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Supplemental Information

Natural Variation in Dauer Pheromone Production and Sensing Supports Intraspecific Competition in Nematodes

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1. Supplemental Figures.

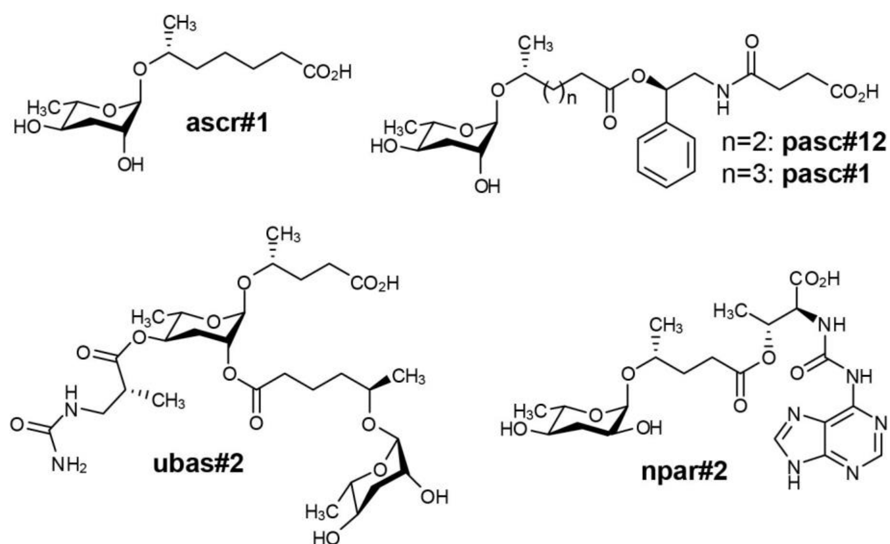

Figure S1. A subset of ascarosides and paratosides previously identified from *P. pacificus*. Related to Figure 1.

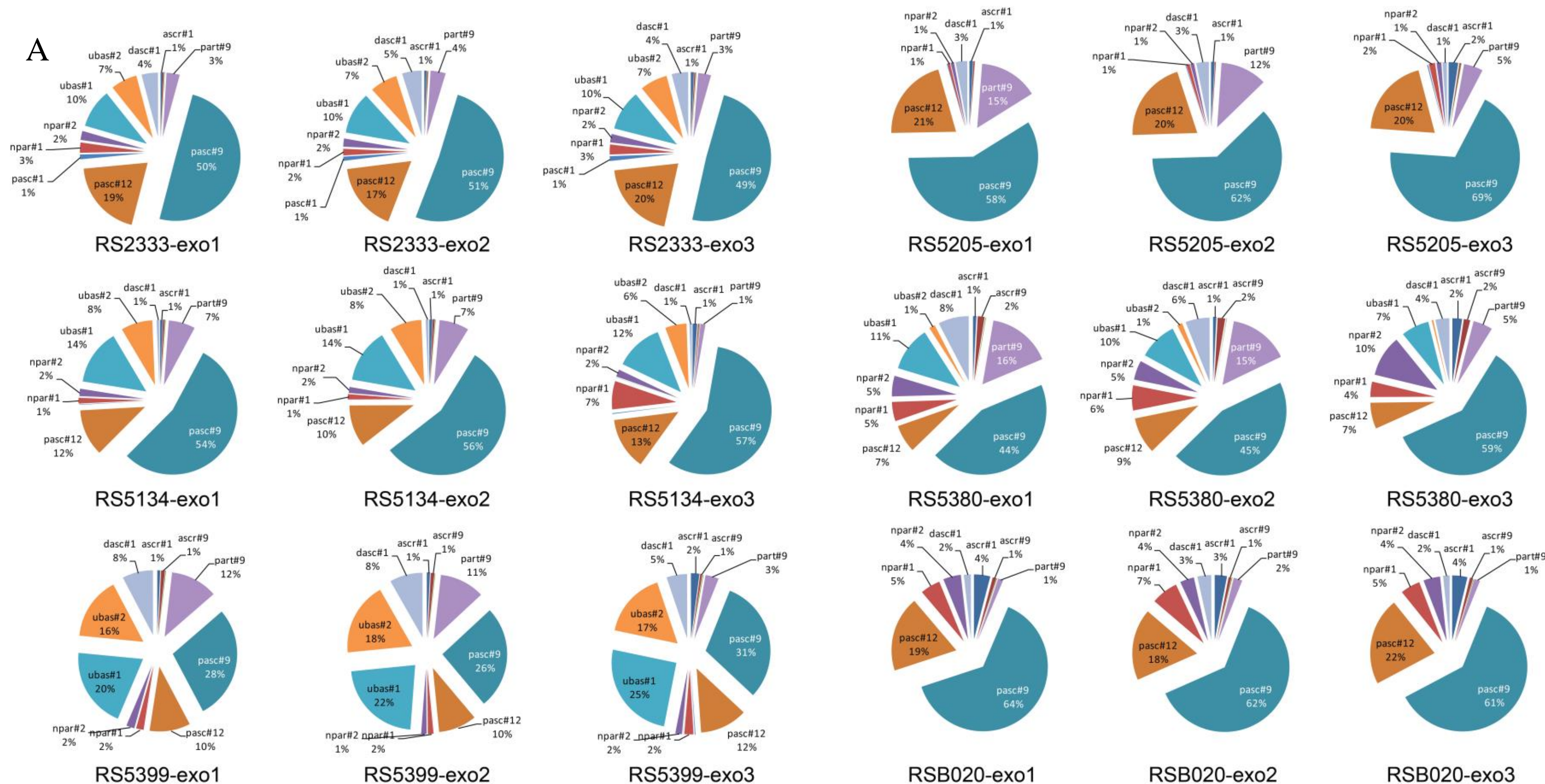

Figure S2. Ascaroside and paratoside profiles of *P. pacificus* wild isolates used in this study. Related to Figure 2. (A) Relative abundances of ascarosides and paratosides in the exo-metabolome of *P. pacificus* wild isolates derived from HPLC-MS analysis. Ascaroside and paratoside profiles for all biological replicates performed for the six strains used in this study are shown here. Profiles for the first replicate from exemplary strains are represented in Figure 2A for comparison with corresponding endo-metabolome profiles.

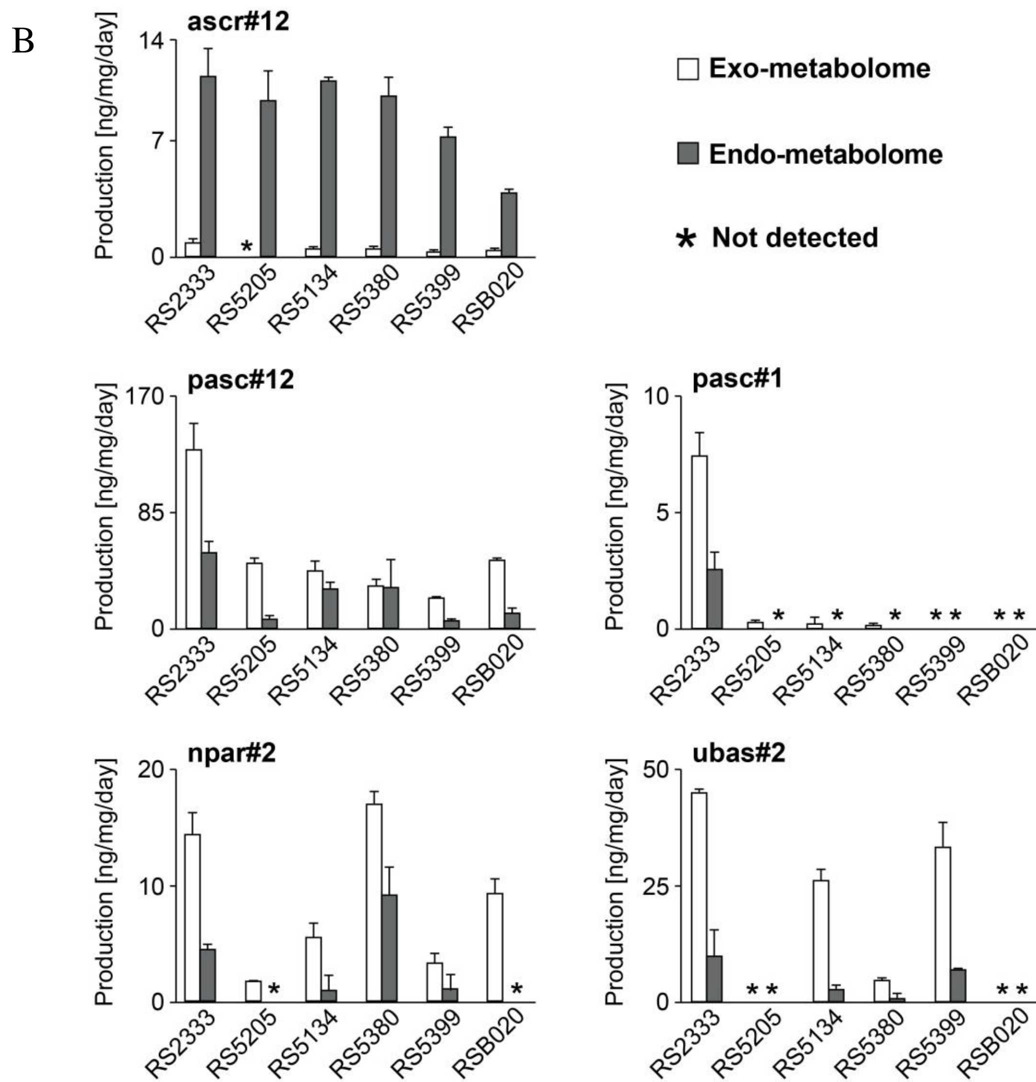

Figure S2. Ascaroside and paratoside profiles of *P. pacificus* wild isolates used in this study. Related to Figure 2.

(B) Comparison of abundances of five minor ascarosides and paratosides in the exo- and endo-metabolomes in six different *P. pacificus* wild isolates, represented in ng/day, and normalized by worm pellet dry weight (see Supplemental Experimental Procedures). Error bars, SD.

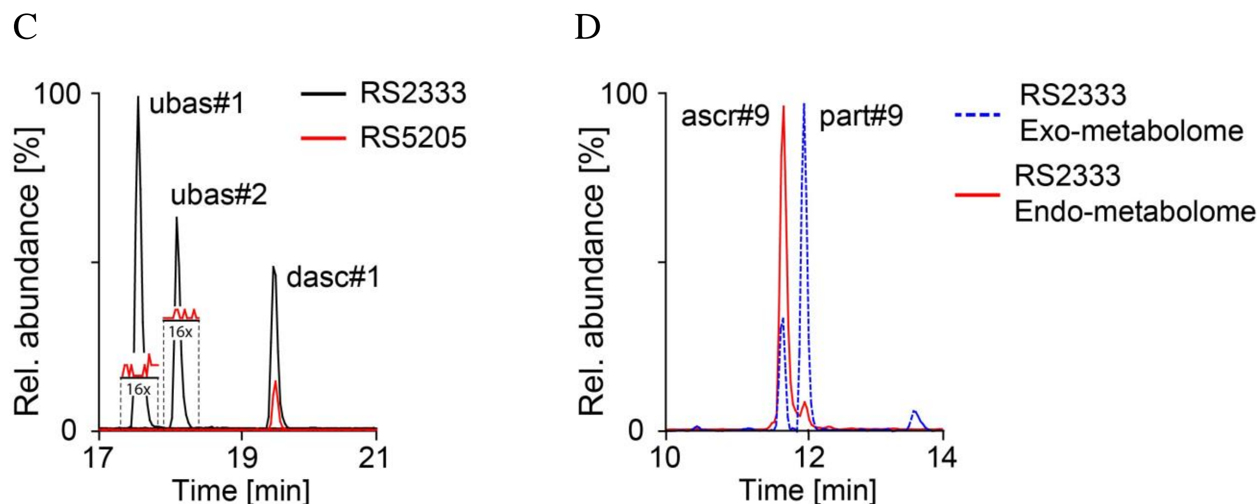

Figure S2. Ascaroside and paratoside profiles of *P. pacificus* wild isolates used in this study. Related to Figure 2.

(C) ESI- HPLC-MS ion traces for ubas#1 (*m/z*=605), ubas#2 (*m/z*=619), and dasc#1 (*m/z*=533) comparing their relative amounts in the exo-metabolomes of RS2333 and RS5205. RS5205 is incapable of producing ubas-compounds under the tested conditions, and its dasc#1 production is reduced as compared to RS2333.

(D) ESI- HPLC-MS ion traces comparing the relative abundances of ascr#9 and part#9 (*m/z*=247) in the RS2333 exo- and endo-metabolomes.

A

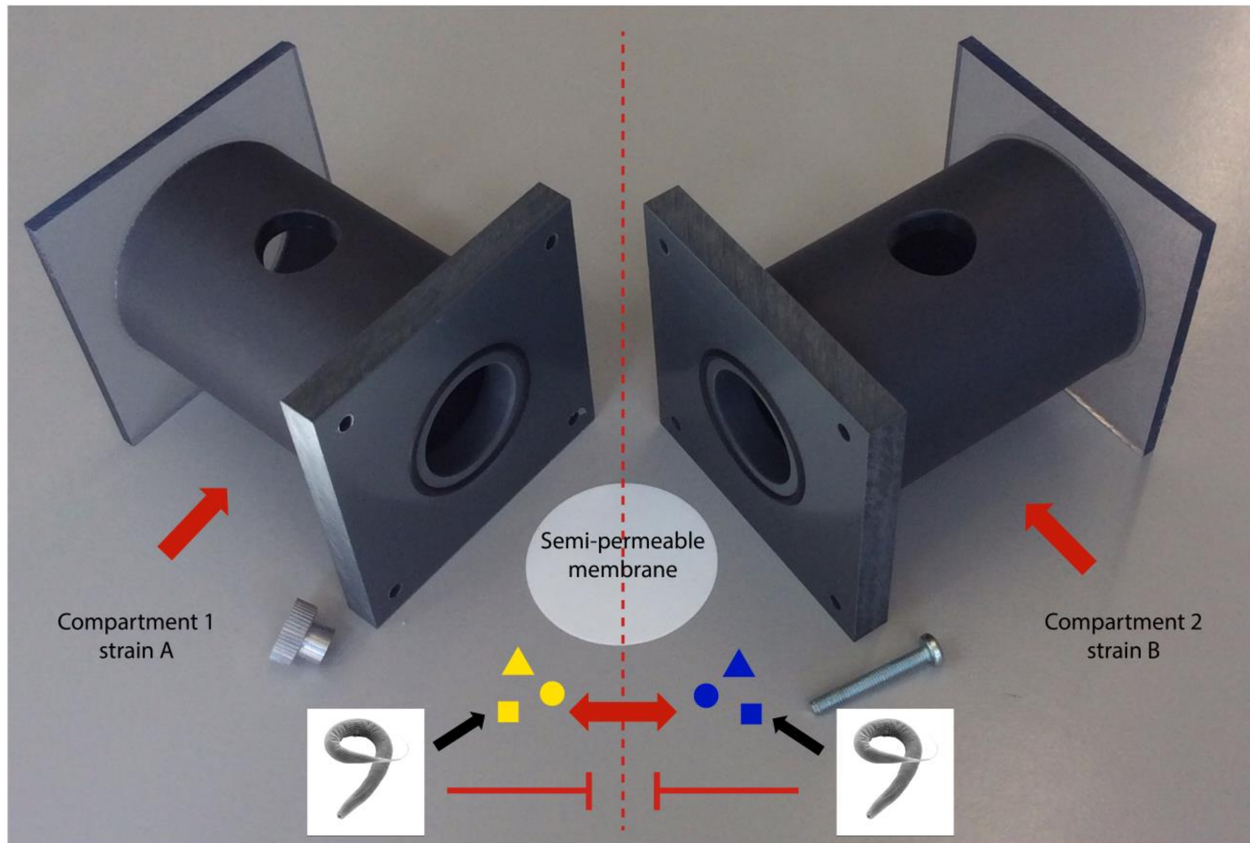

Figure S3. Dauer pheromone competition assay. Related to Figure 4.

(A) Experimental setup. An Ussing chamber consists of two compartments that are separated by a semi-permeable membrane. In the competition experiments, one strain (e.g. RS2333) was grown in liquid culture in one compartment of a chamber, while a different strain (e.g. RS5134) was grown in the other compartment of the same chamber. Since the competition assay was designed to test for dauer formation in a "natural" environment, no synthetic compounds were added. The small molecules excreted by the two strains can pass through the membrane between the two compartments, while the nematodes cannot.

B

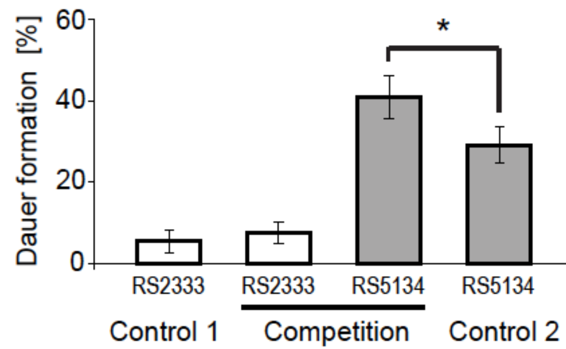

Figure S3. Dauer pheromone competition assay. Related to Figure 4.

(B) Dauer pheromone competition assay with RS2333 and RS5134 grown in Ussing chambers. In control experiments, both compartments of a chamber contained either RS2333 (Control 1) or RS5134 (Control 2) worms. In the competition experiment, RS2333 was grown in one compartment of an Ussing chamber, while RS5134 was grown in the other compartment of the same chamber. The mean dauer formation of three replicates is shown with exact binomial 95% confidence intervals.

Table S1. Statistical analysis of the dauer response using one way ANOVA (strain and compound only) and an ANOVA (GLM) combining both.

	DF	**SS**	**MS**	**F**	**P**
ANOVA (strain)	5	19973	3995	12.51	< 0.001
ANOVA (compound)	6	31644	5274	20.73	< 0.001
ANOVA (GLM) (strain and compound)	5	19973	3896	26.44	< 0.001
	6	31152	5192	35.23	< 0.001

2. Supplemental Experimental Procedures.

2.1. Nematode strains and cultures.

The following wild isolates of *P. pacificus* were used in this study: RS2333 (reference *P. pacificus* strain from California, USA), RS5134 (Ohio, USA), RS5205 (South Africa), RS5399, RS5380, and RSB020 (La Réunion Island). Worms were grown on NGM agar plates seeded with OP50, and liquid cultures of worms were prepared as described previously [S1, S2].

2.2. Preparation of metabolome extracts.

Three biological replicates of 100 ml liquid cultures of each *P. pacificus* wild isolate were prepared. These cultures were harvested, centrifuged, and the resultant supernatant media and worm pellets were frozen over dry ice-acetone slush and lyophilized separately. The lyophilized materials from the supernatant were extracted with 50 ml of 95% ethanol at room temperature for 16 h. The worm pellets were crushed with ~1 g of granular NaCl using a mortar pestle and extracted with 25 ml of 100% ethanol at room temperature for 16 h. The resulting suspensions were filtered, and the filtrate was evaporated *in vacuo* at room temperature, producing supernatant ("exo-metabolome") extracts and worm pellet ("endo-metabolome") extracts.

2.3. HPLC protocol and HPLC-MS analysis.

HPLC-MS was performed using an Agilent 1100 Series HPLC system equipped with an Agilent Eclipse XDB-C18 column (4.6 x 250 mm, 5 μm particle diameter) connected to a Quattro II spectrometer (Micromass/Waters). A 0.1% acetic acid-acetonitrile solvent gradient was used at a flow rate of 1 ml/min, starting with an acetonitrile content of 5% for 5 min which was increased to 100% over a period of 40 min.

Exo- and endo-metabolome extracts were resuspended in 250 μL of methanol, filtered, and used directly for HPLC-MS. The metabolome extracts were analyzed by HPLC-ESI-MS in negative ion mode as reported previously [S3]. Briefly, a capillary voltage of 3.5 kV and a cone voltage of -35 V were used, and the resulting data were analyzed using Waters MassLynx™ 4.1 software.

Quantifications were based on integration of HPLC-MS signals from the corresponding ion-traces. The following ESI- ion traces were used m/z = 247 (ascr#9 and part#9), 261 (ascr#12), 275 (ascr#1), 466 (pasc#9), 480 (pasc#12), 494 (pasc#1), 509 (npar#2), 533 (dasc#1), 605 (ubas#1), 619 (ubas#2), and 641 (npar#1). Absolute concentrations were calculated using response factors determined for synthetic standards. Concentrations for minor compounds, for

which synthetic standards were not available, were based on extrapolation of available standards of closely related structures. To account for the culture duration and worm biomass, ascaroside and paratoside content of the exo- and endo-metabolomes are represented in ng/day, and normalized by mg of worm pellet dry weight.

2.4. Dauer formation assay.

6 cm plates used in the dauer assay modified from [S2] contained NGM agar without peptone or cholesterol but with 50 µg/ml kanamycin (final concentration). Synthetic ascarosides and paratosides were resuspended in ethanol, and an aliquot was added for treatment. The same amount of ethanol was added to the control plates. OP50 from an overnight culture was incubated with kanamycin for 1.5 hours on a shaker at 25 °C and 800 rpm, washed with S-medium without cholesterol, and diluted to a final concentration of 2% in S-medium without cholesterol. The suspension was spotted on the assay plate. In *P. pacificus*, the J1 stage hatches inside the eggshell. Therefore, the J2 stage is essentially the first larval stage in *P. pacificus* that can be collected and manipulated. Nematodes of each strain were washed off from two fully-grown 6 cm agar plates (containing almost exclusively freshly hatched J2 larvae) with S-medium without cholesterol, washed once, and filtered through a 20 µm nylon membrane, allowing only J2 larvae to pass. After centrifugation at 1300 × g for 1 min, the S-medium was reduced to a final volume of 500 µl. From this worm stock solution, 10 µl were applied to each assay plate (~100 worms each). The plates were incubated at 25°C, and after two days dauer versus non-dauer larvae were counted. For all experiments, three independent replicates (three assay plates) for each control and treatment were performed on the same day and with the same worm batch. We repeated the experiments on different days using different worm batches and plates and found the same trend.

2.5. Dauer pheromone competition assay.

In the dauer formation assays described above, each strain was tested individually with each synthetic small molecule and never came in contact with another strain. The competition assay was specifically designed to enable the observation of dauer formation in a more "natural" environment. Therefore, nematodes were grown in liquid culture in Ussing chambers (commercially available from PHYWE®) [S4] without adding any synthetic compounds because we wanted to test for dauer formation in response to the small molecules in their naturally excreted combinations and concentrations. An Ussing chamber consists of two compartments that are separated by a membrane, through which the nematodes cannot pass (Figure S3A). In control experiments, both compartments of a chamber contained the same strain. In the competition experiments, one strain was grown in one compartment of an Ussing chamber, while a different strain was grown in the other compartment of the same chamber. To start the

experiment, the nematodes from one fully-grown 6 cm NGM agar plate (initially containing ten J4 larvae) were washed off into one compartment. For RS2333 and RS5134, three replicates of a sample volume of 30 µl were taken from each compartment after 14 days, and dauer formation was calculated as the percentage of dauers in the sample volume. We repeated all experiments with RS2333 and RS5134 multiple times and obtained similar results. For RS5380, RS5399, and RSB020, four replicates of a sample volume of 10 µl were taken from each compartment after 21 days. The experiments with RS5380, RS5399, and RSB020 were performed three times and showed similar results. Figure 4B shows the mean dauer formation of eight samples obtained from two independent experiments.

2.6. Statistical analyses.

Statistical analysis was performed with the commercial program minitab (http://www.minitab.com). Fisher's exact test was performed to compare control conditions against treatment using the R software [S5]. An ANOVA (GLM) was used to determine the significance of the variation among strains and compounds. The p-value represents significance of the model versus a model in which strain and compound information are not present (Table S1). The statistical significance of differences between treatment and control in the competition assay was tested using Fisher's exact test. All intervals are 95% exact binomial confidence intervals.

3. Supplemental References.

S1. Sommer, R.J., Carta, L.K., Kim, S.Y., and Sternberg, P.W. (1996). Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n. (Nematoda: Neodiplogastridae). Fundamental and Applied Nematology *19*, 511-522.

S2. Ogawa, A., Streit, A., Antebi, A., and Sommer, R.J. (2009). A Conserved Endocrine Mechanism Controls the Formation of Dauer and Infective Larvae in Nematodes. Curr Biol *19*, 67-71.

S3. Bose, N., Ogawa, A., von Reuss, S.H., Yim, J.J., Ragsdale, E.J., Sommer, R.J., and Schroeder, F.C. (2012). Complex small-molecule architectures regulate phenotypic plasticity in a nematode. Angew Chem Int Ed Engl *51*, 12438-12443.

S4. http://www.phywe.com/461/pid/14188/Ussing-chamber.htm

S5. R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.