A p p e n d i x

CAENORHABDITIS ELEGANS CAN USE MECHANOSENSATION TO PREDICT ENVIRONMENTAL COLLAPSE

(This work was done in collaboration with Lee J, Chin-Sang I, and Brugman K)

A.1 Abstract

Animals make decisions to alter aspects of their development based on signals from the environment. The roundworm *Caenorhabditis elegans* can escape environmental collapse by entering a spore-like dauer larval stage. Food, pheromone, and temperature have long been known to input into the dauer entry decision, but some inputs are clearly missing in models of the decision. Here we report a role for mechanosensation as an overlooked input into the decision. We show that gentle, harsh, and piezo touch promote dauer entry, using quantitative entry assays on CRISPR knock-ins and existing mutants in mechanosensation. We demonstrate that touch and pheromone likely work in parallel to promote dauer entry, by examining pheromone sensation and signal transmission in mechanosensation-defective mutants. We confirm that direct mechanical stimulation of *C. elegans* promotes dauer entry, and we provide a plausible role for mechanosensation in sensing dauer-promoting weather and crowding conditions. Our findings reveal that the dauer entry decision is more complex than previously recognized, and illuminates how animals can make robust decisions, even with a numerically simple nervous system.

A.2 Introduction

Most if not all organisms undergo developmental decisions to survive in changing environments (1, 2). By altering aspects of their development, organisms including bacteria (3, 4), insects (5), plants (6, 7), and mammals (8, 9) can adapt their metabolism, physiology, and reproductive strategy to meet resource availability. In this way, *Caenorhabditis elegans* roundworms can escape environmental collapse by becoming dauer larvae (10). Dauers are spore-like, stress-resistant, and capable of long-range dispersal (11-13). In addition, dauers have a remodeled nervous system and cease feeding, reproduction, and aging, making dauer entry one of the most dramatic postembryonic switches to be reported (14-16).

Dauer entry is a complex decision, requiring multiple inputs from food, pheromone, and temperature to assess the quality of the environment (17). Seven amphid sensory neurons (**Figure A.1A**) transduce these signals over an integration period of several hours, presumably to extract trend information on the environment's decline (18-20). Dauer entry is therefore an anticipatory decision that aims to predict whether environmental conditions will continue to support growth.

Despite being one of the best studied life cycle decisions, no satisfying model of dauer entry exists (but see (12, 18)), likely because a complete accounting of all of the inputs into the decision has not been made (21). We therefore investigated the possibility that mechanosensory inputs affect the dauer entry decision. Indeed, mechanosensation is useful for assessing population density in plants and bacteria (7, 22), and can be used to self-assess growth rate in insects (23). In the wild, *C. elegans* is found in rotting vegetation, where it can come into contact with bacteria, fungi, insects, predators, and other nematodes (24). *C. elegans* can use several types of touch, including discriminative gentle touch (25, 26) and

nociceptive harsh touch (27, 28), to help navigate through such complex physical environments (29, 30). Conceivably, information captured by mechanosensation could complement food, pheromone, and temperature signals to assess crowding, nutrition status, or other cues.

Using quantitative dauer entry assays, we demonstrate that CRISPR mutants and existing strains of mechanosensation-defective animals make inaccurate dauer entry decisions. By examining pheromone sensation and signal transmission, we find that pheromone and touch work in parallel pathways to promote dauer entry. Using direct mechanical stimulation, we further demonstrate that mechanosensation promotes dauer entry. Finally, we provide a plausible role for mechanosensation in assessing weather and crowding conditions that promote dauer entry. Our findings reveal that *C. elegans* use mechanosensation to enhance the accuracy of their dauer entry decision, demonstrating that the decision is more complex than previously recognized.

A.3 Results

The dauer entry life cycle decision is modulated by mechanosensation.

Gentle touch in *C. elegans* is sensed by the ALM, AVM, PLM, and PVM touch receptor neurons (TRNs) (25). The MEC-3/LIM homeodomain transcription factor is necessary for the differentiation of the TRNs during development (31). Using pheromone to induce dauer entry (19, 32), we tested the ability of *mec-3(e1338)* null mutants to enter dauer, relative to wild type. We observed that *mec-3(e1338)* entered dauer at a 3.4-fold lower rate than wild type (*mec-3(e1338)* dauer entry rate = 16%, N = 147; wild type dauer entry rate = 55%, N = 245) (**Figure A.1B-C**). This data suggests that MEC-3, and likely the TRNs, promotes dauer entry.

Mechanotransduction in the TRNs relies on the MEC-4/ MEC-10/MEC-2/MEC-6 channel complex (33). The MEC-4 channel subunit is essential for the activity of this complex, and is expressed exclusively in the TRNs (25, 34). Additionally, MEC-4 is believed to be required specifically for mechanotransduction, since other ionic currents are unaffected in *mec-4* nulls (33). Using CRISPR, we knocked in a 43-nucleotide stop cassette (35) into the *mec-4* gene to generate 3 putative null alleles: *sy1124*, *sy1125*, and *sy1126* (**Figure A.2**). We observed that the pheromone-induced dauer entry of these mutants occurred at an average 2.0-fold lower rate than wild type (*e.g. mec-4(sy1124)* dauer entry = 21%, N = 315; wild type dauer entry = 58%, N = 520) (**Figure A.1B-C**, **Figure A.3**).

We also tested the canonical *mec-4(u253)* null allele (36), which demonstrated a 126-fold decrease in dauer entry (*mec-4(u253)* dauer entry = 0%, N = 267; wild type dauer entry = 47%, N = 446). The stronger phenotype of the *u253* allele may indicate that *sy1124*, *sy1125*, and *sy1126* are loss-of-function alleles instead of nulls, or could be due to genetic background

effects in the *mec-4(u253)* strain.

Furthermore, we observed that *mec-4(e1611)* gain-of-function mutants have a 2.0-fold increased dauer entry rate as compared to wild type (*mec-4(e1611)* dauer entry = 79%, N = 228; wild type dauer entry = 37%, N = 167). Although the *e1611* gain-of-function allele causes neurodegeneration in the TRNs through hyperactivity of the mechanotransduction channel (37), the AVM touch neuron is not fully degenerated until adulthood (38). It is therefore likely that mechanotransduction is hyperactive in the AVM during the dauer entry decision in *mec-4(e1611)* animals. These data suggest that MEC-4 promotes dauer entry through the activity of the mechanotransduction channel.

We further confirmed this by testing the MEC-10 subunit of the channel complex, which regulates the ionic activity of the complex (39). We used CRISPR to generate 2 putative null alleles of *mec-10*: *sy1127*, and *sy1129* (**Figure A.2**), and observed that they entered dauer at an average 1.9-fold lower rate than wild type (*e.g. mec-10(sy1127)* dauer entry = 35%, N = 341; wild type dauer entry = 58%, N = 520) (**Figure A.1B-C**).

We also tested the *mec-10(e1515)* point mutant, which dramatically reduces the mechanoreceptor current (MRC) of the transduction complex (39). *mec-10(e1515)* mutants entered dauer at a 37.9-fold lower rate than wild type (*mec-10(e1515)* dauer entry = 1%, N = 181; wild type dauer entry = 42%, N = 241). Furthermore, the loss-of-function allele *mec-10(ok1104)*, which only mildly decreases the peak MRC of the channel complex (39), did not significantly affect dauer entry (*mec-10(ok1104)* dauer entry = 38%, N = 236; wild type dauer entry = 46%, N = 299). These data suggest that MEC-10 promotes dauer entry through the MRC of the transduction complex.

MEC-18/Firefly luciferase-like protein and MEC-19/novel membrane protein modulate

gentle touch (40, 41). We observed that *mec-18(u228)* decreased dauer entry by 5.1-fold (*mec-18(u228)* dauer entry = 9%, N = 167; wild type dauer entry = 46%, N = 418) and *mec-19(ok2504)* modestly decreased dauer entry by 1.4-fold (*mec-19(ok2504)* dauer entry = 44%, N = 233; wild type dauer entry = 60%, N = 430) (**Figure A.1B-C**). These data further indicate that gentle touch promotes dauer entry.

We also tested the role of harsh touch on dauer entry by assaying the *trp-4(sy695)* and *trp-4(sy696)* putative null alleles (42). The TRP-4/TRPN channel subunit is expressed in the ADE, DVA, and PDE harsh touch neurons and regulates posterior harsh touch (27). We observed that *trp-4(sy695)* and *trp-4(sy696)* decreased dauer entry by an average 3.9-fold (*e.g. trp-4(sy695)* dauer entry = 10%, N = 143; wild type dauer entry = 50%, N = 294) (**Figure A.1B-C**). These data suggest that harsh touch mediated by TRP-4 promotes dauer entry.

Since *mec* and *trp-4* mutants disrupt the function of several neurons, we used *ceh-17(np1)* nulls to test the effects of an incomplete nervous system on the dauer entry decision. The CEH-17 transcription factor is necessary for the proper axonal outgrowth of the ALA and 4 SIA neurons (43, 44), neither of which have known functions in dauer entry or mechanosensation. We observed that *ceh-17(np1)* did not significantly affect dauer entry, relative to wild type (*ceh(np1)* dauer entry = 39%, N = 185; wild type dauer entry = 49%, N = 239) (**Figure A.1B**). Therefore, the effects of the *mec* and *trp-4* mutants on dauer entry are likely beyond those of an incomplete nervous system. These data indicate that the dauer entry decision is modulated by gentle and harsh touch.

Touch and pheromone are parallel inputs into the dauer entry decision

To understand how the dauer entry decision is affected in touch mutants, we tested the dauer entry dose-response of *mec-4*, *trp-4*, and *mec-4;trp-4* mutants to pheromone. Using concentrations of 0.25%, 0.75%, and 2.25% pheromone to drive dauer entry, we observed a logarithmic dose-response to pheromone in wild type, as expected (45), with an EC50 of 0.64% ($R^2 = 0.99$) (**Figure A.4A**). *mec-4(sy1124)* mutants demonstrated an EC50 of 2.22% ($R^2 = 0.99$), corresponding to a decreased dose-response to pheromone across 0.75%-2.25%. *trp-4(sy695)* mutants demonstrated an EC50 of 0.98% ($R^2 = 0.99$), corresponding to a modest decrease in dose-response across all concentrations. The *mec-4(sy1124);trp-4(sy695)* double mutant demonstrated a similar dose-response to that of the *mec-4(sy1124)* single, with an EC50 of 2.07% ($R^2 = 0.99$). The decreased dose-response of the mutants suggests that *mec-4* and *trp-4* affect dauer entry by modulating pheromone sensation, or by affecting the decision as a parallel input to pheromone.

Aside from dauer entry, another method for assaying pheromone sensation is to measure *str-3* gene expression in the ASI neuron (46). STR-3 is a chemosensory receptor, and its expression in the ASI is repressed by sensation of pheromone in ASI and ASK. As a result, *str-3::gfp* is useful for identifying mutants that disrupt pheromone sensation and signal transmission (47, 48). We observed that STR-3::GFP fluorescence in the ASI did not vary between L2d animals with wild type *mec-4*, null *mec-4(sy1124)*, and gain-of-function *mec-4(e1611)* (**Figure A.4B-C**). In addition, STR-3::GFP fluorescence was the same between wild type, *mec-4(sy1124)*, and *mec-4(e1611)* young adults (**Figure A.4D**). Furthermore, STR-3::GFP levels did not vary in wild type adults that were mechanically stimulated via drop test (49) (**Figure A.4E**). These data suggest that touch does not affect pheromone sensation or signal transmission. A simple interpretation is that touch affects the dauer entry

decision as a parallel input to pheromone.

mec-4 and *trp-4* act additively with *pezo-1* to promote dauer entry

Despite being the major mechanotransducer in mammals (50, 51), the role of PEZO-1/Piezo in *C. elegans* remains unclear. In addition, *pezo-1* is expressed in neurons but not the TRNs (**Table A.1**). We used CRISPR to generate 3 loss-of-function alleles of *pezo-1*: *sy1184*, *sy1199*, and *sy1200*, and we observed that *pezo-1(sy1199)* decreased dauer entry by 2.0-fold (*pezo-1(sy1199)* dauer entry = 28%, N = 172; wild type dauer entry = 57%, N = 1039) (**Figure A.5**). This data suggest that *pezo-1* acts similarly to the *mec-4* and *trp-4* mechanotransducers and promotes dauer entry.

mec-4(sy1124);pezo-1(sy1200) double mutants decreased dauer entry by 2.5-fold (dauer entry = 23%, N = 137; wild type dauer entry = 57%, N = 1039), though this effect was not significantly different from the effect of the *mec-4* and *pezo-1* single mutants (**Figure A.5**). On the other hand, *mec-4(sy1124);trp-4(sy695);pezo-1(sy1184)* triple mutants decreased dauer entry by 4.2-fold (dauer entry = 14%, N = 190; wild type dauer entry = 57%, N = 1039) (**Figure A.5**). The effect of the *mec-4;trp-4;pezo-1* triple mutant was significantly greater than the effect of the single mutants, as well as the *mec-4;trp-4* double. These data suggest that *mec-4* and *trp-4* act additively with *pezo-1* to modulate dauer entry.

Direct mechanical stimulation promotes dauer entry

We investigated whether direct mechanical stimulation of animals could drive them into dauer entry. We used two methods for inducing mechanosensation: (1) we added 150-212 um glass beads to dauer entry plates to increase the roughness of the culture surface, and (2)

we used a servo shaker to gently agitate culture plates every 10 to 20 seconds.

We observed that the addition of 0.2 to 0.6 mg/cm^2 glass beads did not affect wild type dauer entry (dauer entry without beads = 64%, N = 215; dauer entry with beads = 64%, N = 325) (**Figure A.6A**). However, we observed that gently agitating sensitized *daf-2(e1370)* mutants—which enter dauer mildly at room temperature (52)—increased dauer entry by 1.7-fold (*daf-2(e1370)* dauer entry = 59%, N = 76; *daf-2(e1370)* with vibration = 100%, N = 44%) (**Figure A.6B**). These results suggest that direct mechanical stimulation, at least from vibration, can promote the dauer entry decision.

A.4 Discussion

Developmental decisions allow organisms to survive in changing environments (2). One of the best studied developmental decisions is *C. elegans* dauer entry. The principal regulators of this decision have been identified through genetic analysis of dauer-constitutive and -defective mutants, which highlighted the major inputs of food and pheromone (53-58). However, no satisfying model of the entry decision exists, likely because all of the inputs have not been identified (21).

Indeed, the known inputs into the dauer entry decision—food, pheromone, and temperature—are not the only cues that nematodes are exposed to in the wild, and in some cases these cues may be unreliable for assessing the environment. For instance, pheromones may be quenched by organic matter in soils (59), and may be used as dishonest signals to manipulate other nematodes into disadvantageous dauer decisions (60, 61).

Here we have demonstrated a role for mechanosensation as an overlooked modulator of the dauer entry decision. *C. elegans* can sense several types of touch, presumably to help navigate its natural environments where it can come into contact with bacteria, fungus, insects, carriers, predators, and other nematodes (62). These types of touch include gentle touch, harsh touch, nose touch, and food texture sensation (30). Gentle touch is likely analogous to low-threshold, discriminative touch in humans, which helps to detect light touch, hair movements, vibrations, quivering, and social touch (26, 63, 64). On the other hand, harsh touch is likely analogous to high-threshold nociception, which detects physically damaging forces (26-28). Curiously, the major mechanotransducers in nematodes are MEC-4/10 and TRP-4, while the major mechanotransducer in mammals in Piezo.

Using quantitative dauer entry assays on CRISPR knock-ins and existing mutants of

gentle touch (*mec-3*, *mec-4*, *mec-10*, *mec-18*, and *mec-19*), harsh touch (*mec-3* and *trp-4*), and piezo touch (*pezo-1*), we showed that mechanosensation promotes the dauer entry decision. We further confirmed this using direct mechanical stimulation, and demonstrated that vibration can promote dauer entry. We mostly did not observe large effect sizes for the mechanosensation-defective single mutants, and this is to be expected since the principal regulators of the decision have already been identified. Therefore, mechanosensation is a modulator of the decision, much like temperature which enhances pheromone-induced dauer entry (17).

Because of the moderate effect size of *trp-4(sy695)* on dauer entry, the *mec-4(sy1124)*;*trp-4(sy695)* phenotype could not be used to determine if *mec-4* and *trp-4* act additively or in the same pathway (65). However, close connections between the harsh touch and gentle touch neurons suggest it is likely that *mec-4* and *trp-4* act in the same circuit pathway to modulate dauer entry: The harsh touch PDE neuron is directly gap junctioned to the gentle touch PVM, and is gap junctioned to the gentle touch PLM via PVC (66, 67). In addition, the harsh touch DVA is gap junctioned to the gentle touch ALM and PLM via PVR and PVC/PVR, respectively. On the other hand, we demonstrated that *mec-4* and *trp-4* act additively with *pezo-1* to promote dauer entry, indicating that there are parallel pathways for mechanosensation to input into the decision.

We propose that mechanosensation could be used to assess at least two conditions that correlate with dauer entry: humidity and crowding. First, humidity is sensed, in part, by MEC-10 (68), and has been suggested by some groups to promote dauer entry (21). Moreover, moisture has been shown to affect the dispersal of parasitic nematodes (69), suggesting it may affect dauer dispersal as well. Indeed, we and others have shown that

dauers and parasitic nematodes share common strategies for dispersal (32, 70). Thus, while dauers can survive dessication for a few days (13), it may be advantageous for *C. elegans* to enter dauer when humidity levels are favorable for dispersal.

Second, *C. elegans* can sense crowding via pheromone signals (71), which can be inaccurate (59-61). We speculate that *C. elegans* could also measure crowding via contact-dependent signaling, such as in bacteria (22), plants (7), and insects (5). We have shown that touch and pheromone likely act in parallel to affect the dauer entry decision, and it is conceivable that they might jointly assess crowding in order to increase the accuracy of the decision.

The input of mechanosensation into dauer entry has revealed the decision to be more complex than previously recognized. This growing complexity raises the intriguing possibility that other cues such as light, O_2/CO_2, pH, and osmotic stress may input into the decision as well (**Figure A.7**). This hypothesis is supported by recent findings that the dauer entry decision is modulated by noxious stimuli, which may facilitate pheromone signaling (48). It is plausible that multiple inputs assessing various aspects of the environment may be crucial for making robust developmental decisions in *C. elegans*. Finally, since mechanosensation is important for growth and development in invertebrates to vertebrates (72), and is used to make developmental decisions in fungi (73), plants (7), and insects (5), we speculate that mechanosensation may be a common input into developmental decisions across biology.

A.5 Materials and Methods

Animal strains

C. elegans strains were grown using standard protocols with *Escherichia coli* OP50 as a food source (74). The wild type strain was N2 (Bristol). Strains obtained from the *Caenorhabditis* Genetics Center (CGC) include CB1515 *mec-10(e1515)*, RB1115 *mec-10(ok1104)*, TU228 *mec-18(u228)*, RB1925 *mec-19(ok2504)*, and IB16 *ceh-17(np1)* 3x outcrossed. TQ526 *mec-3(e1338)* 4x outcrossed, TQ253 *mec-4(u253)*, and TQ1243 *mec-4(e1611)* 6x outcrossed were gifts from the Xu laboratory. PS4492 *trp-4(sy695)* 7x outcrossed and PS4493 *trp-4(sy696)* 6x outcrossed were generated in the Sternberg laboratory.

CRISPR-generated strains

CRISPR alleles of *mec-4*, *mec-10*, and *pezo-1* were generated by knocking in the 43-nucleotide stop cassette:
GGGAAGTTTGTCCAGAGCAGAGGTGACTAAGTGATAAgctagc (35).

PS7913 *mec-4(sy1124)*, PS7914 *mec-4(sy1125)*, and PS7915 *mec-4(sy1126)* were generated using the guide RNA ACGACGTGCCGGTTTTGTGG. Flanking sequences

(Left) CCGAACCACCCACCACCCCTGCACCCACCA

(Right) CAAAACCGGCACGTCGTCGAGGAAAACGTG.

PS8039 *trp-4(sy695)*;*mec-4(sy1124)* was generated by crossing PS7913 males to PS4492.

PS7916 *mec-10(sy1127)* and PS7918 *mec-10(sy1129)* were generated using the guide RNA TATACAATTTATCAATCAGG. Flanking sequences

(Left) TTCTAATCTGTGCTATACAATTTATCAATC

(Right) AGGCGGTCGCTGTGATTCAGAAGTATCAGA.

PS8111 *pezo-1(sy1199)*, PS8112 *pezo-1(sy1200);mec-4(sy1124)*, and PS8084 *trp-4(sy695);pezo-1(sy1184);mec-4(sy1124)* were generated using the guide RNA CCAGAAGCTCGTAAGCCAGG. Putative flanking sequences

(Left) CGCTGTTTCTGAACCAGAAGCTCGTAAGCC

(Right) AGGAGGCACTGAAGAAACGGATGGTGATGA.

Dauer entry assay

Pheromone-induced dauer entry assays were performed as previously described (32). The conditions used to induce dauer entry were: 20 uL of 8% w/v heat-killed OP50 and incubation at 25.5^{o}C for 48 hours, with approximately 50 animals per plate. For phenotypic screening (**Figure A.1B**), we used 1.5% pheromone to induce approximately 50% dauer entry in wild type in order to detect increased or decreased dauer entry in mutants.

Mechanical perturbation of animals

Glass beads: 2 to 6 mg of autoclaved glass beads (Millipore Sigma G1145, 150-212 um) were added to the surface of 0.75% pheromone dauer entry plates, to an approximate density of 0.2 to 0.6 mg/cm^2. Dauer entry was assayed as above.

Vibration assay: We used the *daf-2(e1370)* sensitized mutant, which enters dauer modestly at room temperature (52). We attached culture plates containing *daf-2(e1370)* animals to a servo shaker and gently agitated every 10 to 20 seconds at room temperature for 48 hours.

Drop test assay

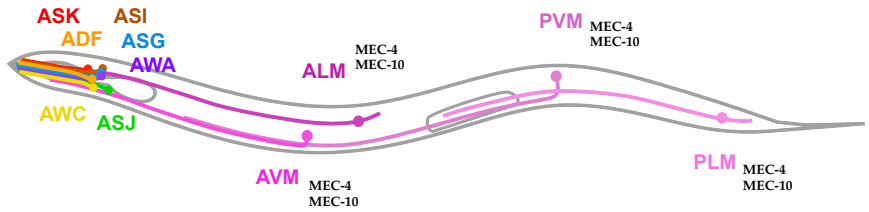
Culture plates were parafilmed, put in a cardboard box, and dropped as in (49): from a height of 5 cm, 30 times, with a 10 second interstimulus interval.

Pheromone sensitivity assay

For measurements in L2d, larvae were grown on 2.25% pheromone dauer entry plates for 23 to 27 hours at 25.5^{o}C. For measurements in young adults, 20 L4 animals were picked onto seeded NGM plates the day before the assay. For the drop test assay, 15 L4 animals were picked the day before the assay. Fluorescence measurements of STR-3::GFP in the ASI neuron were made using ZEISS ZEN software. Average fluorescence intensities were obtained from regions drawn around the ASI and image backgrounds, and fluorescence was corrected by subtracting the background. All fluorescence intensities were normalized to measurements from the same-day CX3596 *str-3::gfp* control.

A.6 Figures and tables

A



B

Genotype	Function Effect	Mutant Entry %	WT Entry %	Relative Entry (WT % / Mutant %)	Adjusted *P*	Trials Tested	Mutant N_{tested}	WT N_{tested}
mec-3(e1338) x4 out.	null	16	55	3.4	***	3	147	245
mec-4(sy1124)	putative null	21	58	2.7	***	6	315	520
mec-4(sy1125)	putative null	29	54	1.9	***	4	279	419
mec-4(sy1126)	putative null	41	58	1.4	***	4	261	520
mec-4(u253)	null	0	47	126.3	***	4	267	446
mec-4(e1611) x6 out.	gf	79	37	0.5	***	4	228	167
mec-10(sy1127)	putative null	35	58	1.6	***	6	341	520
mec-10(sy1129)	putative null	28	58	2.1	***	4	165	520
mec-10(e1515)	gf	1	42	37.9	***	3	181	241
mec-10(ok1104)	lf	38	46	1.2	n.s.	4	236	299
mec-18(u228)	unknown	9	46	5.1	***	3	167	418
mec-19(ok2504)	putative null	44	60	1.4	***	4	233	430
trp-4(sy696) x6 out.	putative null	19	50	2.7	***	3	176	294
trp-4(sy695) x7 out.	putative null	10	50	5.1	***	3	143	294
ceh-17(np1) x3 out.	null	39	49	1.3	n.s.	3	185	239

C

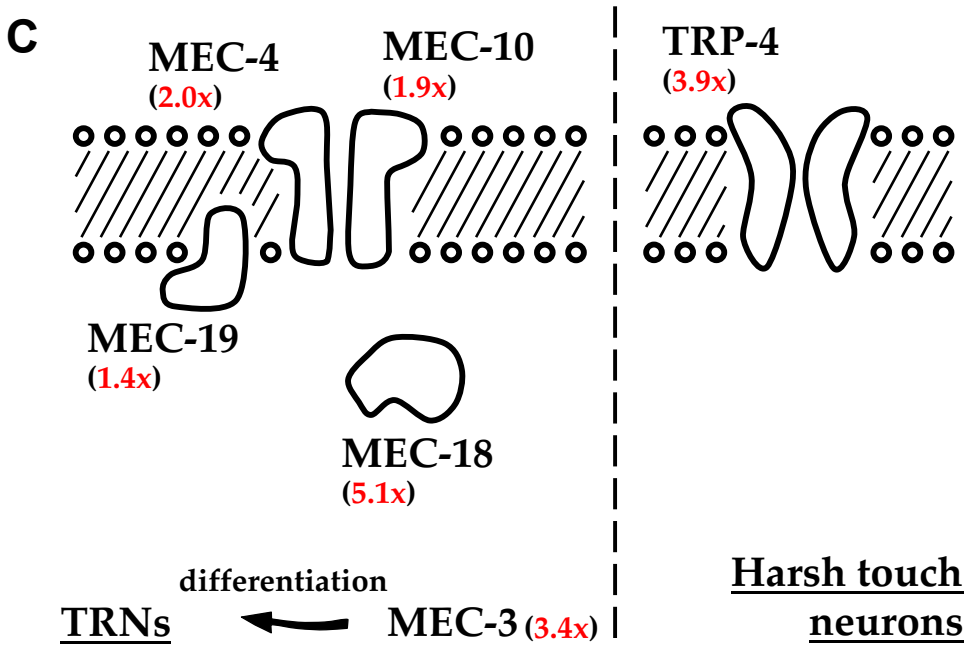
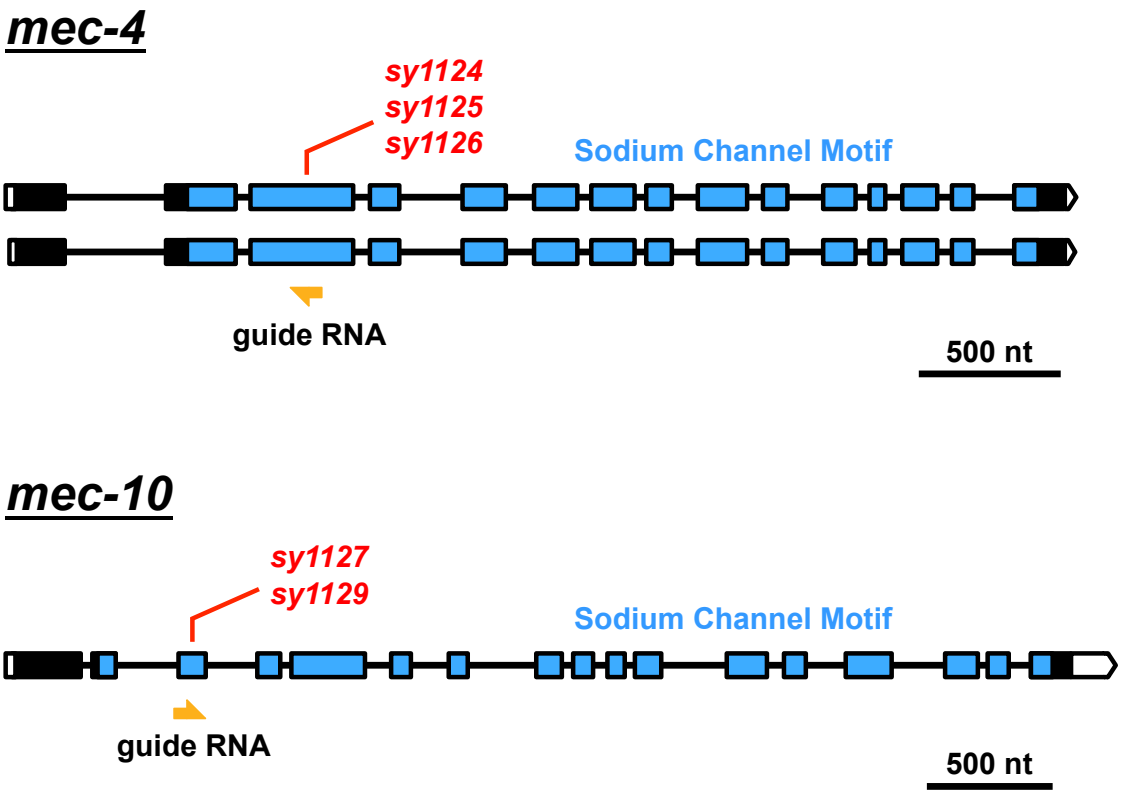


Figure A.1. The dauer entry life cycle decision is modulated by mechanosensation. (A) Schematic of the gentle touch mechanosensory neurons (magenta) and amphid neurons (rainbow) of *C. elegans*. The expression of MEC-4 and MEC-10 mechanoreceptors in the gentle touch neurons is indicated. **(B)** Dauer entry rates of *mec* mutants. *P* calculated via nonparametric permutation test and adjusted using Bonferroni correction. out., outcrossed. **(C)** Schematic of gentle (left) and harsh (right) touch neurons. Top, ECM; bottom, cytoplasm. Numbers in parentheses represent the relative dauer entry rate of wild type to mutant. Red, dauer entry promoting.



sy1124 – 1126, 1127, 1129
STOP-IN allele: GGGAAGTTTGTCCAGAGCAGAGGTGACTAAGTGATAAgctagc

Figure A.2. *mec-4* and *mec-10* CRISPR alleles are putative nulls. Gene models of *mec-4* and *mec-10*. The location of the *sy* CRISPR alleles are indicated in red. White, untranslated regions; black, exons; blue, sodium channel-encoding exon regions; lines, introns. Arrow indicates the direction of the guide RNA.

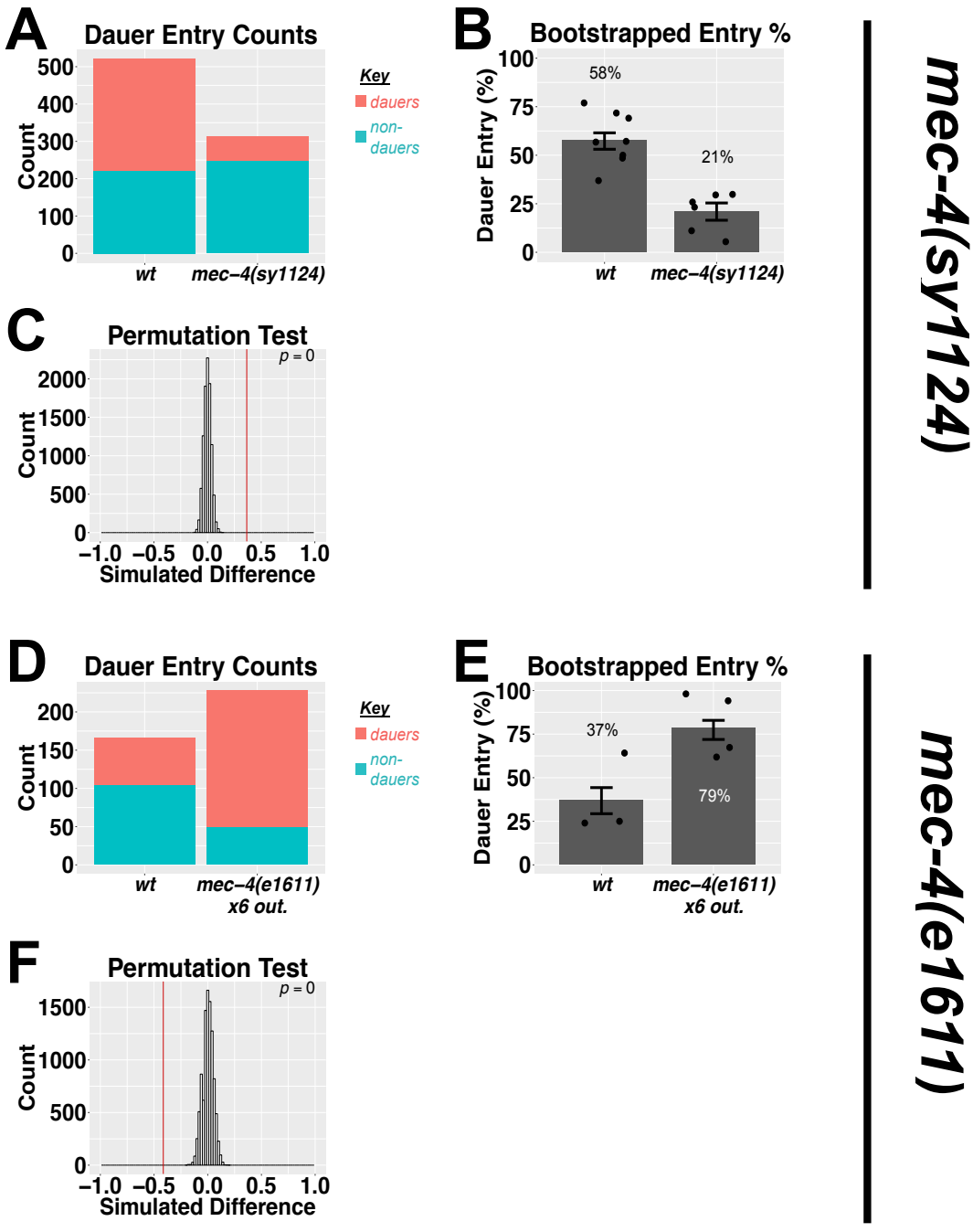
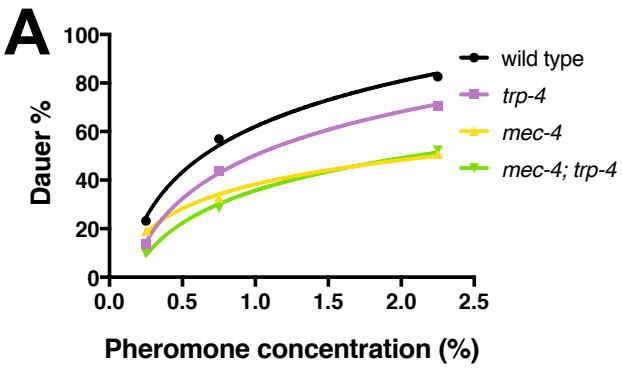

A
Dauer Entry Counts
Count
500
400
300
200
100
0
wt
mec–4(sy1124)
Key
dauers
non-dauers
B
Bootstrapped Entry %
Dauer Entry (%)
100
75
50
25
0
58%
21%
wt
mec–4(sy1124)
C
Permutation Test
p = 0
Count
2000
1500
1000
500
0
–1.0 –0.5 0.0 0.5 1.0
Simulated Difference
mec-4(sy1124)
D
Dauer Entry Counts
Count
200
150
100
50
0
wt
mec–4(e1611) x6 out.
Key
dauers
non-dauers
E
Bootstrapped Entry %
Dauer Entry (%)
100
75
50
25
0
37%
79%
wt
mec–4(e1611) x6 out.
F
Permutation Test
p = 0
Count
1500
1000
500
0
–1.0 –0.5 0.0 0.5 1.0
Simulated Difference
mec-4(e1611)

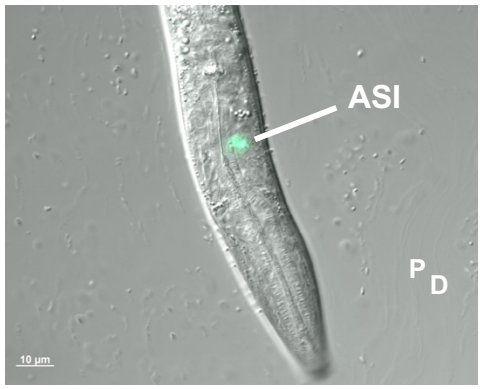
Figure A.3. *mec-4* promotes dauer entry. (A, D) The number of animals that decided to enter dauer (red) or reproductive development (blue) for the wild type control, (A) *mec-4(sy1124)* nulls, and (D) *mec-4(e1611)* gain-of-function mutants. **(B, E)** Representation of dauer entry counts as percentages. Points, independent trials; bar, bootstrapped dauer entry percentage; whiskers, 95% confidence interval. **(C, F)** Histogram of the 9,999 simulated differences between wild type and (C) *mec-4(sy1124)* nulls or (F) *mec-4(e1611)* gain-of-function mutants in non-parametric permutation tests. Red line, observed difference.

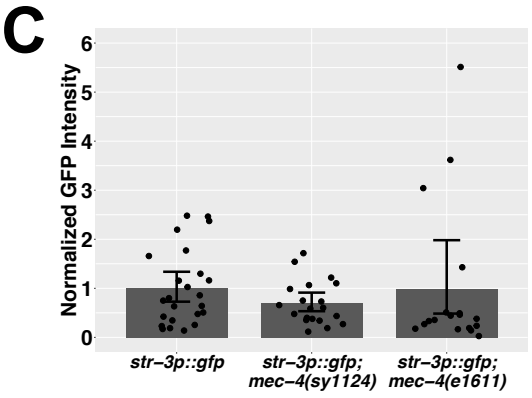


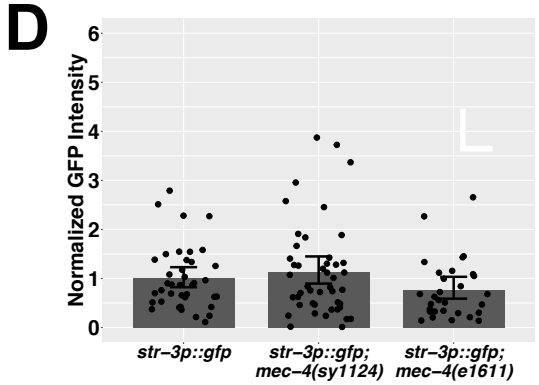
0.25%	wild type	*mec-4*	*trp-4*	*mec-4; trp-4*
wild type				
mec-4	n.s.			
trp-4	*	n.s.		
mec-4; trp-4	*	n.s.	n.s.	

0.75%	wild type	*mec-4*	*trp-4*	*mec-4; trp-4*
wild type				
mec-4	***			
trp-4	**	*		
mec-4; trp-4	***	n.s.	**	

2.25%	wild type	*mec-4*	*trp-4*	*mec-4; trp-4*
wild type				
mec-4	***			
trp-4	***	***		
mec-4; trp-4	***	n.s.	***	







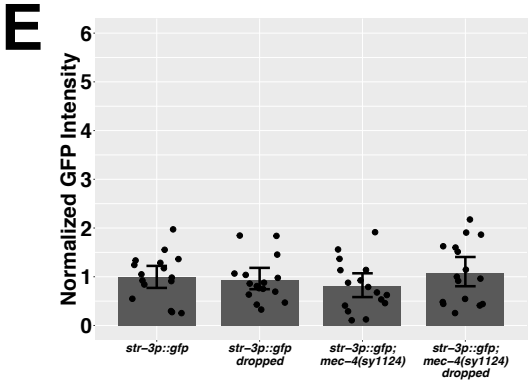
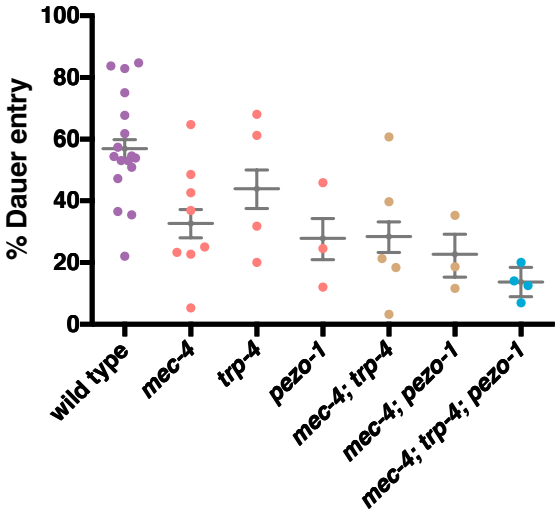


Figure A.4. Touch and pheromone are parallel inputs into the dauer entry decision. (A) Pheromone dose-response curve of dauer entry for wild type, *mec-4(sy1124)* nulls, *trp-4(sy695)* nulls, and *mec-4(sy1124)*;*trp-4(sy695)* double mutants. Points represent averages from 3-17 independent trials. Pairwise adjusted *P* values are indicated in the matrices corresponding to each pheromone concentration point. Shades of green, increasing statistical confidence. **(B)** Representative image of *str-3::gfp* fluorescence in the ASI neuron of *mec-4(wt)* L2d larvae. **(C-D)** STR-3::GFP intensity in (C) L2d and (D) adult animals. **(E)** STR-3::GFP intensity in adults mechanically stimulated via dropping. Points, individual animals; bar, bootstrapped mean intensity; whiskers, 95% confidence interval.

Gene	Protein Type	Expression	Strain	Allele	Protein Effect	Function Effect	Phenotype	Citation
mec-3	LIM homeodomain protein	AIZ, ALM, AVM, FLP, PLM, PVD, PVM, VNC	TQ526	*mec-3(e1338)*	Insertion and frameshift	Putative loss-of-function or null	TRNs fail to differentiate	Way & Chalfie 1989; Xue, Tu, & Chalfie 1993; Bounoutas *et al*. Chalfie 2009; Kubanek *et al*. Goodman 2018
mec-4	DEG / ENaC channel	ALM, AVM, PLM, PVM	TU253	*mec-4(u253)*	Deletion	Null	Abolished mechanoreceptor currents	Hong, Mano, & Driscoll 2000; O'Hagan, Chalfie, & Goodman 2005
			TQ1243	*mec-4(e1611)*	T442A	Gain-of-function	Touch insensitivity, touch cell degeneration	Driscoll & Chalfie 1991
			CB1339	*mec-4(e1339)*	G230E	Loss-of-function	Partially touch insensitive	O'Hagan, Chalfie, & Goodman 2005; Chalfie & Sulston 1981
mec-10	DEG / ENaC channel	ALM, AVM, FLP, PLM, PVD, PVM, tail neuron	CB1515	*mec-10(e1515)*	S105F	Gain-of-function	Touch insensitive (but weaker than *u20*, *u390*, *u332*, *e1715*)	Huang & Chalfie 1994; Arnadottir *et al*. Chalfie 2011
			RB1115	*mec-10(ok1104)*	Deletion	Loss-of-function	Partially touch insensitive (weaker than *e1515*)	Arnadottir et al. Chalfie 2011
mec-18	Firefly luciferase-like	ALM, AVM, PLM, PVM	TU228	*mec-18(u228)*	Uncurated	Unknown	Partial abnormality in mechanosensation	WormBase; CGC
mec-19	Novel membrane protein	ALM, AVM, FLP, PLM, PVD, PVM	RB1925	*mec-19(ok2504)*	Deletion	Putative null	Enhanced *mec-4(d)* degeneration	Barstead *et al*. Zapf 2012; Chen *et al*. Chalfie 2016
pezo-1	Piezo-type mechanosensitive ion channel	head neurons, HOA, HOB, male tail interneurons, PCS, CAN, ray neurons, spermatheca, vulval muscle	PS8111	*pezo-1(sy1199)*	Insertion, stop, and frameshift	Putative loss-of-function or null	Male mating defective (falling off), reduced fecundity	Brugman & Sternberg *unpublished*
trp-4	TRPN channel pore-forming subunit	ADE, CEP, DVA, DVC, PDE	PS4492	*trp-4(sy695)*	Deletion	Putative null	Abnormal body bends	Li *et al*. Xu 2011
			PS4493	*trp-4(sy696)*	Deletion	Putative null	Abnormal body bends	Li et al. Xu 2011
ceh-17	Q_{50} paired-like homeodomain protein	ALA, DA8, DB5, DNC, head muscle, RMED, SIA, SIBV, VNC	IB16	*ceh-17(np1)*	Deletion	Null	ALA and SIA axonal outgrowth impaired	Pujol et al. Brunet 2000; Buskirk & Sternberg 2007

Table A.1. Expression pattern and allele effects of mechanosensation genes. Magenta, gentle touch receptor neurons; Orange, harsh touch receptor neurons.



0.75%	wild type	*mec-4*	*trp-4*	*pezo-1*	*mec-4; trp-4*	*mec-4; pezo-1*	*mec-4; trp-4; pezo-1*
wild type							
mec-4	***						
trp-4	**	*					
pezo-1	***	n.s.	**				
mec-4; trp-4	***	n.s.	**	n.s.			
mec-4; pezo-1	***	n.s.	***	n.s.	n.s.		
mec-4; trp-4; pezo-1	***	***	***	*	**	n.s.	

Figure A.5. *mec-4* and *trp-4* act additively with *pezo-1* to promote dauer entry. Dauer entry *mec-4*, *trp-4*, and *pezo-1* at 0.75% pheromone. Points, independent trials; center line, bootstrapped dauer entry percentage; whiskers, 95% confidence interval.

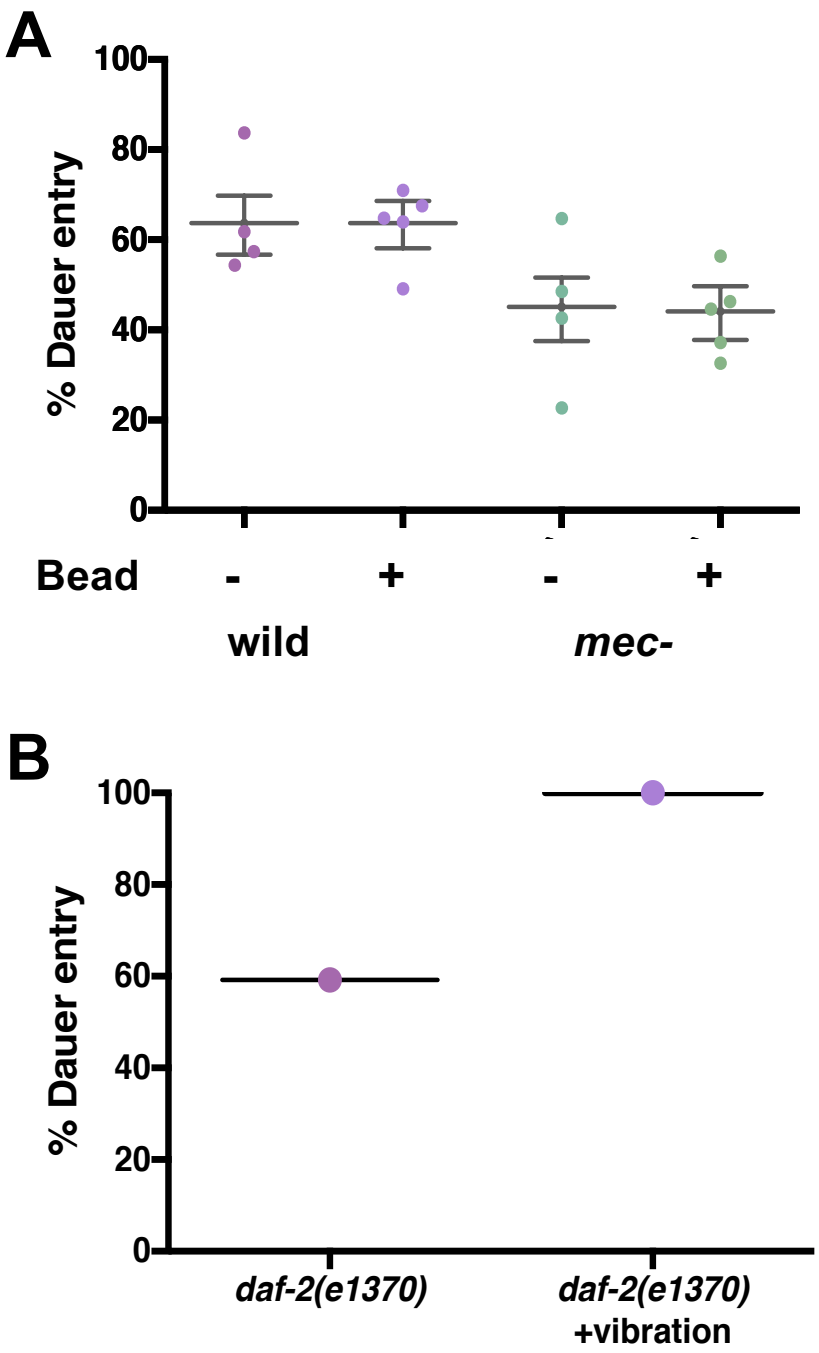


Figure A.6. Direct mechanical stimulation promotes dauer entry. (A) Dauer entry percentages for wild type animals grown with glass bead perturbation. **(B)** Dauer entry for *daf-2(e1370)* with vibration perturbation. Points, independent trials; bar, bootstrapped dauer entry percentage.

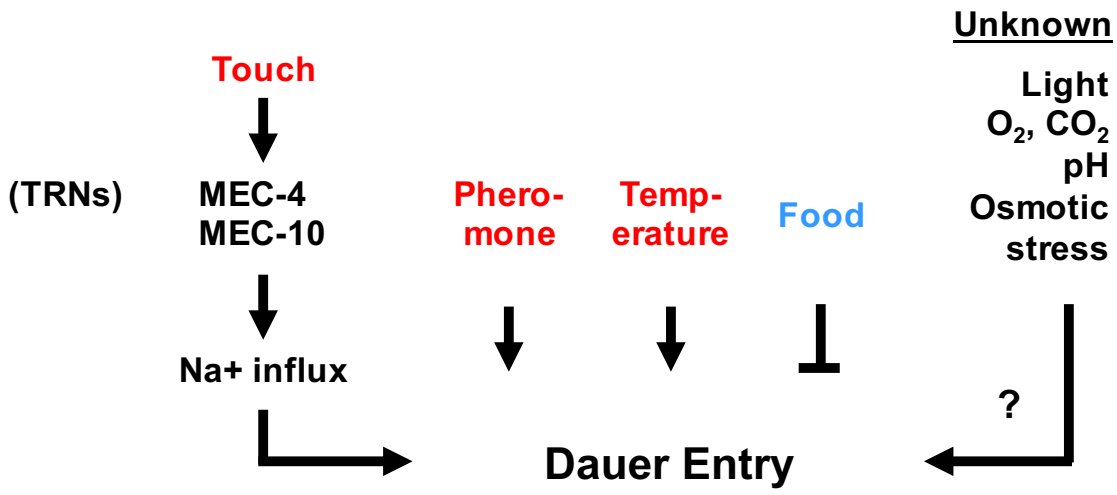


Figure A.7. Model of the complex dauer entry decision. Red, dauer-promoting inputs; blue, dauer-inhibiting.

A.7 References

1. Ackermann M (2015) A functional perspective on phenotypic heterogeneity in microorganisms. *Nat Rev Microbiol* 13(8):497-508.
2. Fusco G & Minelli A (2010) Phenotypic plasticity in development and evolution: facts and concepts. Introduction. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 365(1540):547-556.
3. Perkins TJ & Swain PS (2009) Strategies for cellular decision-making. *Mol Syst Biol* 5:326.
4. Tagkopoulos I, Liu YC, & Tavazoie S (2008) Predictive behavior within microbial genetic networks. *Science* 320(5881):1313-1317.
5. Simpson SJ, Sword GA, & Lo N (2011) Polyphenism in insects. *Current biology : CB* 21(18):R738-749.
6. Gruntman M, Gross D, Majekova M, & Tielborger K (2017) Decision-making in plants under competition. *Nat Commun* 8(1):2235.
7. de Wit M*, et al.* (2012) Plant neighbor detection through touching leaf tips precedes phytochrome signals. *Proceedings of the National Academy of Sciences of the United States of America* 109(36):14705-14710.
8. Ptak GE*, et al.* (2012) Embryonic diapause is conserved across mammals. *PloS one* 7(3):e33027.
9. Renfree MB & Fenelon JC (2017) The enigma of embryonic diapause. *Development* 144(18):3199-3210.

10. Cassada RC & Russell RL (1975) The dauerlarva, a post-embryonic developmental variant of the nematode Caenorhabditis elegans. *Developmental biology* 46(2):326-342.

11. Felix MA & Duveau F (2012) Population dynamics and habitat sharing of natural populations of Caenorhabditis elegans and C. briggsae. *BMC biology* 10:59.

12. Fielenbach N & Antebi A (2008) C. elegans dauer formation and the molecular basis of plasticity. *Genes & development* 22(16):2149-2165.

13. Kiontke K & Sudhaus W (2006) Ecology of Caenorhabditis species. *WormBook : the online review of C. elegans biology*:1-14.

14. Albert PS & Riddle DL (1983) Developmental alterations in sensory neuroanatomy of the Caenorhabditis elegans dauer larva. *The Journal of comparative neurology* 219(4):461-481.

15. Klass M & Hirsh D (1976) Non-ageing developmental variant of Caenorhabditis elegans. *Nature* 260(5551):523-525.

16. Vanfleteren JR & Braeckman BP (1999) Mechanisms of life span determination in Caenorhabditis elegans. *Neurobiol Aging* 20(5):487-502.

17. Golden JW & Riddle DL (1984) The Caenorhabditis elegans dauer larva: developmental effects of pheromone, food, and temperature. *Developmental biology* 102(2):368-378.

18. Avery L (2014) A model of the effect of uncertainty on the C elegans L2/L2d decision. *PloS one* 9(7):e100580.

19. Golden JW & Riddle DL (1984) A pheromone-induced developmental switch in Caenorhabditis elegans: Temperature-sensitive mutants reveal a wild-type

temperature-dependent process. *Proceedings of the National Academy of Sciences of the United States of America* 81(3):819-823.

20. Schaedel ON, Gerisch B, Antebi A, & Sternberg PW (2012) Hormonal signal amplification mediates environmental conditions during development and controls an irreversible commitment to adulthood. *PLoS biology* 10(4):e1001306.
21. Karp X (2016) Working with dauer larvae. *WormBook : the online review of C. elegans biology*:1-19.
22. Blango MG & Mulvey MA (2009) Bacterial landlines: contact-dependent signaling in bacterial populations. *Curr Opin Microbiol* 12(2):177-181.
23. Nijhout HF & Callier V (2015) Developmental mechanisms of body size and wing-body scaling in insects. *Annu Rev Entomol* 60:141-156.
24. Frezal L & Felix MA (2015) C. elegans outside the Petri dish. *eLife* 4.
25. Chalfie M & Sulston J (1981) Developmental genetics of the mechanosensory neurons of Caenorhabditis elegans. *Developmental biology* 82(2):358-370.
26. Lumpkin EA, Marshall KL, & Nelson AM (2010) The cell biology of touch. *J Cell Biol* 191(2):237-248.
27. Li W, Kang L, Piggott BJ, Feng Z, & Xu XZ (2011) The neural circuits and sensory channels mediating harsh touch sensation in Caenorhabditis elegans. *Nat Commun* 2:315.
28. McClanahan PD, Xu JH, & Fang-Yen C (2017) Comparing Caenorhabditis elegans gentle and harsh touch response behavior using a multiplexed hydraulic microfluidic device. *Integr Biol (Camb)* 9(10):800-809.

29. Maguire SM, Clark CM, Nunnari J, Pirri JK, & Alkema MJ (2011) The C. elegans touch response facilitates escape from predacious fungi. *Current biology : CB* 21(15):1326-1330.

30. Schafer WR (2015) Mechanosensory molecules and circuits in C. elegans. *Pflugers Arch* 467(1):39-48.

31. Way JC & Chalfie M (1989) The mec-3 gene of Caenorhabditis elegans requires its own product for maintained expression and is expressed in three neuronal cell types. *Genes & development* 3(12A):1823-1833.

32. Lee JS*, et al.* (2017) FMRFamide-like peptides expand the behavioral repertoire of a densely connected nervous system. *Proceedings of the National Academy of Sciences of the United States of America* 114(50):E10726-E10735.

33. O'Hagan R, Chalfie M, & Goodman MB (2005) The MEC-4 DEG/ENaC channel of Caenorhabditis elegans touch receptor neurons transduces mechanical signals. *Nature neuroscience* 8(1):43-50.

34. Lai CC, Hong K, Kinnell M, Chalfie M, & Driscoll M (1996) Sequence and transmembrane topology of MEC-4, an ion channel subunit required for mechanotransduction in Caenorhabditis elegans. *J Cell Biol* 133(5):1071-1081.

35. Sternberg PW, Wang H, Park H, & Liu J (2018) An efficient genome editing strategy to generate putative null mutants in Caenorhabditis elegans using CRISPR/Cas9. *bioRxiv*.

36. Hong K, Mano I, & Driscoll M (2000) In vivo structure-function analyses of Caenorhabditis elegans MEC-4, a candidate mechanosensory ion channel subunit. *J Neurosci* 20(7):2575-2588.

37. Brown AL, Fernandez-Illescas SM, Liao Z, & Goodman MB (2007) Gain-of-function mutations in the MEC-4 DEG/ENaC sensory mechanotransduction channel alter gating and drug blockade. *J Gen Physiol* 129(2):161-173.

38. Calixto A, Jara JS, & Court FA (2012) Diapause formation and downregulation of insulin-like signaling via DAF-16/FOXO delays axonal degeneration and neuronal loss. *PLoS Genet* 8(12):e1003141.

39. Arnadottir J, O'Hagan R, Chen Y, Goodman MB, & Chalfie M (2011) The DEG/ENaC protein MEC-10 regulates the transduction channel complex in Caenorhabditis elegans touch receptor neurons. *J Neurosci* 31(35):12695-12704.

40. Chen Y, Bharill S, O'Hagan R, Isacoff EY, & Chalfie M (2016) MEC-10 and MEC-19 Reduce the Neurotoxicity of the MEC-4(d) DEG/ENaC Channel in Caenorhabditis elegans. *G3 (Bethesda)* 6(4):1121-1130.

41. Topalidou I, van Oudenaarden A, & Chalfie M (2011) Caenorhabditis elegans aristaless/Arx gene alr-1 restricts variable gene expression. *Proceedings of the National Academy of Sciences of the United States of America* 108(10):4063-4068.

42. Li W, Feng Z, Sternberg PW, & Xu XZ (2006) A C. elegans stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. *Nature* 440(7084):684-687.

43. Pujol N, Torregrossa P, Ewbank JJ, & Brunet JF (2000) The homeodomain protein CePHOX2/CEH-17 controls antero-posterior axonal growth in C. elegans. *Development* 127(15):3361-3371.

44. Van Buskirk C & Sternberg PW (2010) Paired and LIM class homeodomain proteins coordinate differentiation of the C. elegans ALA neuron. *Development* 137(12):2065-2074.

45. Schroeder NE & Flatt KM (2014) In vivo imaging of Dauer-specific neuronal remodeling in C. elegans. *J Vis Exp* (91):e51834.

46. Peckol EL, Troemel ER, & Bargmann CI (2001) Sensory experience and sensory activity regulate chemosensory receptor gene expression in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences of the United States of America* 98(20):11032-11038.

47. Kim K*, et al.* (2009) Two chemoreceptors mediate developmental effects of dauer pheromone in C. elegans. *Science* 326(5955):994-998.

48. Neal SJ*, et al.* (2016) A Forward Genetic Screen for Molecules Involved in Pheromone-Induced Dauer Formation in Caenorhabditis elegans. *G3 (Bethesda)* 6(5):1475-1487.

49. Rose JK, Sangha S, Rai S, Norman KR, & Rankin CH (2005) Decreased sensory stimulation reduces behavioral responding, retards development, and alters neuronal connectivity in Caenorhabditis elegans. *J Neurosci* 25(31):7159-7168.

50. Geng J, Zhao Q, Zhang T, & Xiao B (2017) In Touch With the Mechanosensitive Piezo Channels: Structure, Ion Permeation, and Mechanotransduction. *Curr Top Membr* 79:159-195.

51. Ranade SS*, et al.* (2014) Piezo2 is the major transducer of mechanical forces for touch sensation in mice. *Nature* 516(7529):121-125.

52. Gems D*, et al.* (1998) Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in Caenorhabditis elegans. *Genetics* 150(1):129-155.

53. Gerisch B, Weitzel C, Kober-Eisermann C, Rottiers V, & Antebi A (2001) A hormonal signaling pathway influencing C. elegans metabolism, reproductive development, and life span. *Developmental cell* 1(6):841-851.

54. Gottlieb S & Ruvkun G (1994) daf-2, daf-16 and daf-23: genetically interacting genes controlling Dauer formation in Caenorhabditis elegans. *Genetics* 137(1):107-120.

55. Jia K, Albert PS, & Riddle DL (2002) DAF-9, a cytochrome P450 regulating C. elegans larval development and adult longevity. *Development* 129(1):221-231.

56. Riddle DL, Swanson MM, & Albert PS (1981) Interacting genes in nematode dauer larva formation. *Nature* 290(5808):668-671.

57. Thomas JH, Birnby DA, & Vowels JJ (1993) Evidence for parallel processing of sensory information controlling dauer formation in Caenorhabditis elegans. *Genetics* 134(4):1105-1117.

58. Vowels JJ & Thomas JH (1992) Genetic analysis of chemosensory control of dauer formation in Caenorhabditis elegans. *Genetics* 130(1):105-123.

59. Torr P, Heritage S, & Wilson MJ (2004) Vibrations as a novel signal for host location by parasitic nematodes. *International journal for parasitology* 34(9):997-999.

60. Bose N*, et al.* (2014) Natural variation in dauer pheromone production and sensing supports intraspecific competition in nematodes. *Current biology : CB* 24(13):1536-1541.

61. Diaz SA*, et al.* (2014) Diverse and potentially manipulative signalling with ascarosides in the model nematode C. elegans. *BMC Evol Biol* 14(1):46.

62. Schulenburg H & Felix MA (2017) The Natural Biotic Environment of Caenorhabditis elegans. *Genetics* 206(1):55-86.

63. McGlone F, Wessberg J, & Olausson H (2014) Discriminative and affective touch: sensing and feeling. *Neuron* 82(4):737-755.

64. Tan PL & Katsanis N (2009) Thermosensory and mechanosensory perception in human genetic disease. *Hum Mol Genet* 18(R2):R146-155.

65. Huang LS & Sternberg PW (2006) Genetic dissection of developmental pathways. *WormBook : the online review of C. elegans biology*:1-19.

66. Chen BL, Hall DH, & Chklovskii DB (2006) Wiring optimization can relate neuronal structure and function. *Proceedings of the National Academy of Sciences of the United States of America* 103(12):4723-4728.

67. White JG, Southgate E, Thomson JN, & Brenner S (1986) The structure of the nervous system of the nematode Caenorhabditis elegans. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 314(1165):1-340.

68. Russell J, Vidal-Gadea AG, Makay A, Lanam C, & Pierce-Shimomura JT (2014) Humidity sensation requires both mechanosensory and thermosensory pathways

in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences of the United States of America* 111(22):8269-8274.

69. dos Santos CN*, et al.* (2011) Seasonal dynamics of cyathostomin (Nematoda - Cyathostominae) infective larvae in Brachiaria humidicola grass in tropical southeast Brazil. *Veterinary parasitology* 180(3-4):274-278.

70. Viney ME (2009) How did parasitic worms evolve? *BioEssays : news and reviews in molecular, cellular and developmental biology* 31(5):496-499.

71. Ludewig AH & Schroeder FC (2013) Ascaroside signaling in C. elegans. *WormBook : the online review of C. elegans biology*:1-22.

72. Ardiel EL & Rankin CH (2010) The importance of touch in development. *Paediatr Child Health* 15(3):153-156.

73. Vidal-Diez de Ulzurrun G & Hsueh YP (2018) Predator-prey interactions of nematode-trapping fungi and nematodes: both sides of the coin. *Appl Microbiol Biotechnol* 102(9):3939-3949.

74. Brenner S (1974) The genetics of Caenorhabditis elegans. *Genetics* 77(1):71-94.