of Molecular Biosciences

Northwestern University

4619 Silverman Hall

2205 Tech Drive

Evanston, IL 60208

847-467-4382

[erik.andersen@northwestern.edu](mailto:erik.andersen@northwestern.edu)

ORCIDs:

Erik: 0000-0003-0229-9651

Heather: 0000-0002-5344-2115

Running title: *C. elegans* and *P. scaber* likely interact opportunistically

Keywords: *C. elegans*, *P. scaber*, Chemotaxis, Phoresy

## ABSTRACT

Phoresy is a behavior in which an organism, the phoront, travels from one location to another by 'hitching a ride' on the body of a host as it disperses. Some phoronts are generalists, taking advantage of any available host. Others are specialists and travel only when specific hosts are located using chemical cues to identify and move (chemotax) toward the preferred host. Free-living nematodes, like *Caenorhabditis elegans*, are often found in natural environments that contain terrestrial isopods and other invertebrates. Additionally, the *C. elegans* wild strain PB306 was isolated associated with the isopod *Porcellio scaber*. However, it is currently unclear if *C. elegans* is a phoront of terrestrial isopods, and if so, whether it is a specialist, generalist, or developmental stage-specific combination of both strategies. Because the relevant chemical stimuli might be secreted compounds or volatile odorants, we used different types of chemotaxis assays across diverse extractions of compounds or odorants to test whether *C. elegans* is attracted to *P. scaber*. We show that two different strains – the wild isolate PB306 and the laboratory-adapted strain N2 – are not attracted to *P. scaber* during either the dauer or adult life stages. Our results indicate that *C. elegans* was not attracted to chemical compounds or volatile odorants from *P. scaber*, providing valuable empirical evidence to suggest that any associations between these two species are likely opportunistic rather than specific phoresy.



## INTRODUCTION

A phoretic animal, or phoront, hitches a temporary ride on a host in order to disperse to new locations. It is a commensal relationship between phoront and host, and phoronts can be generalists using numerous host species or specialists with a single or very few specific hosts. Most phoronts are animals that have a limited ability to travel any significant distance under their own power. In order to disperse, they must rely on the movement of a more mobile host, e.g. mites traveling via beetles or lice hitching a ride on hippoboscids flies (Bartlow *et al.*, 2016; Baumann, 2018). Animals with such low mobility often have a fitness advantage if they disperse to new habitats because dispersal reduces competition for food and/or mates, helps individuals avoid predation, and can facilitate increased gene flow between populations thereby reducing an accumulation of deleterious mutations and inbreeding depression. Moreover, when populations are dependent on unstable food sources, animals must disperse to find a new food source or populations will starve. Therefore, phoretic interactions between phoront and host are critical to species survival.

The nematode *Caenorhabditis elegans* has been isolated in association with several terrestrial invertebrate species, including snails, slugs, and isopods (Schulenburg & Félix, 2017). These associations have assumed to capture phoretic relationships where *C. elegans* is using larger invertebrates as vectors for travel. Consistent with this observation, *C. elegans* dauer larvae appear to seek dispersal vectors using a stage-specific behavior (called nictation) in which individuals stand on their tails, move their bodies in a waving motion, and attach themselves to objects passing nearby such as larger invertebrates (Lee *et al.*, 2012). Moreover, genetic differences across natural populations influence this behavior, which suggests that variation in this trait is subject to evolutionary selection (Lee *et al.*, 2017). However, it is unknown whether *C. elegans* seeks out specific invertebrates as phoretic hosts or randomly attaches to whatever organism happens to be nearby. Additionally, although dauer larvae are the life stage most frequently found in association with invertebrates, other life stages have been isolated

(Schulenburg & Félix, 2017; Petersen *et al.*, 2015; Félix & Duveau, 2012), suggesting that phoretic association with invertebrates is not necessarily limited to the dauer stage and to nictation behavior.

*C. elegans* might have specific phoretic hosts similar to what has been observed in other closely related nematode species. For example, phoretic associations between *Pristionchus pacificus* and scarab beetles (Herrmann *et al.*, 2006), *Caenorhabditis japonica* and the shield bug *Parastrachia japonensis* (Kiontke *et al.*, 2002; Tanaka *et al.*, 2010), and the facultative parasite *Phasmarhabditis hermaphrodita* and slugs of the genus *Arion* or *Deroceras* as well as the snail *Helix aspersa* (Rae *et al.*, 2009; Félix *et al.*, 2018; Andrus & Rae, 2019). Previous studies have shown that mucus of the slug *Arion subfuscus* and the snail *Helix aspersa* act as strong chemoattractants for *P. hermaphrodita* (Rae *et al.*, 2009; Andrus & Rae, 2019). This association with slugs and snails has also been observed with *C. elegans*, where nematodes have been recovered from the intestines and feces of *Arion sp.* slugs (Petersen *et al.*, 2015). Taken together, these observations suggest that *C. elegans* might also detect and move toward chemical cues from invertebrates that act as hosts for dispersal.

To determine whether phoretic interactions between *C. elegans* and the terrestrial isopod *Porcellio scaber* are facilitated by a chemical stimulus, we tested the chemotactic behaviors of *C. elegans* toward *P. scaber*. Because the relevant chemical stimuli might be secreted compounds or volatile odorants, we used different types of chemotaxis assays across diverse extractions of compounds or odorants. Additionally, we tested two different genetic backgrounds and different developmental stages. Across all these different conditions, our results indicate that *C. elegans* was not attracted to chemical compounds or volatile odorants from the isopod *P. scaber*, providing valuable empirical evidence to suggest that any associations between these two species are likely opportunistic.

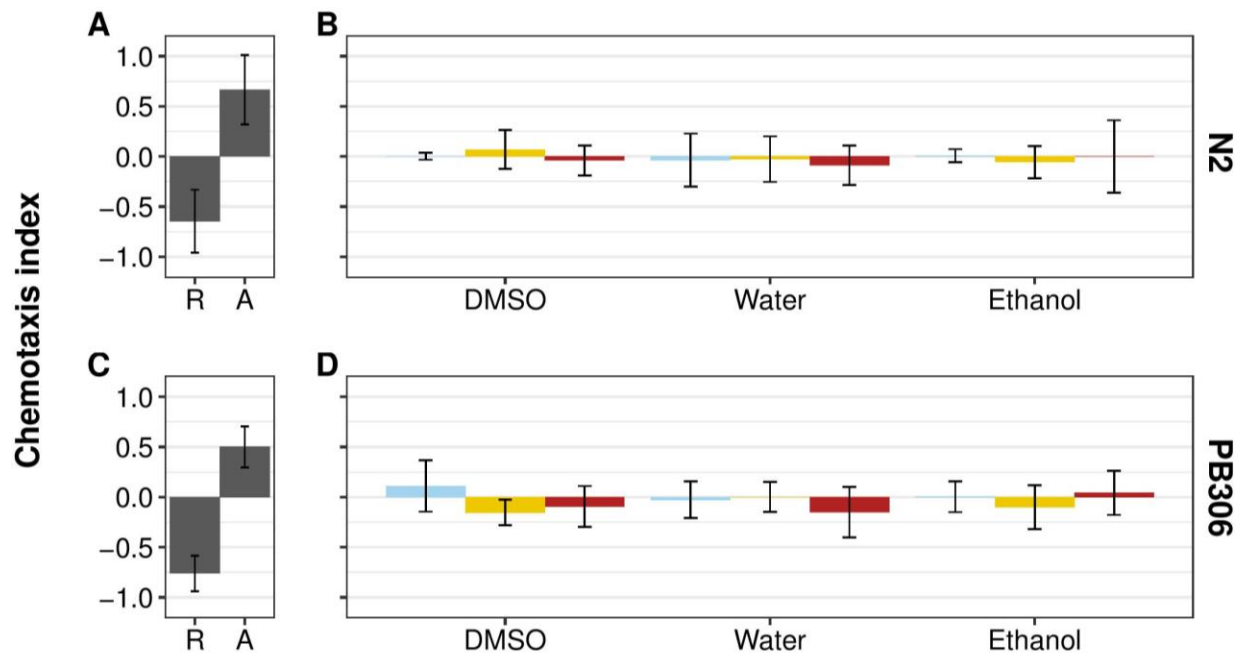
## RESULTS

The canonical *C. elegans* strain, N2, has been continuously domesticated in a laboratory environment since its initial isolation from mushroom compost in 1951 (Sterken *et al.*, 2015). This long-term propagation caused the accumulation of laboratory-derived alleles with associated phenotypic effects, and any research to understand *C. elegans* behavior in the wild must take this potential limitation into consideration. Because it is unlikely that N2 has had any interactions with invertebrates during its domestication in the lab, invertebrate-associated traits could have been lost (Persson *et al.*, 2009; McGrath *et al.*, 2011; Dubeau & Félix, 2012; Andersen *et al.*, 2014). The *C. elegans* strain PB306 is a wild isolate collected in 1998 as dauer juveniles from the body of an isopod (*Porcellio scaber*) and cryopreserved. These isopods were obtained in 1998 from Connecticut Valley Biological Supply by Scott Baird (Rockman & Kuglyak (2009), and the geographic origin of the isopods is not known. As such, it is likely to have invertebrate-associated traits intact and is reasonable to hypothesize that these traits would be observed in interactions with *P. scaber*. For these reasons, we tested both of these strains for chemoattraction towards *P. scaber* compounds that could be secreted or attached to the surface of the isopod. Because the chemical nature of any potential secretions is unknown, a set of three solvents capable of solubilizing both polar and nonpolar compounds was used: ethanol, dimethyl sulfoxide (DMSO), and deionized water. None of these three compounds elicited any chemotactic behaviors (attraction or repulsion, Supplemental Figure 1).

### ***C. elegans* adults are not attracted to compounds from the terrestrial isopod *P. scaber***

If *C. elegans* seeks out specific hosts, then one possible hypothesis is that chemicals secreted by the host into the local environment form a basis for seeking behaviors. The terrestrial isopod *Armadillidium vulgare* uses sex-specific short-distance chemical cues for mate attraction (Beauché & Richard, 2013). This result suggests that another isopod, *P. scaber*, could also secrete different pheromones between the sexes. For this reason, we treated male and female

isopods separately. In order to test whether secretions act as a chemoattractant for *C. elegans*, adult male and female isopods were washed, and the wash solution was used to test chemoattraction in standard assays (Bargmann *et al.*, 1993; Margie *et al.*, 2013). Post-hoc Tukey's Honest Significant Difference (HSD) tests showed that neither N2 nor PB306 had a statistically significant attractive or repulsive behavior towards any isopod wash (Figure 1, Supplemental Figure 2, Supplemental Table 1, Supplemental Table 2). However, both strains were repelled by the control repellent 1-octanol and attracted to the control attractant isoamyl alcohol (Bargmann *et al.*, 1993; Supplemental Figure 1; Supplemental Table 1; Supplemental Table 2). Furthermore, we found no significant differences between male or female isopod washes, indicating a lack of dependence on the sex of any chemical cocktail.



**Figure 1.** *C. elegans* N2 and PB306 strains respond neutrally to *P. scaber* washes with three solvents. (A) Plot of repulsive (R) and attractive (A) reference control chemotactic indices for the *C. elegans* N2 strain. Undiluted 1-octanol was used as the repulsive negative control (left) and isoamyl alcohol diluted to  $10^{-3}$  in ethanol was used as the attractive positive control (right). (B) Plot of chemotactic indices of N2 adult *C. elegans* to undiluted *P. scaber* washes compared to neutral control references. Blue bars represent the neutral control of solvent alone. Yellow bars represent chemotactic indices to washes that were prepared from male isopods. Red bars

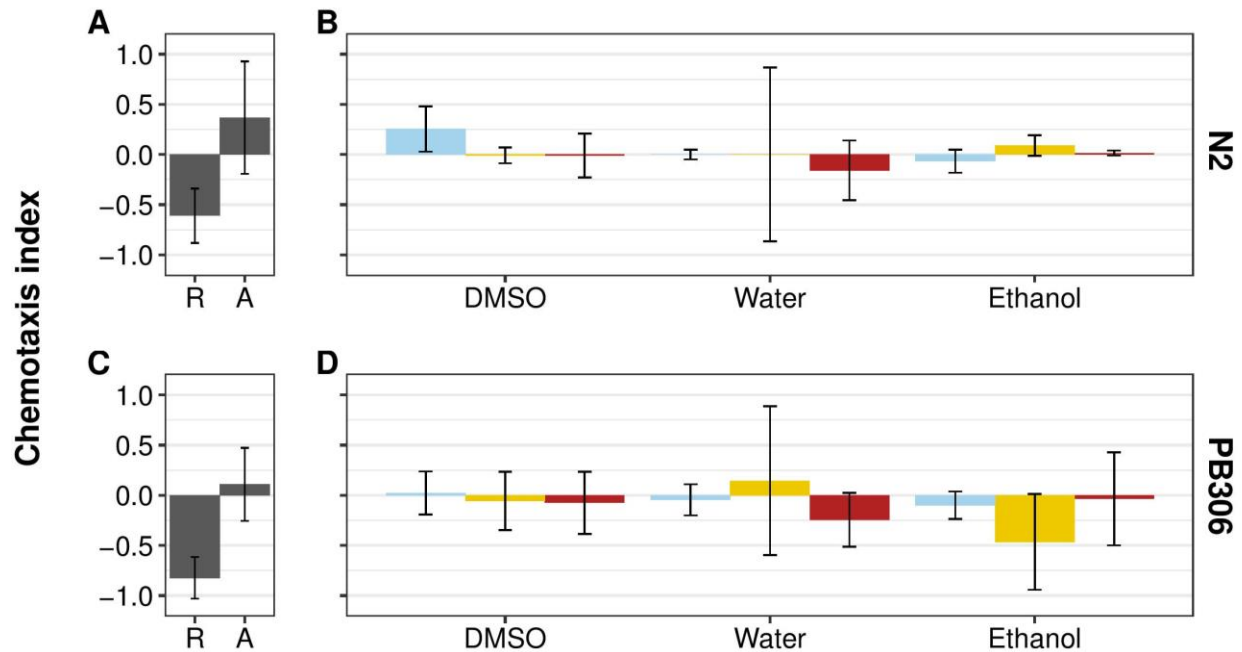
represent chemotactic indices to washes that were prepared from female isopods. From left to right, each grouping of bars represents dimethyl sulfoxide (DMSO), deionized water, and ethanol washes. No isopod wash was significantly different from its corresponding neutral control (see Supplemental Table 5 for *p* values). **(C)** Plot of repulsive (R) and attractive (A) reference control chemotactic indices for the *C. elegans* PB306 strain. Undiluted 1-octanol was used as the repulsive negative control (left), and isoamyl alcohol diluted to  $10^{-3}$  in ethanol was used as the attractive positive control (right). **(D)** Plot of chemotactic indices of PB306 adult *C. elegans* to undiluted *P. scaber* washes compared to neutral control references. Blue bars represent the neutral control of solvent alone. Yellow bars represent chemotactic indices to washes that were prepared from male isopods. Red bars represent chemotactic indices to washes that were prepared from female isopods. From left to right, each grouping of bars represents dimethyl sulfoxide (DMSO), deionized water, and ethanol washes. Chemotactic indices from all isopod washes were not significantly different from the chemotactic indices of its corresponding neutral control (*p* values are listed in Supplemental Table 5).

It is possible that chemoattractants from *P. scaber* are not secreted or solubilized by washes of the isopod surface. For example, fecal matter could contain a chemoattractant that is either not captured or is present in such low density in our wash methodology that it fails to elicit a chemotaxis response. Extractions of physically disrupted, ground whole animals should contain compounds that could be attractive to *C. elegans*. To capture these compounds and test this hypothesis, a whole isopod body was ground into each of the three solvents (DMSO, ethanol, and water) after washing as previously described to make a heterogeneous mixture. Like the previous washes, the N2 strain did not have a statistically significant attraction or repulsion to extractions made with any solvent (Supplemental Figure 2, Supplemental Table 1). Because no significant effects of extractions were observed, we did not test the PB306 strain.

### ***C. elegans* dauers are not attracted to compounds washed from the terrestrial isopod *P. scaber***

Our results suggest that *C. elegans* adults are not attracted to compounds from *P. scaber* in laboratory chemotaxis assays. However, the life stage most commonly found in association with phoretic vectors is the dauer juvenile. This developmentally arrested larval stage is analogous to the 'infective juvenile' stage in entomopathogenic nematodes (Riddle & Albert, 1997; Crook, 2014). As the name implies, this stage has host-seeking behavior, suggesting the

possibility that the *C. elegans* dauer stage is more likely to seek phoretic interactions than adults. Therefore, we tested the hypothesis that dauer individuals are attracted to chemical compounds washed from *P. scaber* in the standard chemotaxis assays used for the adult stage (Figure 2, Supplemental Figure 4, Supplemental Table 3). Post-hoc Tukey's HSD tests showed that both N2 and PB306 dauers did not have statistically significant attractive or repulsive behaviors toward any isopod wash, suggesting that responses to *P. scaber* do not vary based on the developmental stage of *C. elegans*.

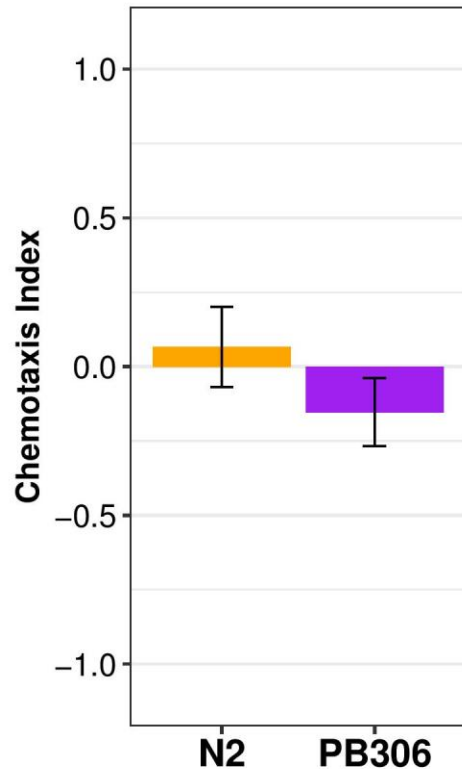


**Figure 2.** *C. elegans* N2 and PB306 dauer animals respond neutrally to *P. scaber* washes with three solvents. **(A)** Plot of repulsive (R) and attractive (A) reference control chemotactic indices for the N2 strain dauers. Undiluted 1-octanol was used as the repulsive negative control (left) and isoamyl alcohol diluted to  $10^{-3}$  in ethanol was used as the attractive positive control (right). **(B)** Plot of chemotactic indices of N2 dauer *C. elegans* to undiluted *P. scaber* washes compared to neutral control references. Blue bars represent the neutral control of solvent alone. Yellow bars represent chemotactic indices to washes that were prepared from male isopods. Red bars represent chemotactic indices to washes that were prepared from female isopods. From left to right, each grouping of bars represents dimethyl sulfoxide (DMSO), deionized water, and ethanol washes. No isopod wash was significantly different from its corresponding neutral control (see Supplemental Table 5 for *p* values). **(C)** Plot of repulsive (R) and attractive (A) reference control chemotactic indices for the PB306 strain dauers. Undiluted 1-octanol was used as the repulsive negative control (left) and isoamyl alcohol diluted to  $10^{-3}$  in ethanol was used as the attractive positive control (right). **(D)** Plot of chemotactic indices of PB306 dauer *C. elegans* to undiluted *P.*

*scaber* washes compared to neutral control references. Blue bars represent the neutral control of solvent alone. Yellow bars represent chemotactic indices to washes that were prepared from male isopods. Red bars represent chemotactic indices to washes that were prepared from female isopods. From left to right, each grouping of bars represents dimethyl sulfoxide (DMSO), deionized water, and ethanol washes. No isopod wash was significantly different from its corresponding neutral control ( $p$  values are listed in Supplemental Table 5).

### ***C. elegans* adults are not attracted to *P. scaber* odorants**

Rather than being attracted to compounds found on the body of a host, some parasitic nematode species are attracted to gaseous components of odorants, such as carbon dioxide (CO<sub>2</sub>), secreted by host invertebrates (Dillman *et al.*, 2012). Moreover, both specialist and generalist entomopathogenic nematodes responded to CO<sub>2</sub>, suggesting attraction to volatile odorants could be common. To test this hypothesis using adult *C. elegans*, we adapted a gas assay (Dillman *et al.*, 2012) to measure chemotaxis in response to volatile odorants from *P. scaber* (Figure 3, Supplemental Table 4). The N2 strain of *C. elegans* was not significantly attracted to *P. scaber* odorants (mean CI = 0.07) and strain PB306 was weakly repulsed (mean CI = -0.15). Overall, *P. scaber* odorants were not an attractant for either *C. elegans* strain to seek isopods.



**Figure 2.** *C. elegans* N2 and PB306 strains respond neutrally to *P. scaber* gas secretions. The orange bar (left) represents the chemotactic indices of N2 adults to *P. scaber* gas secretions from at least three independent experiments. The purple bar (right) represents the chemotactic indices of PB306 adults to *P. scaber* gas secretions from at least three independent experiments (see Supplemental Table 5 for *p* values).

## DISCUSSION

Using chemotaxis assays, our results demonstrate that *C. elegans* is neither attracted to nor repulsed by chemical cues from the isopod *P. scaber* in laboratory-based chemotaxis assays. These results suggest that *C. elegans* phoresy might not be directed toward specific hosts but is instead opportunistic. Moreover, Lee *et al.* (2012) showed that the dauer specific behavior, nictation, is both an opportunistic behavior towards a phoretic host and is necessary for dauer individuals to disperse via the fruit fly *Drosophila melanogaster* (see Lee *et al.* (2017) for a discussion of the genetic basis underlying variation in this behavior in natural populations). This result is consistent with the observation that the dauer life stage is the one most commonly found



in association with invertebrates without any obvious chemical cue. However, this result does not preclude the possibility of chemoattraction to preferred hosts at other life stages but did demonstrate that for dauer individuals dispersal only happened when nictation behavior was present.

In Europe, *C. elegans* has been isolated from habitats shared with *Caenorhabditis briggsae*, including co-isolation on arthropod and mollusk hosts (Félix & Duveau, 2012). In this case, it appears the primary ecological difference between these species is optimal temperature, creating a temporal rhythm where *C. briggsae* dominates when temperatures are warmer and *C. elegans* dominates when temperatures are cooler (Félix & Duveau, 2012; Stegeman *et al.*, 2013). Given that *C. briggsae* has repeatedly been sampled from a variety of mollusk hosts, this result suggests it is possible that at least some *C. elegans* could share an attraction to mollusks. Alternatively, *C. briggsae* could be attracted to hosts and *C. elegans* just follows that species. *P. hermaphrodita* shares habitats and mollusk hosts with *C. elegans* and *C. briggsae* (Rae *et al.*, 2009; Petersen *et al.*, 2015; Schulenburg & Félix, 2017) and has been shown to chemotax toward the mucus, faeces, and volatile odorants of slugs and, in the case of snails, hyaluronic acid (Rae *et al.*, 2006, 2009; Hapca *et al.*, 2007; Small & Bradford, 2008; Nermut *et al.*, 2012; Andrus *et al.*, 2018). This observation suggests that *C. elegans* might prefer mollusks to isopods such as *P. scaber* and testing attraction to these species is a good future step.

## **MATERIALS AND METHODS**

### **Nematode Strains**

PB306 and N2. Strain data including isolation location and isotype information are available from the CeNDR website (<https://www.elegansvariation.org>) (Cook *et al.*, 2017).

### **Bacterial Strains, Growth Conditions, and Media**

Nematodes were grown at 20°C on modified nematode growth media (NGMA) containing 1% agar and 0.7% agarose to prevent burrowing (Andersen *et al.*, 2014) and fed the *Escherichia coli* strain OP50 according to standard methods. Plate-based chemotaxis assays were performed on 6 cm unseeded NGMA plates. Gas chemotaxis assays were performed on 10 cm unseeded NGMA plates.

### **Dauer induction**

Dauers were induced by bleaching gravid adults according to standard methods using K medium in place of 1X M9 solution two days before assays (Stiernagle, 2006). Embryos were titered and added to 1 mL K medium with *E. coli* HB101 lysate (5 g/1 L) and synthetic pheromone mix (5 mL/1 L) (Butcher *et al.*, 2007; Jeong *et al.*, 2005). After growing for 48 hours at 25°C, dauers were identified morphologically by their dark intestines and radially constricted bodies.

### **Isopod culture**

*Porcellio scaber* isopods were ordered as needed from [www.bugsincyberspace.com](http://www.bugsincyberspace.com). Isopods were confirmed as *P. scaber* by the animals' dull as opposed to waxy appearance, and sexed by immobilizing the animals with a short stream of carbon dioxide and examining the abdomen for key sex differences (Oliver & Meecham, 1993). Isopods were then placed into cultures based on methods from Bhella *et al.* (2006). In brief, cultures were prepared by cutting a one-inch hole in the lid of a plastic chamber, filling the chamber with deionized water, and threading a Kimtech

paper towel through the lid of the water-filled chamber and into a one-inch hole in the bottom of a smaller plastic container placed on top. This paper towel was used to wick moisture into and line the bottom of the smaller plastic container before filling the container halfway with dirt sterilized by autoclaving. Autoclaved leaves were then added as food for the isopods, and the containers were covered with a lid with air holes drilled through. Deionized water and autoclaved leaves were added to the appropriate chambers as needed, and isopods were divided into male and female chambers to prevent unwanted mating.

### **Isopod washes and extractions**

Isopods of the appropriate sex were sorted into individual 1 mL microcentrifuge tubes, with one animal per tube. 100  $\mu$ L of the desired solvent (ethanol, dimethyl sulfoxide, or deionized water) was then added to the tube, and washes were prepared by washing the isopods in the solvent for 30 minutes with the tubes rotated on a mutator. In the case of extractions, at the end of the 30 minutes, animals were centrifuged at 3000 RPM for 30 seconds then ground into the solvent in the microcentrifuge tube using a small pestle. One isopod was washed per 100  $\mu$ L of solvent for an undiluted (1X) concentration. Other concentrations were prepared by diluting the 1X stock with the appropriate solvent. New isopod test washes and extractions were prepared for every assay.

### **Chemotaxis assay**

The chemotaxis assay was adapted from Margie *et al.* (2013) and Bargmann *et al.* (1993). Assays were performed on unseeded 6 cm plates (Brenner 1974). Plates were prepared by applying a mask with a 0.5 cm circular center origin and dividing into four quadrants. Each quadrant contained a point labeled as either test or control. For chemotaxis assays of adults (N2 or PB306), 40-50 animals from a synchronized population were pipetted onto the center origin of the plate using a non-stick plastic pipette tip. Immediately following, one microliter of test compound, either wash or extraction, was added to opposing quadrants on the point labeled "test", and 1  $\mu$ L of

solvent was added to the remaining two quadrants labeled “control.” One microliter of 0.5 M sodium azide was pipetted into each of the quadrants as an anesthetic to immobilize the animals. Positive and negative control plates were also prepared, using isoamyl alcohol diluted 1:1000 in ethanol and 1-octanol, respectively, as the test compounds. After worms and compounds were added to the plates, lids were replaced and a timer was set for one hour during which plates were left at room temperature undisturbed. Plates were either scored by hand immediately after the one hour incubation or they were stored at 4°C and scored later the same day according to the following method:

$$\text{Chemotaxis Index} = \frac{(\text{Total \# Animals in Test Quadrants} - \text{Total \# Animals in Control Quadrants})}{(\text{Total \# Scored Animals})}$$

Animals that remained in the center origin, within 1 mm of the origin or the edges of the plates, or on the edges of the plates were not scored. A +1.0 chemotactic index score indicates maximum attraction to the test compound, and an index of -1.0 represents maximum repulsion.

### **Gas assay**

These assays were adapted from Dillman *et al.*, 2012. Assays were performed on unseeded 10-cm standard NGM plates. Plates were divided into halves labeled test and control. Each half contained a point marked 1 cm from the edge of the plate along the axis line that would divide the plate into quadrants. A hole was drilled in the plate lid above each point. For each hole, flexible PVC tubing was attached and connected to a 50 mL syringe. The control syringe was filled with room air, the test syringe contained six live adult *P. scaber* animals. 50-100 adult *C. elegans* of the appropriate strain (N2 or PB306) were pipetted into the center origin with a non-stick pipette tip, covers were replaced, and the syringes were depressed at a rate of 0.5 mL/min for 60 minutes

with a Harvard Apparatus Pump 22 syringe pump. Plates were then scored according to the following:

$$\text{Chemotaxis index} = (\text{Animals in Test Half} - \text{Animals in Control Half}) / (\text{Total Number of Animals})$$

Animals that remained in the center origin, within 1 mm of the origin or the edges of the plates, or on the edges of the plates were not scored. A +1.0 chemotaxis index score indicates maximum attraction to the test gas, and an index of -1.0 represents maximum repulsion.

### **Statistics and plotting**

Assays were performed in triplicate for every combination of nematode strain, isopod sex, and solvent concentration or gas. Mean chemotaxis indices (CI) were reported. Tukey HSD tests were performed comparing data from the test and neutral control plates. T-tests were performed to assess differences between a chemotactic index of zero and the relevant overall strain chemotactic index. All statistics presented in Supplemental Table 5.

### **Acknowledgements**

The authors would like to thank members of the Andersen lab for helpful comments on the manuscript.

### **Financial disclosure statement**

This work was funded by an NSF CAREER Award (1751035) to E.C.A. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## REFERENCES

- Andersen EC, Bloom JS, Gerke JP, Kruglyak L. A Variant in the Neuropeptide Receptor npr-1 Is a Major Determinant of *Caenorhabditis Elegans* Growth and Physiology. *PLoS Genet.* 2014; 10(2):e1004156.
- Andrus P, Ingle O, Coleman T, Rae R. Gastropod Parasitic Nematodes (*Phasmarhabditis* Sp.) Are Attracted to Hyaluronic Acid in Snail Mucus by cGMP Signalling. *J Helminthol.* 2018; 94:e9.
- Andrus P, Rae R. Development of *Phasmarhabditis* Hermaphrodita (And Members of the *Phasmarhabditis* Genus) as New Genetic Model Nematodes to Study the Genetic Basis of Parasitism. *J Helminthol.* 2019; 93(3):319-331.
- Bargmann CI, Hartweg E., Horvitz HR Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell.* 1993; 74:515–527.
- Barrière A, Félix M-A. High Local Genetic Diversity and Low Outcrossing Rate in *Caenorhabditis elegans*. *Curr Biol.* 2005; 15(13):1176-1184.
- Bartlow AW, Villa SM, Thompson MW, Bush SE. Walk or Ride? Phoretic Behaviour of Amblyceran and Ischnoceran Lice. *Int J Parasitol.* 2016; 46(4):221-227.
- Baumann, J. Tiny Mites on a Great Journey – A Review on Scutacarid Mites as Phoronts and Inquilines (Heterostigmatina, Pygmephoroidae, Scutacaridae). *Acarologia.* 2018; 58(1):192-251.
- Beauché F, Richard F-J. The Best Timing of Mate Search in *Armadillidium vulgare* (Isopoda, Oniscidea). *PLoS One.* 2013; 8(3):e57737.
- Bhella S, Fung E, Harrison J, Ing B, Larsen E, Selby R. Genetics of Pigmentation in *Porcellio scaber* Latreille, 1804 (Isopoda, Oniscidea). *Crustaceana.* 2006; 79(8):897-912.
- Brenner S. The Genetics of CAENORHABDITIS ELEGANS. *Genetics.* 1974; 77(1):71–94.
- Butcher RA, Fujita M, Schroeder FC, Clardy J. Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. *Nat Chem Biol.* 2007; 3:420–422.

- Chen J, Lewis EE, Carey J. R., Caswell, H., & Caswell-Chen, E. P. (2006). The ecology and biodemography of *Caenorhabditis elegans*. *Experimental Gerontology*, 41(10), 1059–1065. <http://doi.org/10.1016/j.exger.2006.07.005>
- Choi S, Chatzigeorgiou M, Taylor KP, Schafer WR, Kaplan JM. Analysis of NPR-1 reveals a circuit mechanism for behavioral quiescence in *C. elegans*. *Neuron*. 2013; 78(5):869–880. doi:10.1016/j.neuron.2013.04.002.
- Cook DE, Zdraljevic S, Roberts JP, Andersen EC. CeNDR, the *Caenorhabditis Elegans* Natural Diversity Resource. *Nucleic Acids Res*. 2017; 45(D1):D650-D657.
- Crook M. The dauer hypothesis and the evolution of parasitism: 20 years on and still going strong. *Int J Parasitol*. 2014; 44(1):1–8. doi:10.1016/j.ijpara.2013.08.004
- Denver DR, Dolan PC, Wilhelm LJ, Sung W, Lucas-Lledó JI, Howe DK, et al. A genome-wide view of *Caenorhabditis elegans* base-substitution mutation processes. *PNAS*. 2009; 106(38):16310–16314. doi:10.1073/pnas.0904895106.
- Dillman AR, Guillermin ML, Lee JH, Kim B, Sternberg PW, Hallem EA. Olfaction shapes host-parasite interactions in parasitic nematodes. *PNAS*. 2012; 109(35):E2324-E2333. doi:10.1073/pnas.1211436109.
- Duveau F, Félix M-A. Role of Pleiotropy in the Evolution of a Cryptic Developmental Variation in *Caenorhabditis elegans*. *PLoS Biol*. 2012; 10(1):e1001230.
- Eng MS, Preisse EL, Strong DR. Phoresy of the entomopathogenic nematode *Heterorhabditis marelatus* by a non-host organism, the isopod *Porcellio scaber*. *J Invertebr Pathol*. 2005; 88:173-176.
- Félix M-A, Braendle C. The natural history of *Caenorhabditis elegans*. *Curr Biol*. 2010; 20(22):R965–R969. doi:10.1016/j.cub.2010.09.050.
- Félix M-A, Duveau F. Population Dynamics and Habitat Sharing of Natural Populations of *Caenorhabditis Elegans* and *C. Briggsae*. *BMC Biol*. 2012; 10:59.

- Félix M-A, Ailion M, Hsu JC, Richaud A, Wang J. Pristionchus nematodes occur frequently in diverse rotting vegetal substrates and are not exclusively necromenic, while Panagrellus redivivoides is found specifically in rotting fruits. PLoS ONE. 2018; 13(8):e0200851. doi:10.1371/journal.pone.0200851.
- Frézal L, Félix M-A. C. elegans outside the Petri dish. eLife. 2015; 4:e05849. doi:10.7554/eLife.05849.
- Frøkaer-Jensen C, Ailion M, Lockery SR. Ammonium Acetate Is Sensed by Gustatory and Olfactory Neurons in Caenorhabditis elegans. PLoS ONE. 2008; 3(6):e2467. doi:10.1371/journal.pone.002467.
- Gutteling EW, Riksen JAG, Bakker J, Kammenga JE. Mapping phenotypic plasticity and genotype-environment interactions affecting life-history traits in Caenorhabditis elegans. Heredity. 2007; 98:28-37.
- Hapca S, Crawford J, Rae R, Wilson M, Young I. Movement of the parasitic nematode Phasmarhabditis hermaphrodita in the presence of mucus from the host slug Deroceras reticulatum. Biol Control. 2007; 41(2):223-9.
- Hart AC. Behavior. WormBook, ed. The C. elegans Research Community, WormBook; 2006. doi/10.1895/wormbook.1.87.1. Available from: <http://www.wormbook.org>.
- Harvey SC, Shorto A., Viney ME. Quantitative genetic analysis of life-history traits of Caenorhabditis elegans in stressful environments. BMC Evol Biol. 2008; 8:15. doi:10.1186/1471-2148-8-15.
- Herrmann M, Mayer WE, Sommer RJ. Nematodes of the Genus Pristionchus Are Closely Associated With Scarab Beetles and the Colorado Potato Beetle in Western Europe. Zoology. 2006; 109(2):96-108.
- Hu PJ. Dauer. WormBook, ed. The C. elegans Research Community, WormBook; August 08, 2007. doi/10.1895/wormbook.1.144.1. Available from: <http://www.wormbook.org>.



- Jeong P-Y, Jung M, Yim Y-H, Kim H, Park M, Hong E, et al. Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-Inducing pheromone. *Nature*. 2005; 433:541–545.
- Kammenga JE, Doroszuk A, Riksen JAG, Hazendonk E, Spiridon L, Petrescu A.-J, et al. A *Caenorhabditis elegans* Wild Type Defies the Temperature–Size Rule Owing to a Single Nucleotide Polymorphism in *tra-3*. *PLoS Genetics*. 2007; 3(3):e34. doi:10.1371/journal.pgen.0030034.
- Kiontke K, Hironaka M, Sudhaus W. Description of *Caenorhabditis japonica* n. sp. (Nematoda: Rhabditida) associated with the burrower bug *Parastrachia japonensis* (Heteroptera: Cydnidae) in Japan. *Nematology*. 2002; 4:933-941.
- Kiontke K, Sudhaus W. Ecology of *Caenorhabditis* species. *WormBook*, ed. The *C. elegans* Research Community, *WormBook*; January 09, 2006. doi/10.1895/wormbook.1.37.1. Available from <http://www.wormbook.org>.
- Lee H, Choi MK, Lee D, Kim HS, Hwang H, Kim H, et al. Nictation, a dispersal behavior of the nematode *Caenorhabditis elegans*, is regulated by IL2 neurons. *Nat Neurosci*. 2012; 15(1):107–12.
- Lee D, Lee H, Kim N, Lim DS, Lee J. Regulation of a hitchhiking behavior by neuronal insulin and TGF- $\beta$  signaling in the nematode *Caenorhabditis elegans*. *Biochem Biophys Res Commun*. 2017; 484:323–330.
- Lee D, Yang H, Kim J, Brady S, Zdraljevic S, Zamanian M., et al. The genetic basis of natural variation in a phoretic behavior. *Nat Commun*. 2017; 8:273. doi:10.1038/s41467-017-00386-x.
- Margie O, Palmer C, Chin-Sang I. *C. elegans* Chemotaxis Assay. *JoVE*. 2013; 74.
- McGrath PT, Rockman MV, Zimmer M, Jang H, Macosko EZ, Kruglyak L, Bargmann CI. Quantitative mapping of a digenic behavioral trait implicates globin variation in *C.*

- elegans sensory behaviors. *Neuron*. 2009; 61(5):692–699.  
doi:10.1016/j.neuron.2009.02.012.
- Nermut J, Puza V, Mracek Z. The response of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) and *Steinernema feltiae* (Nematoda: Steinernematidae) to different host-associated cues. *Biol Control*. 2012; 61 201-206.
- Oliver PG, Meecham CJ. *Woodlice: 1-136*. London: Linnean Society of London; 1993.
- Persson A, Gross E, Laurent P, Busch KE, Bretes H, de Bono M. Natural Variation in a Neural Globin Tunes Oxygen Sensing in Wild *Caenorhabditis Elegans*. *Nature*. 2009; 458 (7241):1030-3.
- Petersen C, Hermann RJ, Barg M.-C, Schalkowski R, Dirksen P, Barbosa C, Schulenburg H. Travelling at a slug's pace: possible invertebrate vectors of *Caenorhabditis nematodes*. *BMC Ecol*. 2015; 15(19). doi:10.1186/s12898-015-0050-z.
- Rae RG, Wilson MJ, Robertson JF. The chemotactic response of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) to cues of *Deroceras reticulatum* (Mollusca: Gastropoda). *Nematology*. 2006; 8(2):197-200.
- Rae RG, Robertson JF, Wilson MJ. Chemoattraction and Host Preference of the Gastropod Parasitic Nematode *Phasmarhabditis Hermaphrodita*. *J Parasitol*. 2009; 95(3):517-26.
- Riddle DL, Albert PS. Genetic and environmental regulation of dauer larva development. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR, editors. *C. elegans II*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1997. pp. 739–768.
- Rockman MV, Kruglyak L. Recombinational landscape and population genomics of *Caenorhabditis elegans*. *PLoS Genet*. 2009; 5 e1000419.
- Small RW, Bradford C. Behavioural responses of *Phasmarhabditis hermaphrodita* (Nematoda : Rhabditida) to mucus from potential hosts. *Nematology*. 2008; 10:591-598.

Stegeman GW, de Mesquita MB, Ryu WS, Cutter AD. Temperature-dependent Behaviours Are Genetically Variable in the Nematode *Caenorhabditis briggsae*. *J Exp Biol.* 2013; 216(Pt 5):850-8.

Sterken MG, Snoek LB, Kammenga JE, Andersen EC. The laboratory domestication of *Caenorhabditis elegans*. *Trends Genet.* 2015; 31(5):224–231.  
doi:10.1016/j.tig.2015.02.009

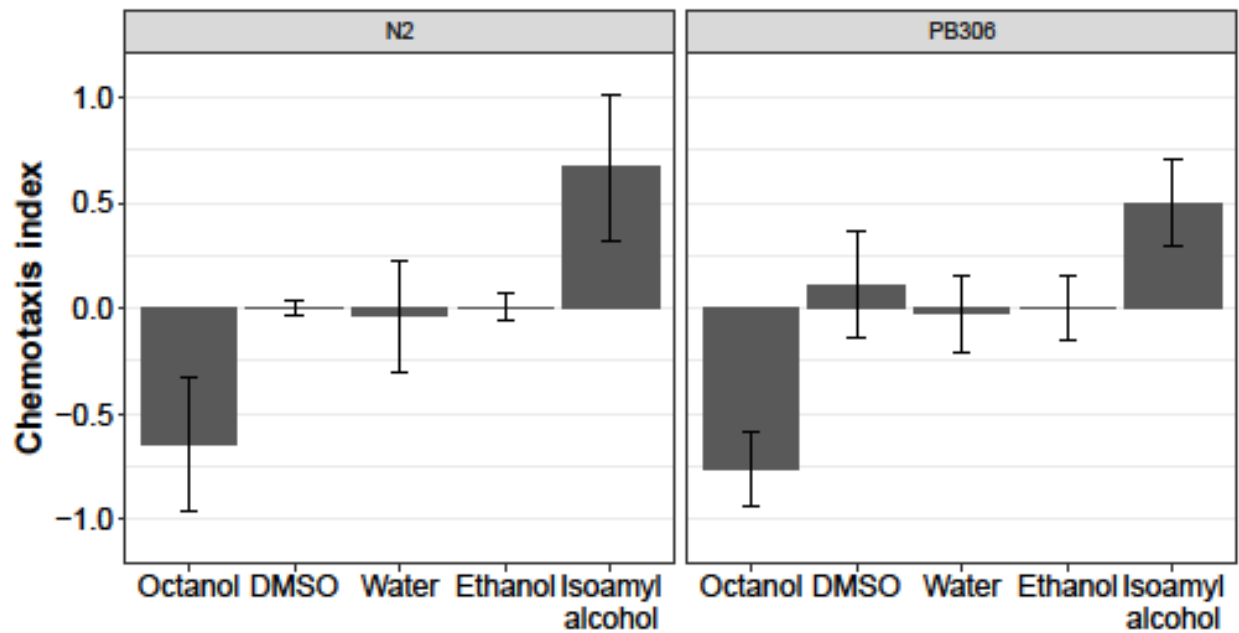
Stiernagle T. Maintenance of *C. elegans*. *WormBook*, ed. The *C. elegans* Research Community, *WormBook*; 2006. doi.org/10.1895/wormbook.1.101.1. Available from <http://www.wormbook.org>.

Styer KL, Singh V, Macosko E, Steele SE, Bargmann CI, Aballay A. Innate immunity in *Caenorhabditis elegans* is regulated by neurons expressing NPR-1/GPCR. *Science.* 2008; 322(5900):460–464. doi:10.1126/science.1163673.

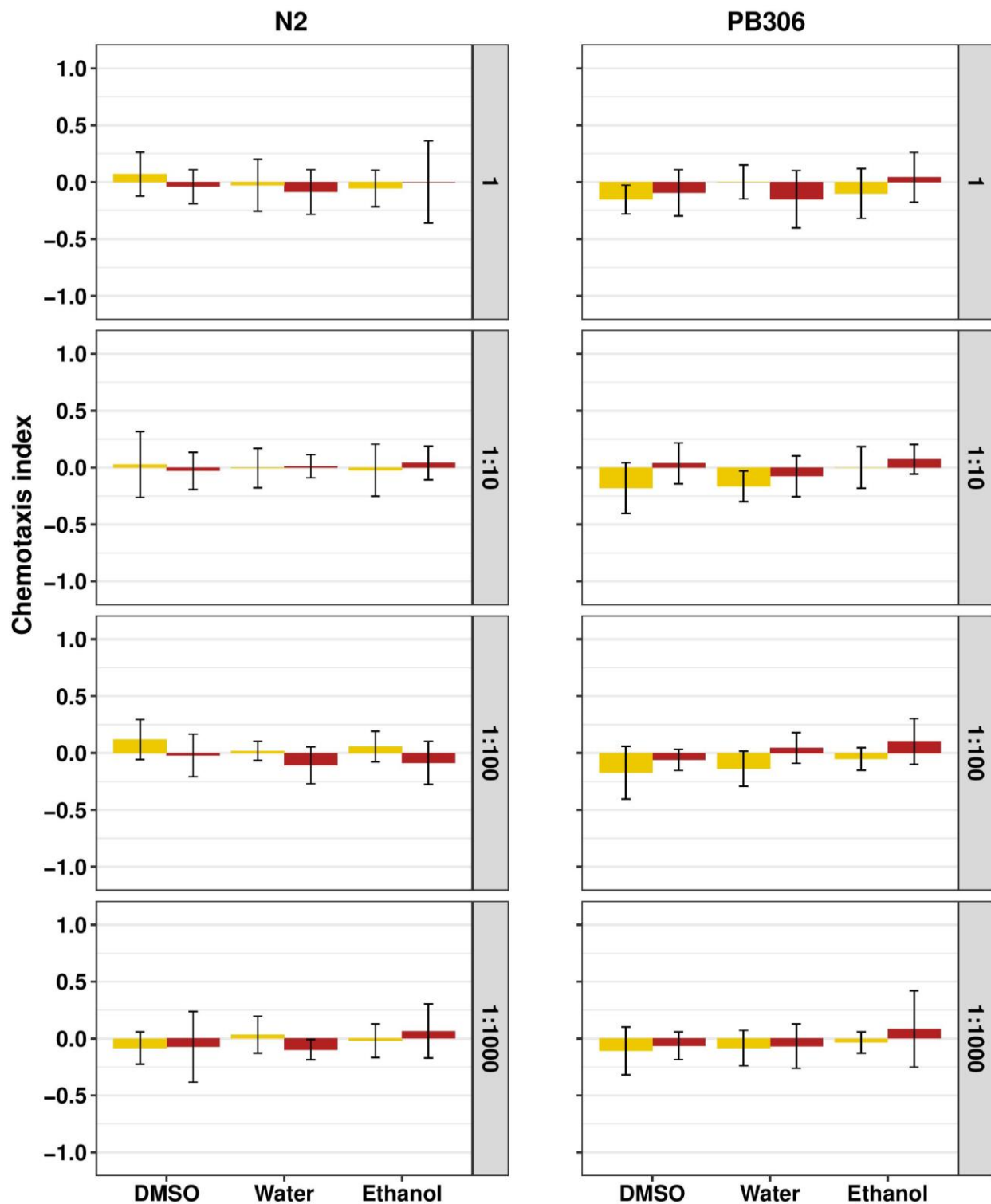
Tanaka R, Okumura E, Yoshiga T. A simple method to collect phoretically active dauer larva of *Caenorhabditis japonica*. *Nematol Res.* 2010; 40:7-12.

Yoshida K, Hirotsu T, Tagawa T, Oda S, Wakabayashi T, Iino Y, Ishihara T. Odour concentration-dependent olfactory preference change in *C. elegans*. *Nat Commun.* 2012; 3:739. doi:10.1038/ncomms1750.

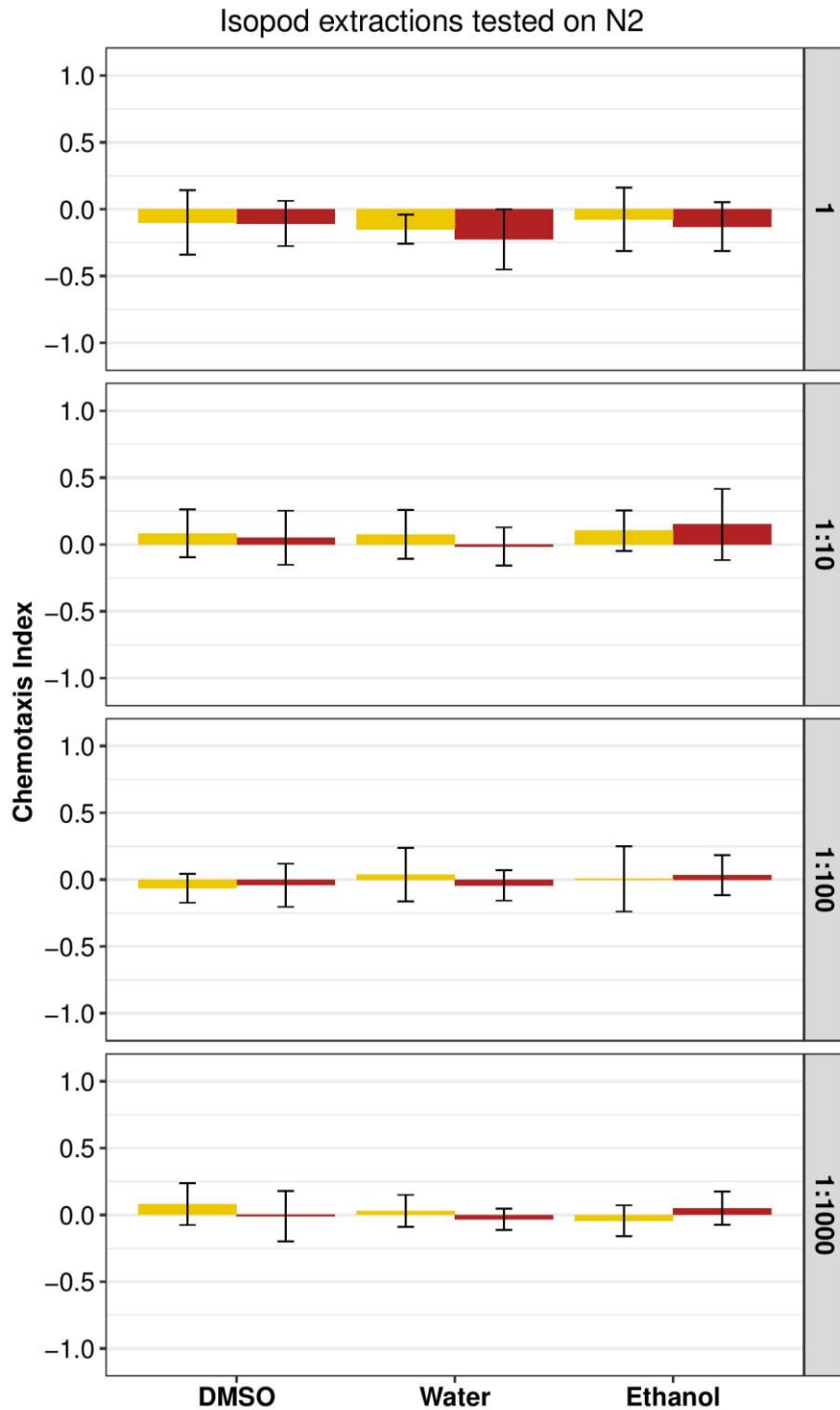
## SUPPLEMENTAL FIGURES



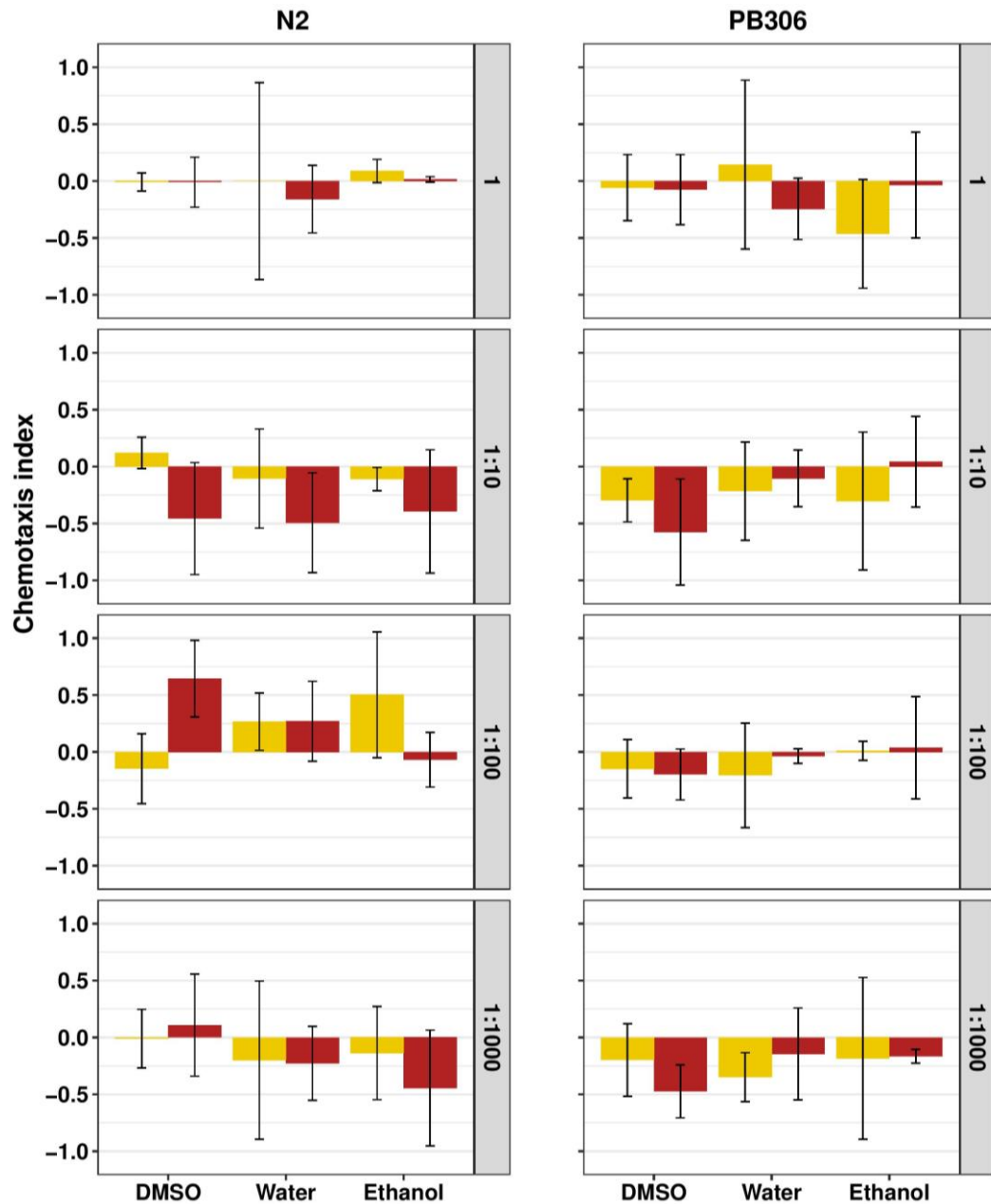
**Supplemental Figure 1.** Isoamyl alcohol (diluted to 1:1000 in ethanol) and 1-octanol serve as control attractant and control repellent, respectively, for both the N2 (left) and PB306 (right) *C. elegans* strains. Both strains respond neutrally to the three solvents used for the isopod washes (Supplemental Table 1, Supplemental Table 2).



**Supplemental Figure 2.** *C. elegans* N2 and PB306 adults respond neutrally to *P. scaber* washes at four different dilutions of the initial wash (1, 1:10, 1:100, and 1:1000). Yellow bars represent washes prepared from male isopods. Red bars represent washes prepared from female isopods. Chemotactic indices from all isopod washes were not significantly different from the chemotactic indices of its corresponding neutral control (Supplemental Table 1, Supplemental Table 2). Significance scores ( $p$  values) are in Supplemental Table 5.



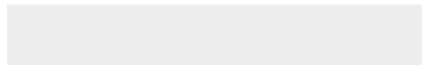
**Supplemental Figure 3.** *C. elegans* N2 adults respond neutrally to *P. scaber* extractions at four different dilutions of the initial extraction (1, 1:10, 1:100, and 1:1000). Yellow bars represent extractions prepared from male isopods. Red bars represent extractions prepared from female isopods. Chemotactic indices from all isopod extractions were not significantly different from the chemotactic indices of its corresponding neutral control (Supplemental Table 1). Significance scores ( $p$  values) are in Supplemental Table 5.



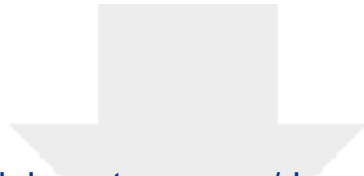
**Supplemental Figure 4.** *C. elegans* N2 and PB306 dauers respond neutrally to *P. scaber* washes at four different concentrations (1, 1:10, 1:100, and 1:1000). Yellow bars represent washes prepared from male isopods. Red bars represent washes prepared from female isopods. Chemotactic indices from all isopod washes were not significantly different from the chemotactic indices of its corresponding neutral control (Supplemental Table 3). Significance scores ( $p$  values) are in Supplemental Table 5.



Click here to access/download  
**Supporting Information**  
TableS1-N2\_chemotaxis.xlsx



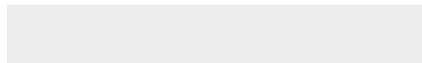


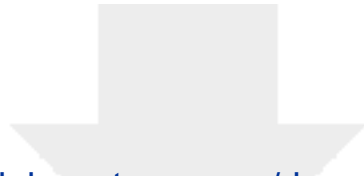


Click here to access/download

**Supporting Information**

TableS2-PB306\_chemotaxis.xlsx





[Click here to access/download](#)

**Supporting Information**

TableS3-dauer\_chemotaxis.xlsx

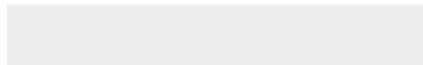


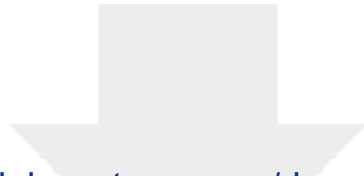


Click here to access/download

**Supporting Information**

TableS4-gas\_chemotaxis.xlsx





Click here to access/download  
**Supporting Information**  
TableS5-statistics.xlsx

