Supplementary Materials for

Neurosensory Perception of Environmental Cues Modulates Sperm Motility Critical for Fertilization

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Materials and Methods

*C. elegans* strains and culture

*C. elegans* strains were cultured with NA22 *E. coli* bacteria, as previously described (7-9). Males were obtained from the *fog-2(q71)* strain (21). The following additional strains were used: N2 (wild type), CB1372 [daf-7(e1372)III], DR62 [daf-7(m62)III], CB1364 [daf-4(e1362)III], DR63 [daf-4(m63)III], DR40 [daf-1(m40)IV], CB1393 [daf-8(e1393)I], DR77 [daf-14(m77)IV], GR1311 [daf-3(mgDf90)X], DA2202 [daf-7(e1372)III;adEx2202(gpa-4p::daf-7+rol-6p::GFP)], CF1038 [daf-16(mu86)I], AA1 [daf-12(rh84)X], RB993 [tdc-1(ok914)II], NU3 [dbl-1(nk3)V], CB1482 [sma-6(e1482)II], NW987 [unc-129(ev554)IV], NL2098 [rrf-1(pk1417)I], and DR86 [daf-19(m86)II]. These strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH Office of Research Infrastructure Programs. The following mutant strains were generated through genetic crosses: daf-1(m40);daf-3(mgDf90), daf-7(m62);daf-3(mgDf90), daf-1(m40);daf-16(mu86);daf-7(m62); daf-1(m40); tdc-1(ok914), and daf-1(m40);daf-12(rh84). Strains were maintained at 16°C, 20°C, or 25°C, as indicated.

For prostaglandin analyses, strains were grown on up to sixty-five 15 cm seeded plates, as previously described (7, 8, 10, 19). L1 synchronized and mixed staged cultures were grown at 16°C and shifted to 25°C for 24 hours prior to collection. For the *daf-1(m40)* allele, the sperm guidance defect is slightly more severe under this condition. Cultures were supplemented with concentrated bacteria. Worms were washed off plates with M9 buffer, collected in polypropylene tubes, and stored at -80°C for lipid extraction. Mixed staged cultures were enriched for adults using gravity sedimentation in M9 buffer. Equal amounts of tissue were analyzed for comparisons.

Sperm guidance assays

MitoTracker Red CMXRos (Invitrogen), a fluorescent dye that selectively stains mitochondria, was used to label male sperm, as previously described (8, 9). 10-20 adult hermaphrodites were anesthetized with 0.1% tricaine and 0.01% tetramisole hydrochloride in M9 buffer for 30 minutes. The anesthetized worms were transferred to a nematode growth media (NGM) plate with a 1 cm drop of *E. coli* bacteria containing 50-100 stained males. Males and hermaphrodites were allowed to mate for 30 minutes. For sperm distribution analysis, the hermaphrodites and males were separated and hermaphrodites were mounted for microscopy one hour later. Sperm distribution was measured by calculating the percent of sperm within three anatomic zones in the gonad between the vulva and spermatheca (Fig. 1A), as previously described (7, 8, 10). Average sperm accumulation in wild-type, mutant, or experimentally manipulated hermaphrodites within zone 3 was compared using a Student’s t-test. For sperm motility analysis, hermaphrodites were allowed to mate with males for 30 minutes and then immediately mounted for microscopy. Time-lapse images were taken in 30-second intervals, and sperm migration distance was measured using Axiovision software, as previously described (7-10). At least 3 videos from different animals were used for quantification.
RNA-mediated interference (RNAi)

RNAi was performed by the feeding method at 25°C, as previously described (8, 9). HT115 bacterial strains were obtained from the feeding library and sequenced for verification. The rrf-1(pk1417) strain is sensitive to RNAi in the germ line and intestine, but not in the somatic gonad, muscles, neurons, etc (20, 22). Neurons are refractory to RNAi by the feeding method (23).

Transgenics and genetic mosaic analysis

To generate transgenic C. elegans, the marker plasmids myo-2p::mCherry (60 ng/µl) or myo-3p::mito::GFP (60 ng/µl) were mixed with myo-3p::daf-1 (60 ng/µl), unc-119p::daf-1 (60 ng/µl), egl-3p::daf-1 (60 ng/µl), glr-4p::daf-1 (60 ng/µl), nmr-2p::daf-1 (60 ng/µl), or tdc-1p::daf-1 (60 ng/µl) and microinjected into the gonads of young adult daf-1(m40) hermaphrodites. The glr-4p::daf-1, egl-3p::daf-1, and tdc-1::daf-1 plasmids were generously provided by the Ashrafi lab (17). The other plasmids were constructed using Promoterome primers (24). Injected worms were incubated for 24 hours, transferred to new NGM plates, and screened for transgenic progeny. Transgenic lines were selected based on the roller phenotype or GFP expression. Multiple independent transgenic lines were generated for all strains. All lines were maintained and scored at 16°C. To conduct genetic mosaic analysis, 10 ng/µl WRM0623aA05 or WRM0615bA06 fosmid DNA containing the daf-1 genomic locus was mixed with 10 ng/µl pTG96 (sur-5p::GFP) plasmid (25) and microinjected into the gonads of daf-1(m40) hermaphrodites. Transgenic lines were selected based on GFP expression. The fosmids rescued the dauer, metabolism, egg retention, and sperm migration defects. For lineage scoring, approximately 12,000 worms were screened. Transgene loss in the AB lineage was scored by GFP loss in head and tail neurons, the nerve cords, and the excretory gland. Transgene loss in the P1 lineage was scored by GFP loss in the intestine, muscle, somatic gonad, hyp11, and embryos. The P2 lineage was scored by GFP loss in body wall muscle cells, hyp11, and embryos, the P3 lineage was scored by GFP loss in body wall muscle and embryos, and the P4 lineage was scored by GFP loss in embryos alone. Transgene loss in the EMS lineage was scored by GFP loss in the intestine and somatic gonad, E lineage was scored by exclusive GFP loss in the intestine, and MS lineage was scored by GFP loss in the somatic gonad. Sperm guidance assays were conducted at 16°C.

Ascaroside synthesis and application

asc-C6-MK and asc–ΔC9 were chemically synthesized, as previously described (26). 2.0 mg dried asc-C6-MK and 2.6 mg dried asc–ΔC9 were diluted in ethanol to a 5 mM concentration. NGM plates made without peptone were seeded with a 1cm diameter drop of heat killed NA22 bacteria (O.D. ~ 0.5). The final ascaroside concentration was 10 µM each, a concentration previously shown to repress DAF-7 expression in adult ASI neurons (12). Control plates contained 0.2% ethanol. Young adult wild-type and mutant hermaphrodites were incubated on plates for 24 hours prior to sperm guidance analysis.

Mouse genetics

All experiments were conducted in accordance with National Institutes of Health guidelines and were approved by the Animal Care and Use Committee at Vanderbilt University. Wild-type and Cox-1−/− mice were generated from heterozygous matings on a
CD-1 genetic background (18, 27, 28). Cox-1−/− and Cox-2−/− mice were mated to generate Cox-1−/−; Cox-2−/− breeding pairs. Cox-1−/−; Cox-2−/− double null offspring were generated by mating Cox-1−/−; Cox-2−/− mice, with rescue of parturition failure by cesarean section. The presence of a vaginal plug was considered day 1 of pregnancy. Adult and newborn mice were killed by cervical transection. Pups were collected and flash frozen.

Lipid extraction
For C. elegans extractions, synchronized adult hermaphrodites (~2.0 g) or mixed-staged hermaphrodites consisting mostly of adults (~6.0 g) were used. Hydrophilic lipids were extracted from frozen worm pellets using a liquid-liquid extraction technique (29), as previously described (7, 8, 19). Briefly, frozen worms were resuspended with 12 ml of ice cold acetone/saline containing 0.005% butylated hydroxytoluene to prevent oxidation. Worms were distributed into plastic tubes for use in the Bullet Blender 5 homogenizer (Next Advance). 0.7-0.8 ml of 0.5 mm diameter Ceria stabilized zirconium oxide beads were added to each tube and the Bullet Blender was run for 2-4 minutes (speed 9). The homogenates were evenly transferred to four 10 ml conical glass tubes. The tubes were centrifuged at 4°C in a clinical centrifuge for 10 minutes at 1000 RCF. The supernatants were transferred to four clean 10 ml conical glass tubes. An equivalent volume of hexane was added to each tube and the tubes were vortexed for 30 seconds. The glass tubes were then centrifuged at 4°C for 10 minutes at 1000 RCF. The upper hexane phase and white debris in the interphase were removed. The lower aqueous phase was acidified to pH 3.5 using 2M formic acid. An equal volume of chloroform was added to each tube. Next, the tubes were vortexed for 30 seconds and centrifuged at 4°C for 10-15 minutes at 1000 RCF. The lower organic phases were transferred to a clean 15 ml conical tube. The extract was flushed with nitrogen gas and stored at -20°C for at least 24 hours. The aqueous top layer was removed and organic phase containing prostaglandins was evaporated in a Teflon-lined capped ½-Dram glass vial under a gentle stream of nitrogen gas. The dried, purged lipids were stored at -20°C for up to 2 weeks. For mass spectrometry analysis, the dried extract was dissolved in 60-200 µl of 80% methanol. The use of internal standards has shown that extraction efficiency is consistent from sample to sample.

Dissected adult wild-type mouse tissues or whole zebrafish were flash frozen and stored at -80°C for less than a week, as previously described (7). Blood was removed from adult mice prior to dissection. Adult Cox-1−/−; Cox-2−/− double mutant tissues from a rare adult mouse that escaped postnatal lethality (~6 months of age) were extracted after about 9 months storage at -80°C. Small segments of brain, small intestine, stomach, and large intestine were analyzed. Storage for extended periods (>1 month) causes prostaglandin breakdown. Hence, prostaglandin levels could not be directly compared to a control. For wild-type and Cox mutant pups, freshly frozen whole pups were weighed and extracted. Liquid/liquid extraction was performed as described above with the following exception: 0.8-1.0 ml of 2.0 mm diameter Ceria stabilized zirconium oxide beads and Bullet Blender speed 10 (5 min) were used for homogenization.
Reverse phase liquid chromatography and mass spectrometry

LC-MS/MS analyses of commercial standards (Cayman Chemical) and extracts were performed as previously described (7, 8, 10, 19), using a system consisting of a Shimadzu Prominence HPLC with refrigerated auto sampler (Shimadzu Scientific Instruments, Inc., Columbia, MD) and API 4000 (Applied Biosystems/MDS Sciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer. The chromatographic separation was performed on a Synergy hydro RP-C18 column pre-equilibrated with 0.1% formic acid. The mobile phase consisted of 0.1% formic acid [A] and acetonitrile containing 0.1% formic acid [B] and was pumped at a flow rate of 0.2 ml/min. The gradient started with 10% B and went up to 80% B from 0-11 min, 80-100% B from 11-14 min, and returned to 10% B at 16 min. The column effluent was introduced into the mass spectrometer using an ESI interface operating in negative ion mode. Nitrogen was used as a nebulizer and curtain gas (CUR = 10). The collision gas, collision energy and temperature were set at 10, -35 eV and 600°C, respectively. BioAnalyst 1.4.2 software controlled the LC-MS/MS system. For comparative analysis, extracts and standards were run consecutively.

To determine prostaglandin concentrations, stock solutions of PGF1α, PGF2α, and 6-keto PGF1α (1 mg/ml in EtOH) were serially diluted with 80% MeOH to obtain 1000, 100, 10, 1, 0.5, and 0.1 ng/ml concentrations. The samples were analyzed by the MRM method using mass transitions m/z 355/311 for PGF1α/CePGF1, m/z 353/193 for PGF2α/CePGF2, and m/z 369/163 for 6-keto PGF1α. The standard curves exhibited excellent linearity in the range of concentration 0.1-100 ng/ml with a correlation coefficient >0.95. Average prostaglandin concentrations in worm and mouse extracts were calculated from at least three MRM analyses. Relative concentrations of 8-epi-PGF2α [and/or 8-epi-15(R) PGF2α] and an unknown PGF2α (peak 3) were also calculated (Figs. 4A and S11). Concentrations were compared using a student’s T-test.
Figure S1. PGF2α synthesis. PGF2α synthesis in mammals (red) and C. elegans (black). In mammals, PGF2α is synthesized from arachidonic acid by the sequential actions of prostaglandin-endoperoxide synthase (Cox) and PGF synthase enzymes. The C. elegans genome lacks Cox homologs, yet PGF2α stereoisomers are still synthesized from arachidonic acid. PGF1α and PGF3α isomers are also synthesized from dihomo-γ linolenic and eicosapentaenoic acids, respectively (7).
Figure S2. Fluorescent (TRITC) wild-type sperm distribution in DAF-7-independent TGF-β pathway mutants one hour after mating. *dbl-1* encodes a TGF-β ligand for the *sma-6* type I TGF-β receptor. *unc-129* encodes a TGF-β ligand important for neuron and gonadal tip cell migration (14). Average zone distributions ± SEM are shown.
Figure. S3. DIC micrographs of wild-type and daf-1 mutant gonads grown at 16°C. Arrows indicate oocytes in meiotic prophase. Bar, 10µm.
Figure. S4. Spermatheca (Z3) targeting of sperm in control and RNAi hermaphrodites one hour after mating. RNAi was initiated in hermaphrodites at the L4 stage by the feeding method (30). Males were untreated. Neurons are largely refractory to RNAi by this method. Error bars are SEM with trial numbers to the right. **, $P < 0.001$. 
Figure S5. Ascaroside structures and effects on sperm guidance. Hermaphrodites were treated with synthetic asc-C6-MK and asc–ΔC9 for 24 hours starting at the young adult stage. Males were untreated. Spermatheca (Z3) targeting was measured one hour after mating. Ascarosides function in complex mixtures that act collectively. High concentration of asc-C6-MK and asc–ΔC9 are used to partially offset the absence of other components (13). Error bars are SEM with trial numbers to the right. *, $P < 0.001$ compared to untreated wild type. **, $P < 0.001$ compared to treated wild type.
Figure. S6. Fluorescent (TRITC) sperm distribution in hermaphrodites one hour after mating. (A) Hermaphrodites without food for 20 hours. (B) Hermaphrodites in the presence of food, but under crowded conditions with high ascaroside levels. (C) daf-19(m86) hermaphrodites grown at 16°C. daf-19 encodes an RFX class transcription factor that is required for sensory neuron cilia formation (31). Males were wild-type and grown in the presence of food under standard conditions. Average zone distributions ± SEM are shown. See Figure 1B and Tables S1-S2 for control values.
Figure. S7. Genetic mosaic analysis of the *daf-1* type I TGF-β receptor. Circles indicate the percentage of wild-type sperm in zone Z3 from individual animals exhibiting *daf-1* loss in major lineages. In *C. elegans*, transgenes expressed from extrachromosomal arrays are spontaneously lost at low frequency during cell division, generating mosaic animals. When these events occur early in development, mosaic animals are generated with transgene losses in neurons, body wall muscle, intestinal cells, somatic gonadal cells, germ cells, or other cell types. A fosmid containing the entire *daf-1* genomic locus was used to generate *daf-1(m40)* transgenic worms. The early embryonic lineages P1, AB, EMS, P2, MS, E, C, P3, D, P4, and their products are shown.
Figure S8. Spermatheca (Z3) targeting of wild-type sperm in mutant hermaphrodites one hour after mating. Error bars are SEM with trial numbers to the right. *, $P < 0.001$ compared to daf-1(m40) single mutants. **, $P < 0.001$ compared to treated daf-7(m62) single mutants. tdc-1 encodes a tyrosine decarboxylase that is required for the synthesis of the neurotransmitters tyramine and octopamine (32). tdc-1 loss suppresses the feeding rate defect of daf-1 mutants (17). daf-16 encodes a FOXO homolog that antagonizes insulin signaling (33). daf-12 encodes a steroid hormone receptor that antagonizes dafachronic acid signaling (34). Z3 targeting in single daf-16(mu86) and daf-12(rh84) mutants is 95% and 88%, respectively (N > 20). daf-3 encodes a co-Smad that antagonizes DAF-7/TGF-β signaling (14) (shown for reference, also see Fig. 1B and Table S1). These data suggest that DAF-7/TGF-β signals are transduced in part via insulin and dafachronic acid ligands, likely originating from head interneurons and/or XXX endocrine cells.
Figure S9. Structures, MRM mass transitions, and chromatography retention times (RTs) of selected prostaglandins. The prostaglandin numbering system is shown for PGF2\(\alpha\). Our reverse phase chromatography program also separates 15\((R)\)-PGF2\(\alpha\) and 5-trans PGF2\(\alpha\) (7, 8, 19). 6-keto PGF1\(\alpha\) is the stable metabolite of prostacyclin and thromboxane B2 is the stable metabolite of thromboxane A2. MRM mass transition m/z 353/193 also detects PGF2\(\alpha\) isomers derived from omega-3 arachidonic acid (7).
Figure S10. Representative MRM chromatograms of wild-type and Cox double mutant pup extracts. (A) MRM chromatogram using mass transition \(m/z\) 369/163, which detects 6-keto PGF1\(\alpha\) isomers. The peak at RT=9.1 is not a prostaglandin. (B) MRM chromatogram using mass transition \(m/z\) 351/189, which detects PGE2 and PGD2 isomers. (C) MRM chromatogram using mass transition \(m/z\) 369/169, which detects thromboxane B2 (TXB2) isomers. Thromboxane B2 is a stable metabolite of Thromboxane A2. Retention times (RTs) of standards are shown. PGD2 and PGE2 were slightly above the detection limit in wild-type pup extracts (~500 to 1500 cps). It is interesting to note that we have thus far been unable to detect PGD2, PGE2, TXB2, and 6-keto PGF1\(\alpha\) isomers in \textit{C. elegans} extracts.
Figure S11. MRM chromatograms of wild-type and Cox double mutant pup extracts, focusing on PGF2α isomers (mass transition m/z 353/193). **, PGF2α and ent-PGF2α standards co-elute (RT=11.8), as do 8-epi-PGF2α and 8-epi-15(R) PGF2α standards (RT=11.4). Peak 3 is an unidentified PGF2α isomer that does not co-elute with commercially available standards. Peak 3 does co-elute with CePGF2b (see Figure 4B).
Figure S12. MRM chromatograms of Cox double mutant adult mouse and C. elegans extracts. (A) MRM chromatogram of adult brain tissue using mass transition m/z 353/193, which detects PGF2α isomers. The three prominent peaks have identical retention times as C. elegans PGF2α isomers derived from arachidonic acid (B). (B) Published chromatogram of fat-1 mutants from Hoang et al. 2013 shown for reference (7). The peak at RT=11.4 is 8-epi-PGF2α and/or 8-epi-15(R) PGF2α, the peak at RT=11.8 is PGF2α and/or ent-PGF2α, and the peak at RT=12.1 is an unidentified PGF2α stereoisomer that does not match commercially available standards (7). (C) MRM chromatogram of adult mouse brain tissue using mass transition m/z 351/189, which detects PGD2 and PGE2 isomers. (D) MRM chromatogram of adult mouse stomach tissue using mass transition m/z 353/193, which detects PGF2α isomers. Similar results were observed for the small intestine. PGF2α isomers were not detected in the large intestine, although degradation could be a factor. These Cox dKO adult tissues were from a rare mouse that escaped postnatal lethality and lived to 6 months of age before being sacrificed. Only small amounts of tissue were available that was stored at -80°C for 9 months.
Figure S13. Representative MRM chromatograms from wild-type adult mouse and zebrafish extracts. Mass transition m/z 353/193, which detects PGF2α isomers, was used. Asterisks indicate peaks that co-elute with 8-epi-PGF2α and 8-epi-15(R) PGF2α standards. Arrows indicate peaks that co-elute with peak 3 from Figure S11. See Figure S9 for structures.
Table S1. Sperm distribution in control or TGF-β mutant hermaphrodites at 16°C.

<table>
<thead>
<tr>
<th>Line</th>
<th>Hermaphrodite Strain</th>
<th>N</th>
<th>Z1 (%)</th>
<th>Z2 (%)</th>
<th>Z3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wild type</td>
<td>36</td>
<td>4.3 ± 1.0</td>
<td>4.7 ± 0.73</td>
<td>91.3 ± 1.4</td>
</tr>
<tr>
<td>2.</td>
<td>daf-7(m62)</td>
<td>16</td>
<td>25.2 ± 3.6</td>
<td>32.6 ± 4.8</td>
<td>42.2 ± 5.3*</td>
</tr>
<tr>
<td>3.</td>
<td>daf-7(e1372)</td>
<td>21</td>
<td>20.8 ± 3.1</td>
<td>14.8 ± 2.4</td>
<td>64.4 ± 4.6*</td>
</tr>
<tr>
<td>4.</td>
<td>daf-4(m63)</td>
<td>16</td>
<td>33.4 ± 4.2</td>
<td>21.3 ± 2.1</td>
<td>45.3 ± 4.1*</td>
</tr>
<tr>
<td>5.</td>
<td>daf-4(e1364)</td>
<td>12</td>
<td>27.3 ± 5.0</td>
<td>23.7 ± 4.0</td>
<td>49.1 ± 7.6*</td>
</tr>
<tr>
<td>6.</td>
<td>daf-1(m40)</td>
<td>50</td>
<td>29.5 ± 3.0</td>
<td>35.2 ± 3.1</td>
<td>35.4 ± 3.5*</td>
</tr>
<tr>
<td>7.</td>
<td>daf-8(e1393)</td>
<td>24</td>
<td>18.0 ± 3.3</td>
<td>14.2 ± 2.7</td>
<td>66.9 ± 5.5*</td>
</tr>
<tr>
<td>8.</td>
<td>daf-14(m77)</td>
<td>20</td>
<td>33.5 ± 3.6</td>
<td>21.8 ± 1.9</td>
<td>45.4 ± 3.5*</td>
</tr>
<tr>
<td>9.</td>
<td>daf-7(m62); daf-3(mgDf90)</td>
<td>16</td>
<td>4.0 ± 1.0</td>
<td>10.2 ± 1.5</td>
<td>85.9 ± 2.1**</td>
</tr>
<tr>
<td>10.</td>
<td>daf-1(m40); daf-3(mgDf90)</td>
<td>30</td>
<td>9.1 ± 1.9</td>
<td>9.6 ± 1.5</td>
<td>81.3 ± 2.4#</td>
</tr>
<tr>
<td>11.</td>
<td>daf-3(mgDf90)</td>
<td>18</td>
<td>5.8 ± 0.9</td>
<td>5.5 ± 1.2</td>
<td>87.7 ± 1.8</td>
</tr>
</tbody>
</table>

Wild-type males were mated to indicated hermaphrodites and separated for 1 hour. Values are average ± SEM.

* P < 0.001 compared to the wild type.

**, P < 0.001 compared to daf-7(m62).

#, P < 0.001 compared to daf-1(m40).
Table S2. Sperm distribution in control or TGF-β mutant hermaphrodites at 25°C.

<table>
<thead>
<tr>
<th>Line</th>
<th>Hermaphrodite Strain</th>
<th>N</th>
<th>Z1 (%)</th>
<th>Z2 (%)</th>
<th>Z3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wild type</td>
<td>22</td>
<td>1.7 ± 0.6</td>
<td>5.7 ± 0.9</td>
<td>92.6 ± 1.3</td>
</tr>
<tr>
<td>2.</td>
<td>daf-7(e1372)</td>
<td>24</td>
<td>16.6 ± 3.1</td>
<td>20.2 ± 3.2</td>
<td>63.2 ± 5.7*</td>
</tr>
<tr>
<td>3.</td>
<td>daf-4(m63)</td>
<td>18</td>
<td>32.6 ± 5.6</td>
<td>19.4 ± 2.1</td>
<td>48.0 ± 6.8*</td>
</tr>
<tr>
<td>4.</td>
<td>daf-1(m40)</td>
<td>19</td>
<td>65.1 ± 5.2</td>
<td>26.8 ± 3.7</td>
<td>8.1 ± 1.9*</td>
</tr>
<tr>
<td>5.</td>
<td>daf-8(e1393)</td>
<td>18</td>
<td>19.1 ± 3.4</td>
<td>14.8 ± 3.8</td>
<td>66.2 ± 6.1*</td>
</tr>
<tr>
<td>6.</td>
<td>daf-14(m77)</td>
<td>18</td>
<td>31.1 ± 3.8</td>
<td>19.5 ± 1.7</td>
<td>49.4 ± 4.5*</td>
</tr>
<tr>
<td>7.</td>
<td>daf-3(mgDf90)</td>
<td>37</td>
<td>10.6 ± 2.8</td>
<td>11.4 ± 2.6</td>
<td>77.8 ± 4.0*</td>
</tr>
</tbody>
</table>

Strains were grown at 16°C and shifted to 25°C for 24 hours at the late L4 stage. Wild-type males were mated to indicated hermaphrodites and separated for 1 hour. Values are average ± SEM.

*, P < 0.001 compared to the wild type.
#, Sperm target the spermatheca (Z3) less efficiently in daf-1(m40) mutants at 25°C compared to 16°C.

Table S3. Sperm motility values in wild-type and mutant hermaphrodite uteri.

<table>
<thead>
<tr>
<th>Line</th>
<th>Hermaphrodite Strain</th>
<th>N</th>
<th>Average Velocity (µm/min)</th>
<th>Average Directional Velocity (µm/min)</th>
<th>Reversal Frequency (rev/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wild type*</td>
<td>95</td>
<td>8.69 ± 0.63</td>
<td>4.39 ± 0.85</td>
<td>1.9</td>
</tr>
<tr>
<td>2.</td>
<td>daf-7(m62)</td>
<td>32</td>
<td>5.52 ± 0.59**</td>
<td>0.56 ± 0.86**</td>
<td>9.8</td>
</tr>
<tr>
<td>3.</td>
<td>daf-1(m40)</td>
<td>43</td>
<td>4.93 ± 0.43**</td>
<td>0.72 ± 0.54**</td>
<td>9.6</td>
</tr>
<tr>
<td>4.</td>
<td>fat-2(wal7) #</td>
<td>94</td>
<td>3.85 ± 0.22**</td>
<td>0.47 ± 0.30**</td>
<td>8.1</td>
</tr>
<tr>
<td>5.</td>
<td>rme-2(b1008) #</td>
<td>75</td>
<td>3.68 ± 0.28**</td>
<td>0.45 ± 0.32**</td>
<td>9.5</td>
</tr>
</tbody>
</table>

The average velocity, average directional velocity toward the spermatheca, and average reversal frequency were determined for wild-type sperm within the uterus (Z2) of wild-type and mutant hermaphrodites. daf-7 and daf-1 mutants were grown at 16°C. Value ± SEM is shown.

* Includes new data and published data from Kubagawa et al., 2006 (9) and Hoang et al., 2013 (7).

**, P < 0.001 compared to the wild type.

#, Published data from Kubagawa et al., 2006 (9) for reference.
References and Notes


