Current Biology, Volume *21*

Supplemental Information

A Sensory Code for Host Seeking in Parasitic Nematodes

Elissa A. Hallem, Adler R. Dillman, Annie V. Hong, Yuanjun Zhang, Jessica M. Yano, Stephanie F. DeMarco, and Paul W. Sternberg

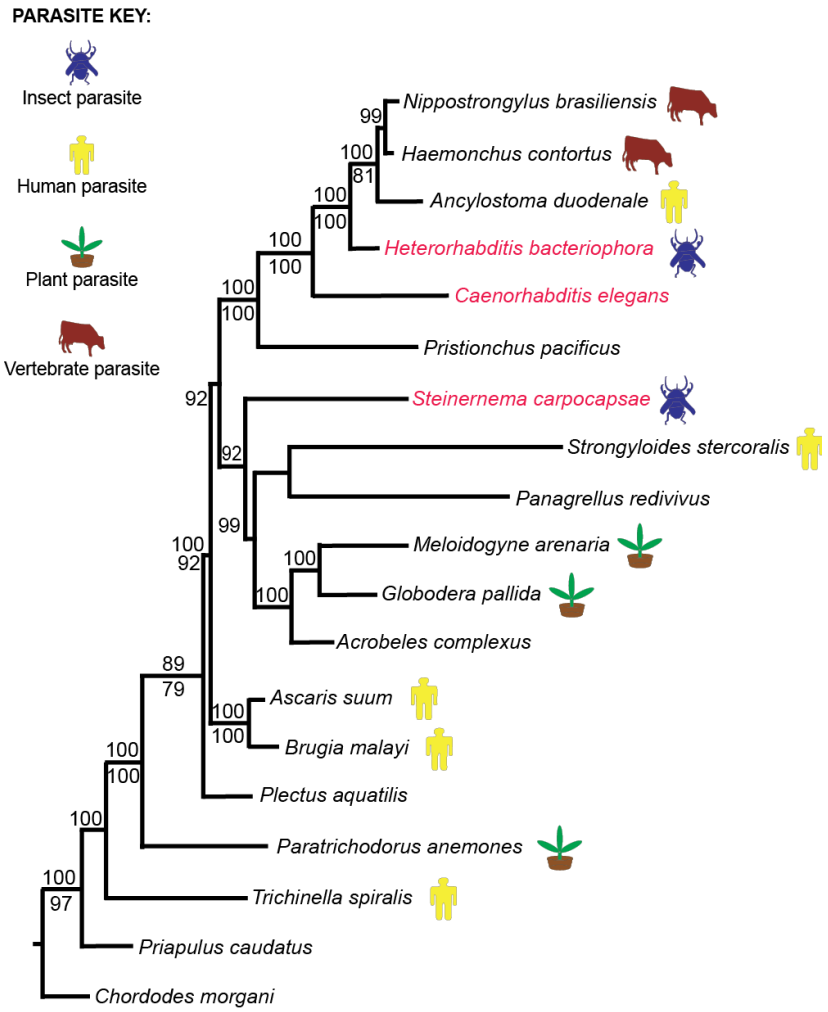


Figure S1. Phylogenetic Relationships of 17 Well-Studied or Representative Species within Nematoda. Relates to Figure 1

Relationships are based on ML and Bayesian analyses of nearly complete SSU sequences. Values above each branch represent Bayesian posterior probabilities; ML bootstrap indices (1000 replicates) appear below each branch. Values lower than 75 are not reported. Both analyses produced concordant tree topologies. The ecologies of parasitic taxa are represented by colored icons. *Priapulus* (a priapulid) and *Chordodes* (a nematomorph) were defined as outgroups.

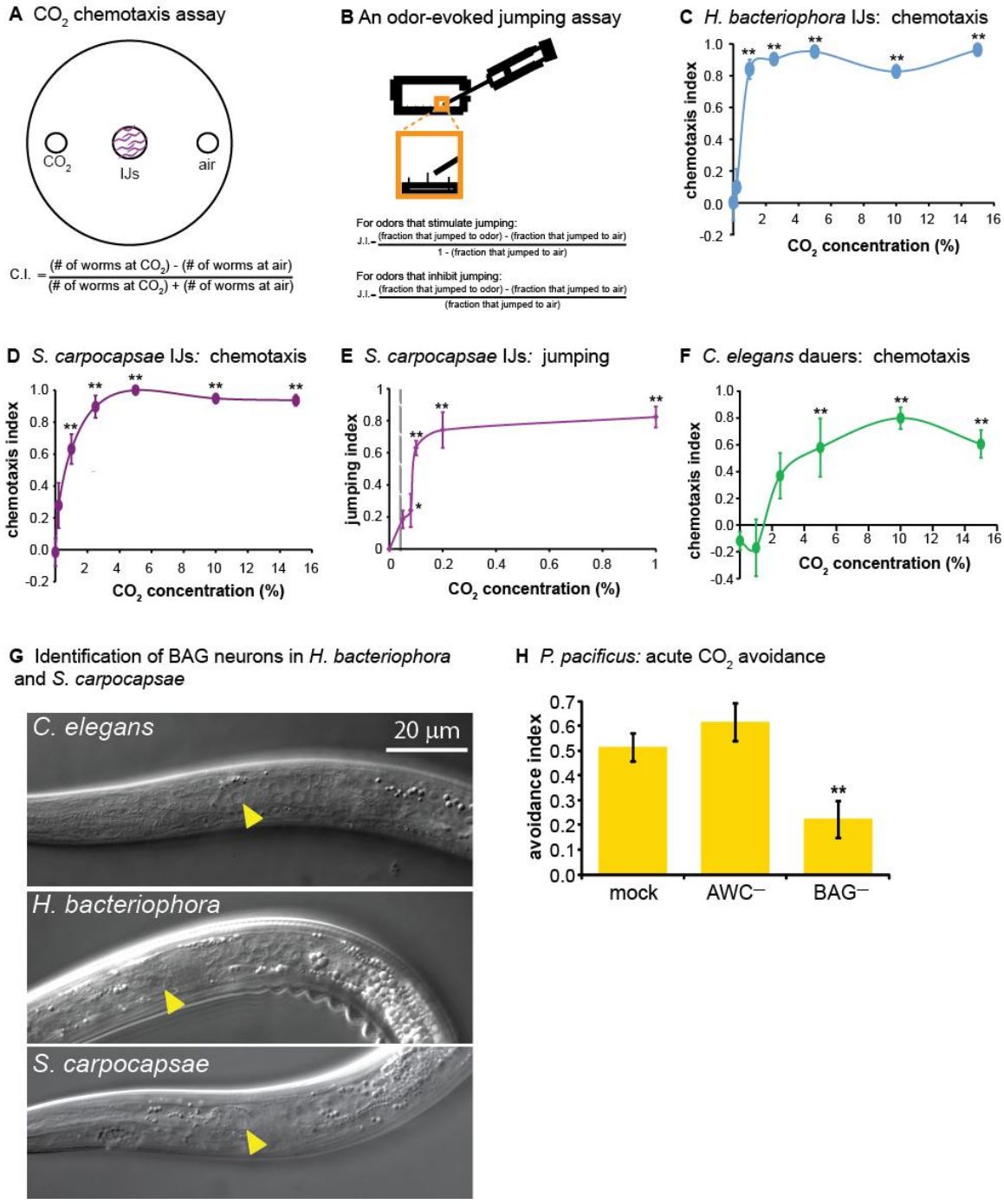


Figure S2. CO_2 Response across Species. Relates to Figure 2

(A) The CO_2 chemotaxis assay. Nematodes are placed in the center of the plate, and allowed to distribute in the CO_2 gradient. The number of worms in each scoring region is then counted. Inner boundaries of scoring regions are indicated by the horizontal lines. The chemotaxis index (C.I.) is calculated as indicated (bottom).

(B) The odor-evoked jumping assay. Individual or populations of nematodes are placed on a piece of filter paper inside a Petri dish. A non-beveled syringe with an attached needle is brought to within 2 mm of an individual nematode that is standing, and a small puff of stimulus is delivered. The percentage of animals that jump within 8 sec. is scored. A jumping index (J.I.) is

then calculated as indicated such that the J.I. is normalized to a scale of -1 to +1. The orange box shows an enlarged view of the standing IJ and stimulus syringe.
(C-D) *H. bacteriophora* and *S. carpocapsae* IJs are attracted to CO_2 across concentrations. n = 6-12 trials.
(E) CO_2 also stimulates jumping in *S. carpocapsae* IJs at concentrations as low as 0.08%. The atmospheric concentration of CO_2 (0.04%) is indicated by the dashed line. Saturation was achieved at 0.1% CO_2; CO_2 concentrations of 0.1% to 15% evoked similar levels of jumping (data not shown). The jumping index was calculated as described in Figure S2B. n = 3-7 trials; for each trial, ~60 individual IJs were tested.
(F) *C. elegans* dauers are attracted to CO_2. n = 5-10 trials. For C-F, *, $P<0.05$; **, $P<0.01$, one-way ANOVA with Dunnett's post-test.
(G) Identification of BAG neurons in *H. bacteriophora* and *S. carpocapsae.* Nomarski images of the left side of a *C. elegans* larva and parasitic IJs. Arrowheads indicate left BAG neurons. Anterior is to the left; dorsal is up. In *C. elegans* as well as the parasites, BAG neuron cell bodies are located laterally within the body just anterior to the nerve ring.
(H) BAG neurons are required for acute CO_2 avoidance in the necromenic nematode *Pristionchus pacificus*. The acute assay for CO_2 avoidance was performed as previously described [1]. The avoidance index was calculated as a.i. = (fraction of worms that reversed in response to CO_2) – (fraction of worms that reversed in response to air control). n = 17-19 worms for each treatment. **, $P<0.01$, Fisher's exact test. For all graphs, error bars represent SEM. We note that for all experiments, assay chambers were open to the external environment; thus the same ambient level of CO_2 (~0.04%) was present in all experiments.

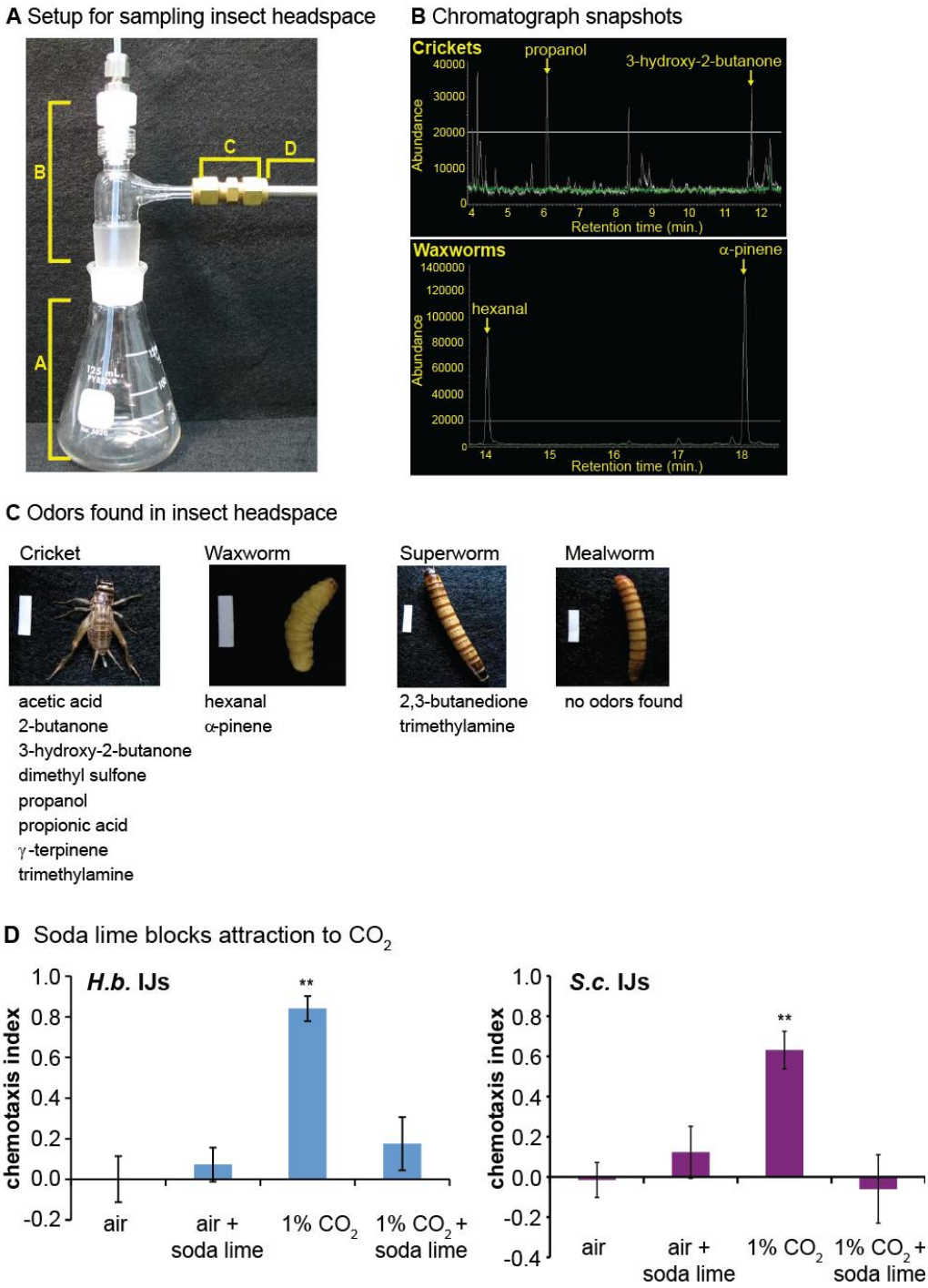


Figure S3. Identification of Insect Volatiles by TD-GC-MS. Relates to Figure 3

Little is known about the odorants emitted by insect larvae. We therefore performed TD-GC-MS to identify odorants emitted by three species of insect larvae (*Galleria mellonella*, *Zophobas morio*, and *Tenebrio molitor*), as well as young adult crickets (*Acheta domesticus*).

(A) The unit used to sample insect headspace. (A) is a 125 ml glass Erlenmeyer flask. (B) is a hand-blown glass adaptor with a ground glass attachment fit into the flask, a Teflon top piece fit to accommodate a 1/8" O.D. Teflon tube for air flow, and a small side neck tapered to 1/4" O.D. (C) is a 1/4" female/female Swagelok compression fitting for the attachment of the thermal

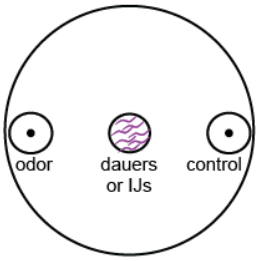
desorption tube to the flask, where air and any volatiles flow out of the set-up. (D) is the thermal desorption tube. For each species, six experimental replicates and three control replicates were obtained. The number of insects sampled in each run, and the average population weights (±SEM), are as follows: *A domesticus:* 50 insects, 8.64 g (±0.15); *G. mellonella:* 100 insects, 28.89 g (±2.79); *Z. morio:* 40 insects, 27.48 g (±0.65); *T. molitor:* 50 insects, 17.0 g (±0.61). Control samples did not contain insects.

(B) Representative snapshots of the ion chromatograph data acquired from cricket (upper trace) and waxworm (lower trace) headspace. White traces represent insect headspace samples and green traces represent controls. Compounds identified in multiple traces at relative abundances of $\geq$20,000, and that were not present in the controls at detectable levels, were then positively identified. Compounds meeting these criteria are indicated with yellow arrows.

(C) Compounds identified from the four insect species tested. Scale bars in insect photographs are 1 cm x 2.5 mm.

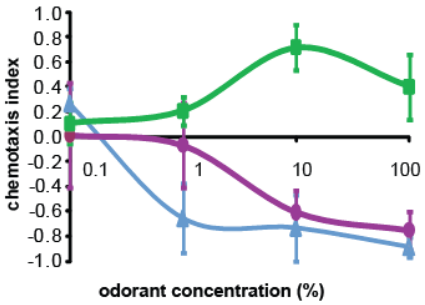
(D) A soda lime assay for examining the responses to host volatiles besides CO_2. The assay is a modified version of the host chemotaxis assay in which the airstream containing host volatiles is passed through a column of soda lime before entering the assay plate. As a control, here we show that for both *H. bacteriophora* IJs (left graph) and *S. carpocapsae* IJs (right graph), soda lime alone does not elicit a behavioral response and passing an airstream containing 1% CO_2 through a column of soda lime eliminates the attractive response to CO_2. Thus, a soda lime column can be used to chemically remove CO_2 from an airstream. n = 8-16 trials. **, $P<0.01$, one-way ANOVA with Dunnett's post-test. Error bars represent SEM.

A Chemotaxis assay

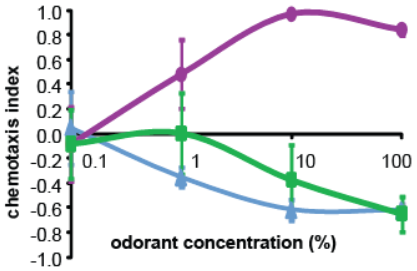


B Chemotaxis across species

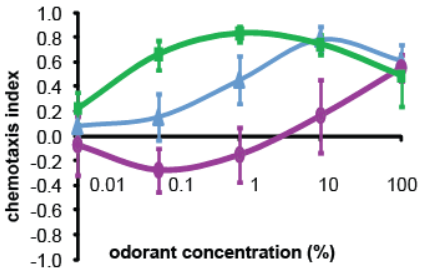
Responses to 2,3-butanedione



Responses to 1-heptanol



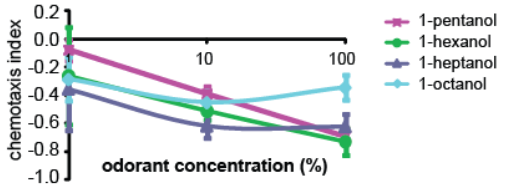
Responses to 4,5-dimethylthiazole



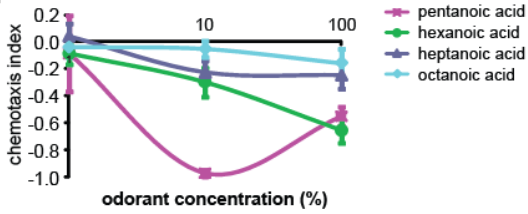
C. elegans dauers
H. bacteriophora IJs
S. carpocapsae IJs

C Chemotaxis by *H. bacteriophora* IJs

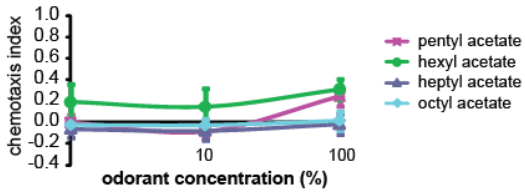
Response to alcohols



Response to acids

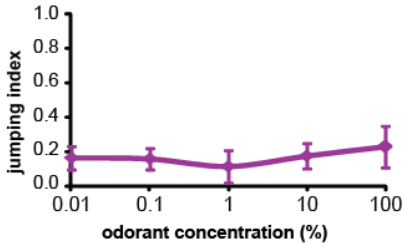


Response to acetates

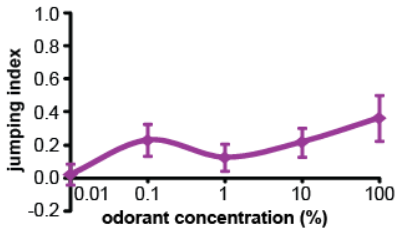


D Jumping by *S. carpocapsae* IJs

Response to 1-heptanol



Response to α-pinene



Response to 2,3-butanedione

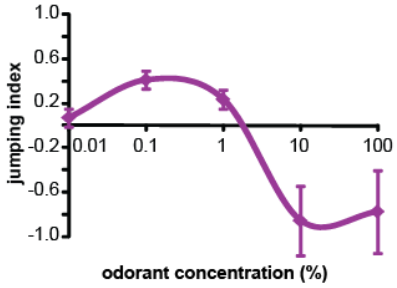


Figure S4. Dose-Response Analysis across Species. Relates to Figure 4

(A) A schematic of the chemotaxis assay. Nematodes are placed in the center of the plate, and allowed to distribute in the odor gradient. After three hours, the number of worms in each scoring region is counted. Scoring regions are indicated by the circles at either side of the plate. The chemotaxis index (C.I.) is then calculated as described in Figure S2A.

(B) Responses to increasing concentrations of 2,3-butanedione, 1-heptanol, and 4,5-dimethylthiazole in a chemotaxis assay. n = 5-33 trials.

(C) Responses of *H. bacteriophora* to alcohols, acids, and acids. n = 5-33 trials. Error bars represent SEM. Responses to 1-heptanol are from B.

(D) Jumping responses of *S. carpocapsae* to increasing concentrations of 1-heptanol, α-pinene, and 2,3-butanedione in a jumping assay. n = 3-8 trials; for each trial, ~60 individual IJs were tested. For all graphs, error bars represent SEM.

Table S1. Odorant Responses across Species. Relates to Figure 4

	C. elegans				***H. bacteriophora***				***S. carpocapsae***						
	C.I.	**n**	**SEM**		**C.I.**	**n**	**SEM**		**C.I.**	**n**	**SEM**		**J.I.**	**n**	**SEM**
ethanol	0.33	5	0.21		-0.22	5	0.12		-0.06	5	0.09		-1.00	3	0.30
1-propanol	0.51	5	0.22		0.36	5	0.13		-0.28	5	0.21		-1.00	3	0.30
1-pentanol	-0.24	7	0.27		-0.66	15	0.06		0.39	5	0.10		-0.92	4	0.31
1-hexanol	-0.61	7	0.27		-0.73	22	0.04		0.83	6	0.08		-0.50	4	0.42
1-heptanol	-0.65	10	0.14		-0.62	33	0.05		0.84	8	0.04		0.16	8	0.10
1-octanol	-0.88	5	0.08		-0.34	15	0.07		0.48	5	0.14		0.24	9	0.12
1-nonanol	-0.79	5	0.11		-0.62	5	0.11		0.53	6	0.08		0.24	8	0.61
isopropyl alcohol	0.18	5	0.29		-0.39	5	0.22		-0.03	5	0.22		-0.68	3	0.43
isoamyl alcohol	0.29	7	0.24		-0.21	5	0.15		-0.05	5	0.05		0.06	6	0.09
linalool	0.38	5	0.14		-0.05	6	0.23		-0.09	5	0.28		0.12	6	0.10
methyl acetate	0.64	5	0.15		-0.38	5	0.09		0.11	5	0.11		-0.71	3	0.34
ethyl acetate	0.36	5	0.23		-0.16	15	0.10		0.26	5	0.13		-0.89	3	0.31
pentyl acetate	-1.00	5	0.00		0.26	13	0.09		-0.09	6	0.10		-1.00	3	0.30
hexyl acetate	-0.70	5	0.29		0.31	5	0.10		0.08	6	0.09		-0.01	8	0.64
heptyl acetate	-0.80	5	0.20		-0.02	14	0.09		0.33	5	0.17		0.08	8	0.10
octyl acetate	-0.37	6	0.31		0.01	14	0.09		0.49	5	0.14		0.37	8	0.14
nonyl acetate	-0.01	5	0.16		-0.25	5	0.15		0.15	5	0.04		0.24	10	0.12
decyl acetate	-0.40	5	0.19		0.10	5	0.20		0.08	5	0.09		-0.89	3	0.31
dodecyl acetate	0.19	5	0.17		0.45	5	0.10		-0.02	6	0.09		0.22	8	0.11
2-butanone	0.83	5	0.11		0.02	6	0.13		-0.18	5	0.24		-0.66	3	2.39
2-pentanone	0.67	5	0.13		-0.29	5	0.13		-0.39	5	0.07		-0.46	5	0.46
2-hexanone	-0.50	5	0.22		-0.36	7	0.15		0.11	5	0.09		-0.89	3	0.31
2-heptanone	-0.99	5	0.01		0.19	5	0.23		0.18	5	0.24		-0.68	3	0.35
2-octanone	-1.00	5	0.00		0.11	5	0.15		0.30	5	0.24		-0.68	3	0.43
2-nonanone	-0.95	5	0.04		0.05	5	0.19		0.52	5	0.12		-0.89	3	0.31
2,3-butanedione	0.40	5	0.26		-0.89	5	0.08		-0.75	5	0.15		-0.89	6	0.31
3-hydroxy-2-butanone	0.25	5	0.12		-0.23	5	0.06		-0.17	5	0.05		-0.74	3	0.33
dimethylsulfone	0.02	5	0.25		0.00	6	0.19		-0.18	5	0.06		0.16	3	0.09
acetic acid	-1.00	6	0.00		-0.66	8	0.23		-0.98	5	0.02		-0.73	3	0.33
propionic acid	-1.00	5	0.00		-1.00	5	0.00		-1.00	5	0.00		0.43	3	0.06
pentanoic acid	-1.00	5	0.00		-0.55	7	0.07		-0.80	5	0.20		0.46	9	0.10
hexanoic acid	-1.00	5	0.00		-0.66	14	0.09		-0.43	7	0.18		0.42	8	0.11
heptanoic acid	-0.90	5	0.10		-0.25	17	0.10		-0.46	5	0.13		0.07	8	0.09
octanoic acid	-0.78	5	0.13		-0.16	14	0.10		-0.53	5	0.19		-0.47	6	0.34
nonanoic acid	-0.68	5	0.19		-0.33	5	0.20		-0.17	5	0.11		0.21	8	0.10
hexadecanoic acid	0.12	5	0.14		-0.06	7	0.24		-0.15	5	0.24		-1.00	3	0.30
octadecanoic acid	0.36	5	0.15		-0.16	5	0.11		-0.02	5	0.20		-1.00	3	0.30

hexanal	-0.70	5	0.14		-0.75	7	0.20		-0.31	7	0.20		0.30	10	0.10
nonanal	-0.45	6	0.06		-0.01	5	0.24		0.09	5	0.10		0.37	9	0.11
undecanal	-0.71	5	0.19		0.24	5	0.16		-0.09	5	0.17		0.01	4	0.06
2-acetylthiazole	0.03	5	0.34		-0.73	8	0.04		-0.15	5	0.25		-0.79	3	0.31
benzothiazole	0.71	5	0.12		-0.06	8	0.05		-0.49	5	0.18		-0.40	6	0.37
2-isobutylthiazole	-0.76	5	0.12		-0.04	8	0.09		-0.04	5	0.29		0.07	8	0.09
4,5-dimethylthiazole	0.49	7	0.25		0.61	6	0.12		0.55	5	0.11		-1.00	6	0.30
benzaldehyde	-0.75	5	0.14		-0.83	5	0.08		0.07	5	0.11		-0.53	5	0.43
methyl salicylate	-0.93	5	0.03		0.86	5	0.02		0.07	5	0.17		-0.79	3	0.36
m-xylene	-0.04	5	0.22		-0.45	5	0.21		-0.05	5	0.03		0.07	6	0.11
m-cymene	-0.04	5	0.17		0.19	7	0.23		-0.10	5	0.17		0.03	5	0.06
p-cymene	-0.30	5	0.20		0.41	5	0.22		-0.10	5	0.07		0.02	8	0.09
β-caryophyllene	0.27	5	0.24		0.06	10	0.07		-0.40	5	0.13		-0.89	3	0.31
α-humulene	0.45	7	0.27		0.00	5	0.25		0.30	5	0.20		-0.04	3	0.30
3-carene	0.48	5	0.25		-0.70	5	0.07		-0.13	6	0.05		-0.89	3	0.31
α-pinene	0.57	5	0.17		-0.63	5	0.12		0.05	5	0.21		0.36	8	0.14
β-pinene	0.30	5	0.17		-0.17	5	0.11		-0.17	5	0.25		0.23	6	0.10
limonene	0.16	5	0.14		-0.64	5	0.12		-0.35	5	0.07		-0.50	5	0.30
γ-terpinene	0.13	7	0.24		-0.02	5	0.14		-0.12	5	0.12		0.26	8	0.10
trimethylamine	0.75	5	0.08		-0.28	5	0.14		0.12	7	0.25		-0.03	3	0.36

Mean values for the chemotaxis index (C.I.) and jumping index (J.I.) of each species in response to each tested stimulus. The number of trials (n) is shown. SEM, standard error of the mean. Odorants were selected based on their chemical diversity and ecological relevance. All of the odorants tested are present either in plants or bacteria [2-5], and many have been shown to elicit responses from free-living adult nematodes [6-9]. Eleven of the odorants were identified in insect headspace (Figure 5). In addition, hexadecanoic and octadecanoic acid have been identified in larval cuticular extracts from insect larvae [10]; acetic, propionic, and pentanoic acids have been identified in cricket excreta [11]; benzaldehyde and linalool have been identified in butterfly wing extracts [12]; and nonanal, undecanal, and nonanol are emitted by adult wax moths [13, 14]. Product numbers are from Sigma-Aldrich, except for ethanol (Pharmco-AAPER), ethyl acetate (Mallinckrodt chemicals), and acetic acid (J.T. Baker).

Supplemental Experimental Procedures

Nematodes

H. bacteriophora were from the inbred strain M31e [15, 16], *S. carpocapsae* were from the inbred strain ALL [17], and *C. elegans* were from the standard N2 ("Bristol") strain, unless otherwise indicated. Other *H. bacteriophora* strains tested were HP88 [18], GPS11 [19], NC1 [20], and a strain we designate as "BU" that was derived from commercially available nematodes that were originally obtained from Becker-Underwood (http://www.beckerunderwood.com). The other *S. carpocapsae* strain tested was an inbred wild isolate that we designate as Base [17]. Other *C. elegans* strains tested were the wild isolate CB4856 ("Hawaii") and CX11697 [*kyIs536[flp-17::p17::s12GFP, elt-2::mCherry]; kyIs538[glb-5::p12::s12GFP, elt-2::mCherry]*], which contains a genetic ablation of the BAG neurons. *P. pacificus* were from the PS312 ("California") strain.

Nematode Culture

H. bacteriophora was cultured at 27°C on either nutrient agar + cholesterol plates (23 g nutrient agar + 1 ml of 5 mg/ml cholesterol in 1 L) or lipid agar + cholesterol plates [21] seeded with either TT01 or RET16 bacteria. RET16 is a GFP-labeled derivative of *P. temperata* strain NC1 mutated with HiMarGM (a hyperactive mariner transposon with gentamicin resistance) [15]. *Photorhabdus* was grown in PP3 broth (20 g proteose peptone #3 (Difco) in 1 L dH_20) and on either nutrient agar + cholesterol plates or lipid agar + cholesterol plates. IJs were stored in 0.85% NaCl (w/v) or dH_20 at room temperature or 15°C prior to use. Prior to behavioral testing, IJs were washed in dH_20. For Figure 1A, *H. bacteriophora* was cultured on plates seeded with GFP-labeled *P. luminescens,* as previously described [16].

S. carpocapsae was cultured as previously described [22]. Briefly, 5 last-instar *Galleria mellonella* larvae (American Cricket Ranch, Lakeside, CA) were placed in a 5 cm Petri dish with a 55 mm Whatman 1 filter paper acting as a pseudo-soil substrate in the bottom of the dish. ≤250 μl containing 500-1000 IJs suspended in water was evenly distributed on the filter paper. After 7-10 days the insect cadavers were placed on White traps [23]. Emerging IJs were harvested, washed for 10 minutes in 0.4% Hyamine 1622 solution (Fluka), and rinsed 3 times with water. To prevent differences in inbreeding between batches of IJs, the same stock population of IJs was used to generate all test batches of IJs. Stock populations were stored at 15°C and propagated in *G. mellonella* every 10 days to produce fresh test batches of IJs. Test batches were stored at room temperature and used in behavioral assays within 12 days of emergence. In some cases, *S. carpocapsae* used for chemotaxis assays were cultured at 27°C on nutrient agar + cholesterol or lipid agar + cholesterol plates as described above, except that plates were seeded with *X. nematophila* strain HGB081 [24]. For Figure 1B, *S. carpocapsae* were cultured on plates seeded with GFP-labeled *X. nematophila,* as previously described [25]. *X. nematophila* was grown in LB broth containing 0.1% sodium pyruvate, and on either nutrient agar + cholesterol or lipid agar + cholesterol plates.

C. elegans was cultured on NGM plates seeded with *E. coli* OP50 according to standard methods [26]. *C. elegans* dauers were grown primarily in liquid culture, although in some cases dauers were collected from the lids of starved plates. For dauers grown in liquid culture, embryos were collected as previously described and diluted to 10 eggs/μl in S complete media [27]. *E. coli* HB101 bacteria was added at a final concentration of 0.5 mg/ml and worms were grown on a carousel at 20°C for 6 days to generate dauers (L.R. Baugh and P.W. Sternberg, unpublished). If necessary, the bacterial concentration was adjusted to generate populations of

nearly 100% dauers. Dauers were stored in dH_2O at 15°C prior to use. For CO_2 assays, dauers were SDS-treated [28]; SDS treatment did not affect CO_2 response. For all other assays, dauers were not treated with SDS; in these cases, a small population sample was treated with SDS, and worms were only used for behavioral assays if nearly all of the sample population survived SDS treatment.

P. pacificus was grown on NGM plates seeded with *E. coli* OP50 bacteria at room temperature.

Population CO_2 Chemotaxis Assay

Assays were performed on standard chemotaxis assay plates [6]. Gases were medical grade certified mixtures (Air Liquide) of either 0%, 0.2%, 1%, 2.5%, 5%, 10%, or 15% CO_2; 10% O_2; and the balance N_2. An O_2 concentration of 10% was chosen to approximate the preferred O_2 concentration of *C. elegans* [29]. 10% CO_2 was used unless otherwise indicated. Two holes were drilled in the plate lid, each 1 cm from the edge along the diameter. Flexible PVC tubing (1/8 inch thickness) was attached to the top of each hole and connected to a 50 ml Hamilton gastight syringe. One syringe was filled with a gas mixture containing CO_2, while the other syringe was filled with a gas mixture without CO_2. Syringes were depressed at a rate of 0.5 ml/min using a syringe pump (PHD 2000, Harvard Apparatus) for the duration of the assay. ~2 μl of worm pellet containing ~50-150 parasitic IJs or *C. elegans* dauers was then placed in the center of the assay plate. Each plate was left undisturbed on a vibration-reducing platform for the duration of the assay. Assay length was 1 hr. unless otherwise indicated. Scoring regions consisted of 2 cm circles on each side of the plate along the diameter, with the center of the circle 1 cm from the edge of the plate. At the end of the assay, the number of worms in each scoring region was counted and a chemotaxis index was calculated as:

$$\text{C.I.} = \frac{(\text{number of worms at } CO_2) - (\text{number of worms at air})}{(\text{number of worms at } CO_2) + (\text{number of worms at air})}$$

Assays were only scored if ≥3 worms moved into the scoring regions, and trials from assay sessions in which all worms exhibited the same directional bias were discarded.

Single Worm CO_2 Chemotaxis Assay

For single-worm assays on laser-ablated and mock-ablated IJs, individual IJs were tested once in a 1 hr assay. IJs were pre-selected by performing a 1 hr CO_2 gradient assay on a population of IJs as described above. IJs that responded to CO_2 were then recovered from the assay plate and used for laser ablation experiments. This pre-selection was used because some IJs in any lab population of parasitic nematodes are unhealthy and unresponsive to stimuli [30]. Following laser ablation treatment, only IJs with normal movement (assayed visually on a plate) were used for subsequent analysis; any IJs that appeared damaged were discarded. After a 1 hr CO_2 gradient assay, the trial was scored as positive if the IJ was in the designated area near the CO_2 source comprising ~3/10 of the plate; otherwise the assay was scored as negative. The percentage of positively-responding IJs for each condition was then calculated.

Jumping Assay

This assay was modified from the jumping assay of Campbell and Kaya [31]. Assays were performed in 5 cm Petri dish arenas with 55 mm Whatman 1 filter paper as a soil-like substrate on the bottom of the dish to absorb and retain moisture while providing a fibrous material to facilitate jumping. A 1.25 mm hole was drilled through the side of the dish and lid to allow odors

to be introduced with the lid on, thus preventing drying or the confounding effects of drafts. Odors were introduced by a 10 ml Hamilton gastight syringe equipped with a Hamilton blunt needle (22s/2"/3) for gases (see below) and insects and by a 10 ml Luer-Lok Becton Dickinson syringe with a Becton Dickinson needle (21 gauge 1.5") with the end clipped to be blunt for odorants (Figure S2B). For host-evoked jumping, a single insect was placed inside the syringe. For odorants, a small piece of filter paper was placed in the syringe and 5 μl of undiluted odorant was placed onto the filter paper. The hole drilled into the dish was covered with Parafilm and punctured only during assays. Nematodes were added to the arena in liquid suspension such that 100 nematodes were suspended in approximately 200 μl of water, which was evenly distributed on the filter paper. For ablation assays or other assays involving individual nematodes, a worm was placed in the middle of the arena by pipetting through a pulled glass capillary; filter paper was pre-moistened with 200 μl of water.

To examine odor-evoked jumping, ≥60 standing individual IJs were randomly selected and presented with a small puff (~0.5 ml volume) of either air control or volatile stimulus. Individuals were tested only once during a standing bout. Cues were introduced by slowly bringing the syringe needle tip to within ~2 mm of the standing individual. Behavioral changes by standing individuals, within an 8 s response interval, were observed and recorded. An 8 s response interval was used because our preliminary experiments revealed that nearly all IJs that were going to jump in response to a stimulus jumped within the first 8 s after the stimulus was given. Responses were divided into 5 categories: (1) no response, (2) jump, (3) waving (the nematode responded by waving back and forth), (4) wave and touch down (the nematode responded by waving and fell back onto the substrate within the time interval), and (5) jump and touch down (the nematode postured to jump but failed). These responses were collapsed and reported as either jump (2 and 5) or no jump (1, 3, or 4). All nematodes were stored and tested at room temperature.

A normalized jumping index (J.I.) was then calculated. For stimuli that evoked higher levels of jumping than the control, the J.I. and SEM were calculated as:

$$\text{J.I.} = \frac{\text{(fraction that jumped to stimulus)} - \text{(fraction that jumped to control)}}{1 - \text{(fraction that jumped to control)}}$$

$$\text{SEM} = \frac{\sqrt{[(\text{SEM for stimulus})^2 - (\text{SEM for control})^2]}}{1 - \text{(fraction that jumped to control)}}$$

For stimuli that evoked lower levels of jumping than the control, the J.I. and SEM were calculated as:

$$\text{J.I.} = \frac{\text{(fraction that jumped to stimulus)} - \text{(fraction that jumped to control)}}{\text{(fraction that jumped to control)}}$$

$$\text{SEM} = \frac{\sqrt{[(\text{SEM for stimulus})^2 - (\text{SEM for control})^2]}}{\text{(fraction that jumped to control)}}$$

This resulted in a normalized J.I. that ranged from -1 to +1. Values obtained for each odorant are listed in Table S1.

To examine the frequency of spontaneous jumping, populations of 100 IJs suspended in water were distributed on a 55 mm Whatman 1 filter paper in a 5 cm Petri dish. The total volume of water, including the suspension of IJs, was 200 μl. The lid was painted with a thin layer of paraffin oil to prevent re-jumping. Worms stuck to the lid were counted after 120 minutes.

Host Chemotaxis Assay

Assays were performed on standard chemotaxis assay plates [6]. Holes were drilled into the plate lids as described above. Live insects (6 insects in the case of waxworms and crickets, and 3 insects in the case of mealworms and superworms) were placed into a 50 ml gastight syringe. A control syringe was filled with room air. Syringes were depressed at a rate of 0.5 ml/min using a syringe pump (PHD 2000, Harvard Apparatus). Assay plates were left undisturbed on a vibration-reducing platform for 1 hour. Assays were then scored as for chemotaxis assays.

Soda Lime Assay

Chemotaxis assays involving soda lime were performed as described above, except that the gas mixture entering one side of the plate was passed through a column containing soda lime. Soda lime columns were constructed using 6 inches of Nalgene (8050-0250) FTP 3/16" OD tubing. The tubing was filled with 2-5 mm soda lime pellets (Sigma-Aldrich 72073), 1.88 g. ± 0.145 g. The column was then capped on either end using fittings 731-8223 and 731-8226 from a BioRad low pressure fittings kit (731-8220). The fittings were made air-tight by wrapping the ends inserted into the column with a thin layer of ½" Teflon tape. The Luer-Lok end of the column was then attached to the gastight syringe while the other end was connected to the PVC tubing that entered the assay plate lid.

Single-Worm Assay for Acute CO_2 Avoidance

Assays were performed as described [1]. Briefly, individual L4 or young adult *P. pacificus* hermaphrodites were placed onto assay plates overnight. Assay plates consisted of thin lawns of *E. coli* OP50 bacteria grown for 1-2 days. Two 50 ml syringes were filled with gas, one with and one without CO_2. The mouths of the syringes were connected to tubes attached to Pasteur pipets, and gases were pumped through the Pasteur pipets using a syringe pump at 1.5 ml/min. Individual worms were exposed to gases by placing the tip of the Pasteur pipet near the head of a forward-moving worm. A response was scored if the worm initiated backward movement within 4 seconds. Worms were tested 15 times with >2 minutes between trials. For each worm, an avoidance index (a.i.) was then calculated by subtracting the fraction of trials the worm reversed in response to air from the fraction of trials the worm reversed in response to CO_2. The a.i. for each treatment was calculated as the mean a.i. for all of the worms subjected to each treatment.

Cell Ablations

Ablations were performed on parasitic IJs and *P. pacificus* L2 and L3 animals essentially as described [32]. Briefly, animals were mounted on glass slides for Nomarski microscopy on a pad consisting of 5% Noble agar in dH_2O with either 20 mM (for *H. bacteriophora*), 40 mM (for *S. carpocapsae*), or 5 mM (for *P. pacificus*) sodium azide as anesthetic. Neurons were ablated by focusing a laser microbeam on the cell. Mock-ablated worms were mounted on glass slides as described above but were not subjected to a laser microbeam. For CO_2 chemotaxis assays and jumping assays, AWC-ablated animals were tested as controls because the AWC neurons are

known to mediate olfactory attraction in *C. elegans* [6]. For host chemotaxis assays, ASI-ablated animals were tested as controls because ASI neurons are sensory neurons but are not known to mediate olfactory attraction [33]. We did not use AWC-ablated animals for this experiment because other host odors besides CO_2 likely contribute to host attraction, and it is possible that the AWC neurons are involved in the detection of these other host odors.

For all ablation experiments, neuron cell bodies were identified based on anatomical position as described [34]. We and others have shown that sensory neural anatomy is highly conserved among nematodes [15, 35-40]. Comparative studies using cell ablations in combination with behavioral analyses have shown that neuron morphology and function are often conserved across species [15, 35, 41-48]). Moreover, developmental studies have demonstrated that cells that are found in the same anatomical position and have similar functions at post-embryonic stages in different species are in many cases derived from analogous embryonic lineages in the different species [49-51]. Thus, one can use the anatomical map of *C. elegans* [52] to identify analogous neurons in other nematodes, including both free-living and parasitic species.

Ablated IJs were allowed to recover on unseeded NGM plates until crawling was observed. IJs were then transferred to dH_2O, stored in wells of a flat-bottom 96 well plate, and tested within 24 hours. Ablated *P. pacificus* larvae were subsequently tested as adults. To identify neurons involved in host chemotaxis, *H. bacteriophora* IJs were cultured in waxworms. IJs were harvested from waxworms using White traps [23] 10-15 days post-infection, and stored in dH_2O. Only newly-emerged IJs (<48 hours after collection in the White trap) were used for ablation experiments.

We note that we did not observe any locomotor or developmental abnormalities as a result of BAG neuron ablation. Furthermore, BAG-ablated *S. carpocapsae* IJs were still capable of nictating and jumping normally, although BAG-ablated IJs showed greatly reduced jumping in response to CO_2.

Chemotaxis Assay

This assay was modified from previously-described chemotaxis assays [6, 53]. Assays were performed on standard 9 cm chemotaxis assay plates [6]. Scoring regions consisted of 2 cm circles on each side of the plate along the diameter, with the center of the circle 1 cm from the edge of the plate. 1 μl of 1 M sodium azide was placed in the center of each scoring region as an anesthetic. 5 μl of odorant was then placed in the center of one scoring region, while 5 μl of a control (either paraffin oil, dH_2O, or ethanol) was placed in the center of the other scoring region. ~2 μl of worm pellet containing ~50-150 parasitic IJs or *C. elegans* dauers was then placed in the center of the assay plate. Each plate was left undisturbed on a vibration-reducing platform. After 3 hours, the number of worms in each scoring region was counted. Assays were only scored if ≥3 worms moved into the scoring regions, and trials from assay sessions in which worms from all assays exhibited a directional bias were discarded. The chemotaxis index (C.I.) was then calculated as:

$$\text{C.I.} = \frac{\text{(number of worms at odorant)} - \text{(number of worms at control)}}{\text{(number of worms at odorant)} + \text{(number of worms at control)}}$$

Odorants were tested undiluted unless otherwise indicated. Solid odorants were dissolved as follows: 3-hydroxy-2-butanone and dimethylsulfone, 1 g in 4 ml dH_2O; hexadecanoic acid, 1 g in 20 ml ethanol; octadecanoic acid, 1 g in 80 ml ethanol. Values obtained for each odorant and

each species are listed in Table S1. For dilution series, odorants were diluted in paraffin oil or dH_2O.

Phylogenetic Analysis

Small subunit ribosomal DNA (SSU rDNA) sequences for all analyses were obtained from GenBank for all taxa included in the present study (accession numbers: AJ920356, EU086375, AF036593, AY268117, U81584, AF083007, AF279916, AF036604, AY284620, AY284621, AY284671, U94367, AF036588, U61761, AF036600, U60231, EU344798, X87984, and AF036639). Most of these sequences have been used in previous phylogenetic analyses [54, 55]. The SSU sequences for *C. elegans*, *S. carpocapsae*, *H. bacteriophora*, and *C. morgani* (a nematomorph) were used for the Neighbor-Joining (NJ) tree in Figure 4C. The sequences were first trimmed to 1783 characters and then aligned using MUSCLE [56]. The subsequent NJ analysis was done using the 'Dnadist' and 'Neighbor' programs from the PHYLIP 3.68 package [57] using default settings with *C. morgani* defined as the outgroup. A total of 17 nematode species and 2 outgroup taxa (a priapulid and a nematomorph) were used in the analyses for Figure S1. In order to facilitate comparison of the SSU sequences of varying lengths, the ends were trimmed by hand, prior to alignment, in MacClade 4 [58] to a maximum length of 1622 characters, which is the length of the taxon with the shortest sequence, *Ancylostoma duodenale*. Sequences were then aligned using MUSCLE [56], resulting in 1933 characters (including gaps). The TIM2+I+G model was selected as the best-fit model of substitution for all analyses using the AIC and AICc model selection criteria in the program jModelTest [59, 60]. Maximum likelihood and bootstrap (1000 replicates) analyses were carried out in PhyML 3.0 [60] using the parameters for base frequencies, substitution rate matrix, proportion of invariable sites, number of substitution categories, and shape distribution parameter determined as the best-fit by jModelTest (freqA = 0.2684, freqC = 0.1835, freqG = 0.2501, freqT = 0.2981, Ra(AC) = 1.6751, Rb(AG) = 2.5642, Rc(AT) = 1.6751, Rd(CG) = 1.0000, Re(CT) = 4.5613, Rf(GT) = 1.0000, p-inv = 0.1710, and gamma shape = 0.5840). Bayesian analysis was carried out using MrBayes 3.1.2 [61]. The number of substitution categories, substitution rate matrix, shape and proportion of invariant sites were based on the parameters determined by jModelTest (as above). The parameters for base frequencies and relative rates were allowed to vary throughout the analysis. The parameters were unlinked to allow for more flexibility in searching tree space. Trees were sampled every 1000 generations. The burn-in value was set to 2000 trees. The total number of generations was set to 8 million. Four parallel chains (one cold and three heated) were used. A majority-rule consensus tree was reconstructed after discarding the burn-in.

Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS)

Appropriately staged insects (adult *Acheta domesticus* and last-instar larvae of *Zophobas morio, Galleria mellonella,* and *Tenebrio molitor*) were placed in a 125 ml glass beaker and sampled for 30 minutes with a stream of air (10% oxygen, 90% nitrogen) flowing into the flask and out through a thermal desorption tube (Sigma-Aldrich 20913-U) at a flow rate of approximately 104 ml/min. Experiments were done in pairs and replicated 3 times, with an empty control flask being run each time. To prevent carry-over of odors between experiments, all tubing used was Nalgene Teflon tubing, connected with Swagelok compression fittings, and flasks were cleaned and sterilized after each use.

The contents of the thermal desorption tubes were transferred to a HP 6890 GC - 5973 MS system (Agilent Technologies, US) with an Eclipse 4660 purge and trap sampler equipped with an airtube desorber accessory (OI Analytical, College Station, TX, US.). Tubes were desorbed at 200°C for 15 minutes and transferred via a flow of helium to an internal trap held at room

temperature. After desorption, the internal trap was heated to 200°C. This trap was brought in line with the GC carrier gas flow as the trap reached 180°C. The trap was then taken offline and subjected to a bakeout procedure. The sample flowed to a GC via a transfer line held at 120°C where it entered a split-splitless injector held at 200°C. The injector was operated in split mode with a split ratio of 30:1, and a 1 mm liner was installed to optimize chromatographic resolution. Separation was achieved with a HP-624 capillary column (30 m x 0.320 mm) where a volumetric flow of 1 ml/min was maintained with electronic pressure control. The transfer line to the mass spectrometer was held at 200°C, the ion source at 250°C and the quadrupole at 100°C. The mass spectrometer is equipped with an electron impact source. Electron energy was set to 70 eV to obtain the best possible library spectrum matches. The quadrupole mass spectrometer was operated with a full width at half maximum of 0.65 m/z. Mass calibration was verified weekly. The GC oven was ramped from 30°C to 260°C and run for 42 minutes. Data was analyzed with both Chemstation and Masshunter software. Mass spectra were searched against the Wiley library (275,000 spectra) of electron impact mass spectra. Only compounds that were found in multiple traces (≥2), with a relative abundance ≥20,000, and not present in the control traces were considered in this study. Compounds identified in this way were then positively confirmed by running the pure compound (Table S1) and comparing the retention time and mass spectra of the assay-identified compound to the known compound. In cases where the retention time was off by ≥0.5 minutes or the mass spectra did not match, the assay-identified compound was considered uncertain and not used in behavioral assays. All insects tested were obtained from commercial sources (American Cricket Ranch, Lakeside CA).

Data Analysis

Statistical analysis was performed using GraphPad InStat. Heat maps and dendrograms were generated using PAST [62].

Supplemental References

1. Hallem, E.A., and Sternberg, P.W. (2008). Acute carbon dioxide avoidance in *Caenorhabditis elegans*. Proc Natl Acad Sci USA *105*, 8038-8043.
2. TNO (2004). Volatile compounds in food: qualitative and quantitative data. http://www.vcf-online.nl.
3. O'Leary W, M. (1962). The Fatty Acids of Bacteria. Bacteriol Rev *26*, 421-447.
4. Malo, E.A., Medina-Hernandez, N., Virgen, A., Cruz-Lopez, L., and Rojas, J.C. (2002). Electroantennogram and field responses of *Spodoptera frugiperda* males (Lepidoptera: Noctuidae) to plant volatiles and sex pheromone. Folia Entomol Mex *41*, 329-338.
5. Ali, J.G., Alborn, H.T., and Stelinski, L.L. (2010). Subterranean herbivore-induced volatiles released by citrus roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. J Chem Ecol *36*, 361-368.
6. Bargmann, C.I., Hartwieg, E., and Horvitz, H.R. (1993). Odorant-selective genes and neurons mediate olfaction in *C. elegans.* Cell *74*, 515-527.
7. Hong, R.L., and Sommer, R.J. (2006). Chemoattraction in *Pristionchus* nematodes and implications for insect recognition. Curr Biol *16*, 2359-2365.
8. Hong, R.L., Svatos, A., Herrmann, M., and Sommer, R.J. (2008). Species-specific recognition of beetle cues by the nematode *Pristionchus maupasi.* Evol Dev *10*, 273-279.
9. Rasmann, S., Kollner, T.G., Degenhardt, J., Hiltpold, I., Toepfer, S., Kuhlmann, U., Gershenzon, J., and Turlings, T.C. (2005). Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature *434*, 732-737.

10. Golebiowski, M., Malinski, E., Bogus, M.I., Kumirska, J., and Stepnowski, P. (2008). The cuticular fatty acids of *Calliphora vicina, Dendrolimus pini* and *Galleria mellonella* larvae and their role in resistance to fungal infection. Insect Biochem Mol Biol *38*, 619-627.
11. McFarlane, J.E., Steeves, E., and Alli, I. (1983). Aggregation of larvae of the house cricket, *Acheta domesticus* (L.), by propionic acid present in the excreta. J Chem Ecol *9*, 1307-1315.
12. Honda, K. (1980). Odor of a papilionid butterfly: odoriferous substances emitted by *Atrophaneura alcinous alcinous* (Lepidoptera: Papilionidae). J Chem Ecol *6*, 867-873.
13. Leyrer, R.L., and Monroe, R.E. (1973). Identification and isolation of the scent of the moth, *Galleria mellonella,* and a revaluation of its sex pheromone. J Insect Physiol *19*, 2267-2271.
14. Romel, K.E., Scott-Dupree, C.D., and Carter, M.H. (1992). Qualitative and quantitative analyses of volatiles and pheromone gland extracts collected from *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). J Chem Ecol *18*, 1255-1268.
15. Hallem, E.A., Rengarajan, M., Ciche, T.A., and Sternberg, P.W. (2007). Nematodes, bacteria, and flies: a tripartite model for nematode parasitism. Curr Biol *17*, 898-904.
16. Ciche, T.A., Kim, K.S., Kaufmann-Daszczuk, B., Nguyen, K.C., and Hall, D.H. (2008). Cell invasion and matricide during *Photorhabdus luminescens* transmission by *Heterorhabditis bacteriophora* nematodes. Appl Environ Microbiol *74*, 2275-2287.
17. Bilgrami, A.L., Gauger, R., Shapiro-Ilan, D.I., and Adams, B.J. (2006). Source of trait deterioration in entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* during *in vivo* culture. Nematol *8*, 397-409.
18. Poiner, G.O., Jr., and Georgis, R. (1990). Description and field application of the HP88 strain of *Heterorhabditis bacteriophora.* Revue Nematol *13*, 387-393.
19. Grewal, P.S., Grewal, S.K., Malik, V.S., and Klein, M.G. (2002). Differences in the susceptibility of introduced and native white grub species to entomopathogenic nematodes from various geographic localities. Biol Contr *24*, 230-237.
20. Stock, S.P., and Kaya, H.K. (1996). A multivariate analysis of morphometric characters of *Heterorhabditis* species (Nemata: Heterorhabditidae) and the role of morphometrics in the taxonomy of species of the genus. J Parasitol *82*, 806-813.
21. Vivas, E.I., and Goodrich-Blair, H. (2001). *Xenorhabdus nematophilus* as a model for host-bacterium interactions: rpoS is necessary for mutualism with nematodes. J Bacteriol *183*, 4687-4693.
22. Kaya, H.K., and Stock, S.P. (1997). Techniques in insect nematology. In Manual of techniques in insect pathology, L. Lacey, ed. (San Diego, CA: Academic Press Limited).
23. White, G.F. (1927). A method for obtaining infective nematode larvae from cultures. Science *66*, 302-303.
24. Richards, G.R., Vivas, E.I., Anderson, A.W., Rivera-Santos, D., Gilmore, S., Suen, G., and Goodrich-Blair, H. (2009). Isolation and characterization of *Xenorhabdus nematophila* transposon insertion mutants defective in lipase activity against tween. J Bacteriol *191*, 5325–5331.
25. Martens, E.C., Heungens, K., and Goodrich-Blair, H. (2003). Early colonization events in the mutualistic association between *Steinernema carpocapsae* nematodes and *Xenorhabdus nematophila* bacteria. J Bacteriol *185*, 3147-3154.
26. Brenner, S. (1974). The genetics of *Caenorhabditis elegans.* Genetics *77*, 71-94.
27. Lewis, J.A., and Fleming, J.T. (1995). Basic culture methods. In Methods in cell biology, Volume 48, H.F. Epstein and D.C. Shakes, eds. (San Diego: Academic Press), pp. 3-29.
28. Cassada, R.C., and Russell, R.L. (1975). The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol *46*, 326-342.

29. Gray, J.M., Karow, D.S., Lu, H., Chang, A.J., Chang, J.S., Ellis, R.E., Marletta, M.A., and Bargmann, C.I. (2004). Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. Nature *430*, 317-322.
30. Campbell, J.F., Koppenhofer, A.M., Kaya, H.K., and Chinnasri, B. (1999). Are there temporarily non-infectious dauer stages in entomopathogenic nematode populations: a test of the phased infectivity hypothesis. Parasitol *118 (Pt 5)*, 499-508.
31. Campbell, J.F., and Kaya, H.K. (2000). Influence of insect-associated cues on the jumping behavior of entomopathogenic nematodes (*Steinernema* spp.). Behavior *137*, 591-609.
32. Bargmann, C.I., and Avery, L. (1995). Laser killing of cells in *Caenorhabditis elegans*. Methods Cell Biol *48*, 225-250.
33. Bargmann, C.I. (2006). Chemosensation in *C. elegans*. In WormBook, ed. The *C. elegans* Research Community, WormBook. doi/10.1895/wormbook.1.123.1, http://www.wormbook.org
34. Bargmann, C.I., and Horvitz, H.R. (1991). Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans.* Neuron *7*, 729-742.
35. Srinivasan, J., Durak, O., and Sternberg, P.W. (2008). Evolution of a polymodal sensory response network. BMC Biol *6*, 52.
36. Bumbarger, D.J., Wijeratne, S., Carter, C., Crum, J., Ellisman, M.H., and Baldwin, J.G. (2009). Three-dimensional reconstruction of the amphid sensilla in the microbial feeding nematode, *Acrobeles complexus* (Nematoda: Rhabditida). J Comp Neurol *512*, 271-281.
37. Fine, A.E., Ashton, F.T., Bhopale, V.M., and Schad, G.A. (1997). Sensory neuroanatomy of a skin-penetrating nematode parasite *Strongyloides stercoralis.* II. Labial and cephalic neurons. J Comp Neurol *389*, 212-223.
38. Ashton, F.T., Bhopale, V.M., Fine, A.E., and Schad, G.A. (1995). Sensory neuroanatomy of a skin-penetrating nematode parasite: *Strongyloides stercoralis.* I. Amphidial neurons. J Comp Neurol *357*, 281-295.
39. Li, J., Ashton, F.T., Gamble, H.R., and Schad, G.A. (2000). Sensory neuroanatomy of a passively ingested nematode parasite, *Haemonchus contortus:* amphidial neurons of the first stage larva. J Comp Neurol *417*, 299-314.
40. Li, J., Zhu, X., Ashton, F.T., Gamble, H.R., and Schad, G.A. (2001). Sensory neuroanatomy of a passively ingested nematode parasite, *Haemonchus contortus:* amphidial neurons of the third-stage larva. J Parasitol *87*, 65-72.
41. Ashton, F.T., Bhopale, V.M., Holt, D., Smith, G., and Schad, G.A. (1998). Developmental switching in the parasitic nematode *Strongyloides stercoralis* is controlled by the ASF and ASI amphidial neurons. J Parasitol *84*, 691-695.
42. Ashton, F.T., Zhu, X., Boston, R., Lok, J.B., and Schad, G.A. (2007). *Strongyloides stercoralis*: Amphidial neuron pair ASJ triggers significant resumption of development by infective larvae under host-mimicking *in vitro* conditions. Exp Parasitol *115*, 92-97.
43. Bhopale, V.M., Kupprion, E.K., Ashton, F.T., Boston, R., and Schad, G.A. (2001). *Ancylostoma caninum:* the finger cell neurons mediate thermotactic behavior by infective larvae of the dog hookworm. Exp Parasitol *97*, 70-76.
44. Li, J., Zhu, X., Boston, R., Ashton, F.T., Gamble, H.R., and Schad, G.A. (2000). Thermotaxis and thermosensory neurons in infective larvae of *Haemonchus contortus,* a passively ingested nematode parasite. J Comp Neurol *424*, 58-73.
45. Forbes, W.M., Ashton, F.T., Boston, R., Zhu, X., and Schad, G.A. (2004). Chemoattraction and chemorepulsion of *Strongyloides stercoralis* infective larvae on a sodium chloride gradient is mediated by amphidial neuron pairs ASE and ASH, respectively. Vet Parasitol *120*, 189-198.
46. Ketschek, A.R., Joseph, R., Boston, R., Ashton, F.T., and Schad, G.A. (2004). Amphidial neurons ADL and ASH initiate sodium dodecyl sulphate avoidance responses in the

infective larva of the dog hookworm *Anclyostoma caninum*. Int J Parasitol *34*, 1333-1336.
47. Nolan, T.J., Brenes, M., Ashton, F.T., Zhu, X., Forbes, W.M., Boston, R., and Schad, G.A. (2004). The amphidial neuron pair ALD controls the temperature-sensitive choice of alternative developmental pathways in the parasitic nematode, *Strongyloides stercoralis*. Parasitol *129*, 753-759.
48. Lopez, P.M., Boston, R., Ashton, F.T., and Schad, G.A. (2000). The neurons of class ALD mediate thermotaxis in the parasitic nematode, *Strongyloides stercoralis*. Int J Parasitol *30*, 1115-1121.
49. Sternberg, P.W., and Horvitz, H.R. (1981). Gonadal cell lineages of the nematode Panagrellus redivivus and implications for evolution by the modification of cell lineage. Dev Biol *88*, 147-166.
50. Sternberg, P.W., and Horvitz, H.R. (1982). Postembryonic nongonadal cell lineages of the nematode Panagrellus redivivus: description and comparison with those of Caenorhabditis elegans. Dev Biol *93*, 181-205.
51. Horvitz, H.R., and Sternberg, P.W. (1982). Nematode postembryonic cell lineages. J Nematol *14*, 240-248.
52. White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phil Trans Royal Soc London B *314*, 1-340.
53. O'Halloran, D.M., and Burnell, A.M. (2003). An investigation of chemotaxis in the insect parasitic nematode *Heterorhabditis bacteriophora*. Parasitol *127*, 375-385.
54. Blaxter, M.L., De Ley, P., Garey, J.R., Liu, L.X., Scheldeman, P., Vierstraete, A., Vanfleteren, J.R., Mackey, L.Y., Dorris, M., Frisse, L.M., et al. (1998). A molecular evolutionary framework for the phylum Nematoda. Nature *392*, 71-75.
55. Meldal, B.H., Debenham, N.J., De Ley, P., De Ley, I.T., Vanfleteren, J.R., Vierstraete, A.R., Bert, W., Borgonie, G., Moens, T., Tyler, P.A., et al. (2007). An improved molecular phylogeny of the Nematoda with special emphasis on marine taxa. Mol Phylogenet Evol *42*, 622-636.
56. Edgar, R.C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics *5*, 113.
57. Felenstein, J. (1989). PHYLIP - Phylogeny Inference Package (Version 3.2). Cladistics *5*, 164-166.
58. Maddison, W.P., and Maddison, D.R. (1989). Interactive analysis of phylogeny and character evolution using the computer program MacClade. Folia Primatol *53*, 190-202.
59. Posada, D. (2009). Selection of models of DNA evolution with jModelTest. Methods Mol Biol *537*, 93-112.
60. Guindon, S., and Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biol *52*, 696-704.
61. Huelsenbeck, J.P., and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics (Oxford, England) *17*, 754-755.
62. Hammer, Ø., Harper, D.A.T., and Ryan, P.D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontol Electronica *4*, 9pp.