

Male behavior wins: converging circuits resolve conflicting parallel sensory inputs

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Highlights

- *C. elegans* hermaphrodites avoid pheromones that are highly attractive to males
- Two pairs of sensory neurons, ASI and ASJ, mediate avoidance behavior
- Sex differences in attraction neurons, but not in ASI and ASJ, determine avoidance
- Attraction interneurons resolve conflicting attraction and avoidance sensory input

Summary

Sex-specific behaviors arise from differences in the male and female nervous systems, but the nature of the differences remains elusive. Sex pheromones are highly attractive to male *C. elegans*, but not to the opposite sex, the hermaphrodite. Here, we show that rather than being indifferent, hermaphrodites strongly avoid sex pheromones. Hermaphrodite avoidance is mediated by two pairs of sensory neurons, ASI and ASJ. Males with feminized ASI and ASJ avoidance neurons are still attracted to pheromones, but feminizing attraction neurons instead of avoidance neurons reveals avoidance behavior. If attraction behavior is disabled, males avoid pheromones. Thus, males experience conflicting attraction and avoidance sensory information in parallel. A set of interneurons common to both sexes resolves the conflict to express attraction over avoidance, prioritizing default and dominant behaviors. Because circuit configurations are variable, but behavioral outcome is not, this priority-switch ensures failsafe behavior.

Introduction

Animals are often presented with situations that require alternate behaviors: stay or run away, look for food here or go elsewhere, sing to find a mate or to defend territory. How are alternate behaviors selected? The behavior that is expressed depends on context: external factors (social cues, food availability) and internal factors (nutritional status, age) both influence behavioral outcomes (Palmer and Kristan, 2011). The sexual identity of an animal may be treated as simply another context for behavior, so sexually dimorphic behaviors provide a tractable framework for addressing how a particular behavior is selected from among different behavioral options. Restated simply, the sex of an animal is a clear and invariant context for behavior. Innate sexually dimorphic behaviors further simplify the question, because two different readouts of the genome—male and female—specify two different but related nervous systems that give rise to two different behavioral outcomes: the same genes specify two sets of molecules, neurons, and connections that specify two behaviors. Comparing males and females should highlight differences important for particular behaviors, and thus show how one behavior is selected from among various behavioral options.

C. elegans allows experimental access to all levels that are key to understanding sexually dimorphic innate behaviors, from genes to cells to circuits. There are two sexes, males and self-fertilizing hermaphrodites; hermaphrodites are functionally females as far as the males are concerned (Herman, 2005). The nervous system of each sex is compact and well-described: males have 383 neurons and hermaphrodites have 302 (Sulston and Horvitz, 1977; Sulston, 1983; Sulston et al., 1983; for review see Hobert, 2005). Of these neurons, males and hermaphrodites share a core nervous system of 294 neurons common to both sexes, based on lineage (Sulston and Horvitz, 1977; Sulston et al., 1983), anatomy (White et al., 1986), and often expression of the same genes (Lee and Portman, 2007). The connectome is complete at the synaptic level for the hermaphrodite (White et al., 1986; Chen et al., 2006) and partly complete for the male (Jarrell et al., 2012). In addition to their highly similar nervous systems, males and hermaphrodites often display similar chemosensory behaviors (Dusenberry, 1976). However, there are some innate chemosensory behaviors that are sexually dimorphic: odor preference (Lee and Portman, 2007), exploratory behavior (Lipton et al., 2004), and behaviors elicited by pheromones (Jang et al., 2012; Macosko et al., 2009; Srinivasan et al., 2008).

There are at least two classes of *C. elegans* pheromones: DAF22-dependent and DAF-22-independent. The DAF-22-dependent mediate both development and behavior, and elicit different behaviors depending on concentration (Srinivasan et al., 2008; Macosko et al., 2009; Jang et al., 2012; Srinivasan et al., 2012). In contrast, the DAF-22-independent pheromones function as potent, male-specific sexual attractants at all concentrations (White et al., 2007). Importantly, this class is not attractive to *C. elegans* hermaphrodites at any testable concentration.

Sexual attraction behavior in males is mediated by a distributed set of core sensory neurons, the AWA, AWC, and ASK sensory neurons, present in both sexes (White et al., 2007; White and Jorgensen, 2012). If present, the male-

specific CEM sensory neurons also contribute, but the core neurons are sufficient (White et al., 2007). The attraction sensory neurons have the capacity to compensate for one another, an unusual feature for a *C. elegans* sensory circuit (White et al., 2007). An important consequence of compensation is that the attraction sensory circuit is degenerate, meaning not that the neurons degenerate (deteriorate physically), but rather that different configurations of sensory neurons yield the same effective behavior (Marder and Goaillard, 2006). In addition to the sensory neurons, sexual attraction is mediated by a distributed set of interneurons (White and Jorgensen, 2012). The attraction interneurons are primary synaptic targets of the attraction sensory neurons (Chen et al., 2006; White et al., 1986), and both sets must have a male sexual identity to generate attraction behavior (sexual identity is a cell-intrinsic property in *C. elegans*; Herman, 2005). Although sexual attraction is typically a male-specific behavior, hermaphrodites have latent sexual attraction (White and Jorgensen, 2012). When revealed, sexual attraction in hermaphrodites requires the same core sensory neurons (AWA, AWC, and ASK) with the same capacity to compensate for one another. Normally, sexual attraction behavior in hermaphrodites is repressed. Sexual repression is established during development via TGF- β signaling. Mutant *daf-7* hermaphrodites lacking DAF-7/TGF- β have impaired repression, and so are attracted to sex pheromones. For sexual attraction behavior to arise, the attraction neurons themselves—both sensory neurons and interneurons—must have a male sexual identity. A female sexual identity in either set engages repression and abolishes attraction. Because both pre-synaptic sensory neurons and post-synaptic interneurons must be male, it is likely that a constellation of matched connections between sensory and interneurons generate attraction behavior, and repression impairs the formation or function of these connections (White and Jorgensen, 2012). Thus, a male specific behavior emerges directly from a male-specific structure in the core nervous system—the attraction connections.

Rather than being wired during development, other sexually dimorphic *C. elegans* chemosensory behaviors arise from acute modulation of behaviors that exist in both sexes (Barrios et al., 2012; Jang et al., 2012). For example, sexually dimorphic behaviors mediated by DAF-22 dependent pheromones are a consequence of modulation of the relative activities of dedicated attractive and aversive sensory neurons (the attractive ASK and aversive ADL neurons Jang et al., 2012). Similarly, male-specific exploratory behavior (Lipton et al., 2004) is actively maintained in adults by neuropeptide signaling (Barrios et al., 2012). In these cases, it is not clear how sexual identity contributes to a sexually dimorphic behavioral outcome; the sex differences might be either in the modulation, or in the core circuits themselves.

Since hermaphrodites are self-fertilizing and do not need to locate a mate, they might be indifferent to sex pheromones. However, we show here that hermaphrodites strongly avoid sex pheromones, so the behaviors that sex pheromones elicit in males and hermaphrodites are opposite and mutually exclusive. The sex differences necessary for avoidance in hermaphrodites are within the neurons required for attraction in males. If the attraction neurons are

disabled in males, they avoid pheromone, indicating both attraction and avoidance sensory channels exist in parallel. A set of interneurons common to both sexes resolves conflicting sensory information to prioritize attraction behavior over avoidance. Resolution in the interneurons is essentially a decision with an outcome predetermined by sex.

Results

Hermaphrodites avoid C. elegans sex pheromones

Pheromones that elicit sexual attraction behavior in *C. elegans* males (White et al., 2007) elicit avoidance behavior in hermaphrodites (Figure 1). When males perceive a source of sex pheromone, they move toward it and linger there once they reach it (White et al., 2007, Supplemental Figure 1). Hermaphrodites that encounter a pheromone source tend to stop, sample the environment by swinging their heads, and then move away by changing course or reversing (Figure 1B). Some *C. elegans* pheromones, the ascarosides, evoke either attraction or avoidance responses depending on concentration (Srinivasan et al., 2008; Srinivasan et al., 2012; Macosko et al., 2009; Jang et al., 2012), but sex pheromones elicit only attraction in males, and only avoidance in hermaphrodites, at all testable concentrations (White et al., 2007, Figure 1 and Supplemental Figure 1). Thus, sex pheromones elicit opposite behaviors in *C. elegans* hermaphrodites and males.

C. elegans sex pheromones elicit sexually dimorphic behaviors in wild strains and diverse nematode species

Avoidance behavior could be a dispersal behavior. In laboratory strains, dispersal is due to a reduced tendency to aggregate and is the result of domestication (McGrath et al., 2009; Weber et al., 2010). To test whether pheromone avoidance arose from laboratory domestication, we examined avoidance behavior in wild *C. elegans* strains. Hermaphrodites from at least seven different wild *C. elegans* strains avoid pheromones, at frequencies comparable to laboratory strains (Figure 2A). Furthermore, males in these strains (and all strains tested) are consistently attracted to hermaphrodite pheromones from the laboratory strain N2 Bristol. Thus, it is unlikely that hermaphrodite pheromone avoidance is a dispersal behavior that arose from domestication. More broadly, because both behaviors are strong in all wild strains tested, they must be ecologically important.

Sexual attraction allows males to find their mates, and sex pheromone entices them to linger in regions where they are most likely to achieve mating success. However, *C. elegans* hermaphrodites are self-fertilizing, so the species does not need to mate. This mode of reproduction evolved relatively recently, and most related nematode species reproduce by obligatory male-female mating (Kiontke et al., 2011). To investigate the evolutionary basis for pheromone-evoked attraction and avoidance, we evaluated both behaviors in both sexes of diverse nematode species (Figure 2B). Males of all species tested display vigorous attraction to *C. elegans* pheromones. Hermaphrodite and female behavior does not correlate with reproductive mode or phylogenetic divergence. Hermaphrodites from closely related *C. briggsae* display neither attraction nor avoidance behavior in response to *C. elegans* pheromones. Females from closely related *C. remanei* and more distantly related *C. sp. 15* and *C. sp. 14* are ambivalent; they display either mild avoidance or attraction (although *C. remanei* female attraction behavior is not strongly significant in this experiment,

it fits the general pattern). Species that are yet more distant display more certain behavior; females from *C. sp. 6* and hermaphrodites from *Pristionchus pacificus* consistently avoid *C. elegans* pheromones, demonstrating that phylogenetic distance does not predict pheromone behavior. Because attraction and avoidance are present in species that reproduce by obligate mating, it is possible that these behaviors evolved to subserve mating and dispersal and persist in hermaphroditic species.

Abolishing or repressing sexual attraction reveals avoidance behavior

In wild-type hermaphrodites, attraction behavior is repressed (White and Jorgensen, 2012). However, *daf-7* mutant hermaphrodites are de-repressed and express latent sexual attraction. In males, the AWA, AWC, and ASK sensory neurons are required for sexual attraction (White et al., 2007). In de-repressed hermaphrodites, ablation of these neurons abolishes attraction (White and Jorgensen, 2012). However, rather than rendering de-repressed hermaphrodites atactic, ablation of the attraction neurons restores avoidance behavior (Figure 3A). A straightforward explanation of this result is that the circuitry for pheromone avoidance behavior is still present in de-repressed hermaphrodites, but expression of avoidance is masked by sexual attraction. Thus, abolishing sexual attraction reveals avoidance.

Consistent with this explanation, restoring repression restores avoidance. Restoring DAF-7/TGF- β signaling in *daf-7* mutant hermaphrodites by expressing DAF-7 in the AWC and ASE sensory neurons restores repression and reveals avoidance (Figure 3B). Similarly, restoring DAF-7/TGF- β signaling in *daf-7* mutant hermaphrodites using the *daf-3* mutation to genetically suppress the *daf-7* mutation restores repression and reveals avoidance (Figure 3B). Thus, experimentally repressing sexual attraction behavior reveals avoidance.

A simple interpretation of these results is that hermaphrodites possess both attraction and avoidance circuitry. Unless it is repressed, attraction takes priority over avoidance. In a wild-type hermaphrodite, attraction is repressed and she avoids pheromones. In a hermaphrodite with impaired DAF-7/TGF- β signaling repression is not engaged, attraction takes priority over avoidance, and she is attracted to pheromones. In a mutant hermaphrodite with restored DAF-7/TGF- β signaling, repression is engaged, and she avoids pheromones. If a hermaphrodite is not capable of sexual attraction because the attraction-promoting sensory neurons have been ablated, she avoids pheromones regardless of whether repression is engaged.

Pheromone avoidance and sexual attraction require distinct sets of sensory neurons

To identify the sensory neurons required for avoidance, we began by ablating different candidates. Hermaphrodites lacking the AWA, AWC, and ASK sensory neurons still avoid pheromones, so these sensory neurons are not required. Avoidance is an aversive behavior, but concurrent ablation of the canonical aversive sensory neurons, ASH,

AWB, and ADL, together with the ascaroside-sensing ASK neurons (Kim et al., 2009; Macosko et al., 2009; Srivastava et al., 2008) also did not detectably impair pheromone avoidance (Supplemental Figure 2). Furthermore, ablation of the ADF sensory neurons, which promote hyperoxia avoidance (Chang et al., 2006), did not detectably impair sex pheromone avoidance (Supplemental Figure 2). Thus, elimination of obvious aversive sensory neurons failed to disrupt pheromone avoidance.

Identification of the sensory neurons required for avoidance is potentially complicated by two factors: 1) repression must be engaged for hermaphrodites to display avoidance, and the same sensory neurons could be required to both engage repression and promote avoidance, 2) possible degeneracy or compensation, similar to sexual attraction (White et al., 2007). To circumvent the first complication, we used the *daf-3* mutation. In *daf-3* mutant hermaphrodites, repression is constitutively engaged, so *daf-3* hermaphrodites avoid. The *daf-3* mutation therefore separates effects on repression from effects on avoidance. To circumvent the second complication, we used a *tax-4* mutation. TAX-4 is a subunit of a cGMP-gated transduction channel that is required for sensory transduction in a broad number of sensory neurons, including AWA, AWC, ASK (Coburn and Bargmann, 1996a; Komatsu et al., 1996; Peckol et al., 1999). Pheromone avoidance is strongly reduced in *tax-4* mutant hermaphrodites (Figure 4A), but TAX-4 is required for ASI activity, and the ASI neurons repress attraction (White and Jorgensen, 2012). That is, *tax-4* mutant hermaphrodites are de-repressed and display sexual attraction behavior. A *tax-4*; *daf-3* double-mutant strain simultaneously addresses both complications: repression is constitutively engaged and pheromone avoidance behavior is strongly impaired.

We selectively restored TAX-4/cGMP-channel function in *tax-4*; *daf-3* double mutant hermaphrodites to identify sensory neurons sufficient for avoidance. Restoring TAX-4 using either a pan-neural promotor (*Prab-3*) or the *Ptax-4* promotor fully restores avoidance behavior (Figure 4A). More selective expression in the ASI, ASJ, and ASK neurons using the *Pgcy-27* promotor also fully restores avoidance. Of these three classes, expression in the ASI neuron pair (*Pstr-3*) fully restores avoidance, expression in the ASJ pair (*Psrh-11*) substantially restores avoidance, and expression in the ASK pair (*Psra-9*) does not detectably restore avoidance (Figure 4A). Thus, the ASI and ASJ sensory neurons function to promote avoidance behavior. Because activity in either pair is sufficient for pheromone avoidance, ASI and ASJ are degenerate and may be redundant. Taken together, these and previous results (White and Jorgensen, 2012) indicate that the ASI neurons function in hermaphrodites to both establish repression and promote avoidance.

Concurrent ablation of the ASI and ASJ neurons in *daf-3* mutant hermaphrodites significantly impairs avoidance behavior, indicating that these neurons are required for pheromone avoidance (Figure 4B). The contributions of ASI and ASJ to pheromone avoidance do not appear to be additive, because hermaphrodites missing both ASI and ASJ

pairs are only slightly more impaired than animals missing either pair alone (Figure 4B). To test that the requirement for the ASIs is not a consequence of the *daf-3* mutant background, we ablated the ASIs in wild-type adults; because the ASI neurons are not necessary to maintain repression in adults, this experiment separates developmental functions of the ASIs from direct sensory functions. Ablation of ASI in wild-type adult hermaphrodites impairs avoidance, indicating that ASI directly senses sex pheromone (Figure 4B). Because the ASI-ASJ double ablation (Figure 4B) is not as severe as the *tax-4* mutant phenotype (Figure 4A), it is possible that additional TAX-4-dependent sensory neurons contribute to avoidance behavior. However, we have been unable to identify this cell by selective TAX-4/CNG channel expression, so it may be that additional sensory neurons are recruited when ASI and ASJ are absent. Such recruitment would be conceptually similar to compensation among sexual attraction sensory neurons (White et al., 2007). Regardless, the ablation of ASI and ASJ impairs avoidance, indicating these neurons are required for the behavior. Taken together, the ablation and TAX-4 expression experiments demonstrate that ASI and ASJ are important sensory neurons for pheromone avoidance.

Avoidance requires sexualization of attraction neurons, not avoidance neurons

Because different sets of sensory neurons mediate male sexual attraction (AWA, AWC, and ASK) and hermaphrodite pheromone avoidance (ASI and ASJ), we investigated whether sex differences in sensory neurons contribute to sexualized behaviors. To localize the neural sex differences necessary for pheromone avoidance, we feminized sets of neurons in animals that were otherwise male. Male animals in which the entire nervous system is feminized using *Prab-3*) avoid pheromones (Figure 5A, B, and C). This result demonstrates that the sexual identity of the nervous system determines avoidance behavior. Male animals in which both attraction and avoidance sensory neurons are feminized (using either *Podr-4* or a combination of neuron-selective promotors, Figure 5C) also avoid pheromones. This result suggests that sex differences in sensory neurons might impart sexualized behavior. We therefore tested whether sex differences in the avoidance sensory neurons ASI and ASJ were sufficient for avoidance behavior. Counterintuitively, male animals in which ASI and ASJ are selectively feminized remain attracted to pheromones (Figure 5C). This result indicates that even though these neurons are required for avoidance, a female sexual identity in ASI and ASJ is not sufficient to impart feminized behavior. However, male animals in which the attraction sensory neurons AWA, AWC, and ASK are selectively feminized do avoid pheromones (Figure 5C). That is, these males acquire feminized behavior even though this set of neurons is not required for avoidance. Together, these results demonstrate that sex differences necessary for pheromone avoidance reside not in the avoidance neurons themselves, but rather in neurons required for male sexual attraction.

Sexual attraction depends on male sexual identity not only in the attraction sensory neurons AWA, AWC, and ASK, but also concurrently in a distinct set of interneurons (White and Jorgensen, 2012). Therefore, we investigated if

sexualization of these interneurons also determined hermaphrodite avoidance behavior. Male animals in which this distinct set of interneurons are feminized using a combination of the *Pglr-2*, *Pglr-5*, and *Pser-2b* promotors acquire feminized behavior and avoid pheromones (Figure 5C). Thus, feminization of the attraction sensory neurons is not exclusively necessary for feminized behavior (avoidance); feminization of either attraction sensory neurons or attraction interneurons is sufficient to impart avoidance behavior.

Taken together, these results demonstrate that repression acts only on attraction neurons with a female sexual identity. In wild-type hermaphrodites, the attraction neurons are female, allowing repression to engage, and the animals avoid pheromones. In wild-type males, the attraction neurons are male, so repression cannot engage, and the animals are attracted to sex pheromones, regardless of whether avoidance is active. In males with feminized attraction neurons—either sensory neurons or interneurons—repression engages on the female side of the synapse so the attraction connections are mismatched and the transgender animals avoid pheromones. Furthermore, males with feminized attraction neurons demonstrate that repression acts on the attraction neurons directly, because no other cells have a female sexual identity, yet repression is engaged. Because wild-type males display attraction behavior and not avoidance, either activation of the attraction circuit turns off the avoidance circuit, or both circuits are active in parallel but attraction predominates.

Males possess latent pheromone avoidance behavior

If both attraction and avoidance circuits are active in parallel, eliminating sexual attraction in males should reveal underlying avoidance behavior. We therefore examined behavior in males in which sexual attraction was eliminated either by removing the necessary sensory neurons or by genetic mutation. First, males without CEM, AWA, AWC, and ASK neurons lack all of the sensory neurons required to promote sexual attraction, and attraction is strongly impaired (White et al., 2007, Figure 6A). When assayed for avoidance behavior, these males avoid pheromones (Figure 6A). Second, a mutation in the *pdk-1* gene was identified in a forward genetic screen for mutants with defective sexual attraction in males, but intact pheromone avoidance in hermaphrodites. Accordingly, mutant males lacking the PDK-1 3-phosphoinositide-dependent kinase have strongly impaired sexual attraction. Intriguingly, inositol signaling is required for localization of synaptic components and integration within interneurons to control thermotaxis behavior (Tanizawa et al., 2006). Importantly, when assayed for avoidance behavior, *pdk-1* mutant males strongly avoid sex pheromones (Figure 6B). In two cases, when sexual attraction behavior is eliminated males are not simply atactic, but instead avoid pheromone. Thus, males possess the latent capacity to avoid pheromones, but sexual attraction normally predominates. This result indicates that males resolve parallel, conflicting attraction and avoidance sensory input so that attraction behavior wins.

Discussion

C. elegans sex pheromones elicit opposite behaviors in each sex: sexual attraction in males and avoidance in hermaphrodites. Although each sex expresses only one behavior, both sexes are capable of either (White and Jorgensen, 2012 and Figure 6). The underlying circuitry for each behavior exists in parallel in males and hermaphrodites, and each circuit can be engaged experimentally to generate either behavior in each sex. If both circuits are intact, attraction overrides avoidance and the animal displays attraction (White and Jorgensen, 2012 and Figure 3). If attraction is disabled or repressed, the subordinate behavior, avoidance, is revealed (Figures 3 and 6). Thus, two key mechanisms act to establish sex-specific behavior: switching the attraction circuit on or off, and ensuring that if attraction is on, it takes priority over avoidance (Figure 7).

The attraction circuit is turned off by DAF-7/TGF- β -mediated repression (White and Jorgensen, 2012). Whether repression is engaged or not depends on the sexual identity of only the attraction neurons—both the attraction interneurons and sensory neurons (White and Jorgensen, 2012 and Figure 5). The sexual identity of the avoidance neurons does not matter (Figure 5). Female attraction neurons are sensitive to repression, male neurons are not. Intriguingly, the ASI avoidance sensory neurons are required during development to establish repression. This cross-regulation may prevent interference between attraction and avoidance behaviors. Because both attraction sensory neurons (AWA, AWC, ASK) and interneurons (a distributed set including AIA, AIB, AIY, and AIZ) must be male to generate attraction behavior (White and Jorgensen, 2012 and Figure 5), we favor a model in which attraction is generated by a constellation of connections that must be matched across the sensory neuron-interneuron synapses—either additional physical synapses or adjustment of the strengths of synapses common to both sexes—the “attraction connections”. Regardless of their exact nature, the attraction connections are disabled in female neurons by DAF-7/TGF- β mediated repression, turning attraction off.

If attraction is on, it takes priority over avoidance. Because the sensory neurons for attraction (AWA, AWC, and ASK, with the CEM neurons contributing in males) and avoidance (ASI and ASJ) are distinct, there are two independent channels of sensory input. The mechanism that gives the attraction channel priority must be implemented prior to the motor neurons, otherwise they would receive conflicting commands—attraction and avoidance are mutually exclusive. One possible mechanism is that activity in the attraction sensory neurons (SNs) directly turns off the avoidance sensory channel, either by altering the activity of the avoidance sensory neurons or preventing sensory information from being transduced to post-synaptic cells. However, direct regulation solely between the attraction and avoidance sensory neurons is difficult to envision, because attraction sensory neurons with a male sexual identity are insufficient to turn off avoidance behavior; both the attraction sensory and interneurons must be male for attrac-

tion to override avoidance. A more likely scenario is that the attraction interneurons integrate information from both sensory channels. The hermaphrodite connectome (White et al., 1986; Chen et al., 2006) shows connections between the attraction interneurons and avoidance sensory neurons; the ASI sensory neurons are connected directly, and ASJ is connected indirectly (via ASK). If attraction is off (the attraction connections are absent or mis-matched), the interneurons initiate avoidance. In this case, the attraction interneurons essentially make a decision, although the capability to make the decision is hard-wired; set during development and dependent on sex.

Conceptually, sexual attraction and pheromone avoidance are less like other *C. elegans* pheromone-mediated behaviors (Jang et al., 2012; Macosko et al., 2009) and more similar to *Drosophila* mating behavior. Other *C. elegans* pheromone-mediated behaviors (aggregation and solitary behavior) are selected by neuropeptide-mediated modulation of sensory neuron gain: turning up an attractive sensory neurons relative to aversive sensory neurons, or the converse (Jang et al., 2012; Macosko et al., 2009). In this case, the nervous system essentially switches between separate, dedicated channels for attraction or aversion by modulating the relative sensitivity of each channel. Oxygen and CO₂ in the environment, feeding state, and sex all contribute to the modulation, and switching between the attraction and aversive states is reversible in adults (Jang et al., 2012). In contrast, male-specific structures in *Drosophila* ensure that only males can make the decision to mate; male-mating behavior depends on male-specific [(*fruM+*)] command neurons, such as the P1, pIP10, and dPR1 neurons (Kimura et al., 2008; von Philipsborn et al., 2011), that are necessary and sufficient to initiate male-specific mating programs. Here, the male-specific structures are not specialized neurons deep in the brain, but a specialized set of connections—the attraction connections, according to our model—that ensure that only males have the capability to make the decision to find their mates.

Why might the nervous system generate sexual attraction behavior as the output of a pre-determined decision, rather than as a simple reflex? Finding a mate is ecologically important and most likely under strong selective pressure (Figure 2). It may be that a conflict-resolution mechanism is more robust than other strategies. The attraction circuit is robust because it is degenerate—several physically different (experimentally altered) configurations of neurons can generate attraction behavior. Specifically, an attraction circuit that is missing one or more of the AWA, AWC, ASK, or CEM sensory neurons generates consistent attraction behavior because these neurons can compensate for one another (White et al., 2007). Likewise, the attraction interneurons must have a male sexual identity to drive attraction, but masculinizing different overlapping subsets of interneurons is sufficient (White and Jorgensen, 2012). A neural circuit configured to make a decision—even one that is pre-determined—may better accommodate multiple neural arrangements to give the same output. We suggest that such conflict-resolution circuits may drive behaviors in other organisms that need to be robust and polarized, such as mating, dominance, aggression or parenting.

Experimental Procedures

Strains and species. Nematodes were cultivated at 20-22 °C. Wild strains of *Caenorhabditis elegans* were obtained from the *Caenorhabditis* Genetic Center. Diverse nematode species were obtained from Michael Ailion (Kiontke et al., 2011). Strain information is provided in Supplemental Table 1.

Behavior. Assays for hermaphrodite pheromone avoidance are the same format as for sexual attraction (White et al., 2007). Single animals were released on the plate and scored after 18-22 hours. All assays were blind for strain and for pheromone vs. control. Assays were scored based on track pattern. A characteristic track pattern leaves a zone of exclusion around the pheromone source with reversals at the edge of the source (Figure 1). The data are categorical (attraction or no attraction) and all data are shown. The number of assays for each condition are indicated in the Figures.

Statistics. Comparisons were made using Fisher's exact test at 90% confidence with the Bonferroni-Holm correction for multiple comparisons. For comparisons, α was taken at 0.05 unless otherwise indicated. Exact p values after correction are given in each Figure.

Laser ablations. Ablations were performed with a MicroPoint laser system as described (Fang-Yen et al., 2012; White et al., 2007) in, L3 larvae or young adults. Operated animals were assayed as one-day old adults, or for adult ablations after one day of recovery. Strains contained GFP markers to assist in neuron identification. Ablations were verified post-assay anatomically or by checking for the absence of GFP, if appropriate. All strains for ablations are listed in Supplemental Table 1.

Molecular biology. For neuron-specific expression of TAX-4 and DAF-7, the Multisite Gateway system (Cheo et al., 2004) was used to fuse different neuron-selective promotors in a 4-1 Entry vector to a standard expression cassette in a 1-2 Entry vector and a standard 3' UTR in a 2-3 Entry vector. The expression cassette contained a cDNA encoding either *tax-4* or *daf-7* in an artificial operon with either EGFP or mCherry. Promotors, reported expression patterns, primers, and references are given in Supplemental Table 2.

Feminization of neural subsets. The Multisite Gateway system (Cheo et al., 2004) was used; Entry vectors were slightly different than with *tax-4* and *daf-7* (above). 4-1 Entry vectors contained different neuron-selective promotors, the 1-2 Entry vector contained a cDNA encoding the intracellular fragment of TRA-2 (Lum et al., 2000, provided by Bill Mowrey and Doug Portman), and the 2-3 Entry vector contained an artificial operon, mCherry, and a generic 3' UTR from the *unc-54* gene. See Supplemental Tables 2 and 3 for promotor information.

Genetic screen for sexual attraction mutants. One thousand clonal F2 lines were established from ENU-mutagenized *him-5(e1490)* or *him-8(e1489)* hermaphrodites. Approximately 500 lines were generated from each

mutagenized background. The *him* mutations cause an increased frequency of spontaneous males, so males were present in the clonal lines at high frequency. Each line was assayed for both avoidance and attraction behaviors, and mutants that were defective for one or the other, but not both, were rescreened multiple times. Putative behavior mutants were screened for normal sensory endings using a dye filling assay (Perkins et al., 1986), only lines that had normal sensory endings were kept for further analysis. Fifteen lines were identified that were mutant for only hermaphrodite avoidance, and two were mutant only for male attraction. *ox349* is an outcrossed strain of one of the attraction mutant lines.

Identification of *ox349*. *ox349* was mapped to a narrow region on X using SNPs (Davis et al., 2005), and a mutation in *pdk-1* identified using whole-genome resequencing (Sarin et al., 2008). *ox349* is a C→T mutation (acatggacgccgtgcatgCaggtggagctaaaaactcg) in *pdk-1* at genomic position X:1326422 (WormBase *C. elegans* release WS234) that is predicted to eliminate a splice site and introduce an early stop into the predicted protein. *ox349* animals (both males and hermaphrodites) are long, have a dark intestine, and the hermaphrodites have a low brood size; these phenotypes are described for the *sa680* strong null allele of *pdk-1* (Paradis et al., 1999).

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Figures

Figure 1: *C. elegans* hermaphrodites avoid sex pheromones released by wild-type and ascaroside-deficient hermaphrodites.

- A. Avoidance assay. Tracks on agar assay plates left by wild-type hermaphrodites (one hermaphrodite, sixteen hours elapsed) show that they avoid a well containing media conditioned with hermaphrodite pheromones. The same conditions elicit sexual attraction in *C. elegans* males (White et al., 2007).
- B. Computer tracking. Ten hermaphrodites were recorded for two hours in an updated assay format (Experimental Procedures) and their positions analyzed by particle tracking once per second. The amount of time a particular position is occupied is indicated by the color code. The tracks indicate that when a hermaphrodite senses sex pheromone, she veers away or performs a complete reversal, depending on the angle of approach. The result is a zone of exclusion around the pheromone source.
- C. Quantitation. Hermaphrodites avoid sex pheromone from both wild-type hermaphrodites and ascaroside-deficient mutants. Sex pheromones released from either wild-type or *daf-22* mutant hermaphrodites were assayed as in (B) at different concentrations. The plot shows the observed frequency that wild-type hermaphrodites avoid the pheromone source in blind assays (Experimental Procedures) multiplied by (-1) to indicate aversion (“pheromone avoidance”). P values were calculated *vs.* no-pheromone control assays using Fisher’s Exact Test with the Bonferroni-Holm correction for multiple comparisons. Sex pheromone sources at these concentrations elicit attraction behavior in males (Supplemental Figure 1).

Figure 2: *C. elegans* sex pheromones elicit sexually dimorphic attraction and avoidance behaviors in wild strains and diverse nematode species.

- A. Hermaphrodites from aggregating wild *C. elegans* strains avoid sex pheromones, and males from aggregating wild strains are attracted to them. The plot shows the response of wild *C. elegans* strains to pheromones released by hermaphrodites from the domestic laboratory strain N2 Bristol. From each strain, males were assayed for attraction (blue bars) and hermaphrodites were assayed for avoidance (red bars). Most wild strains are social, which means that individuals aggregate in clumps on agar plates. P values are calculated *vs.* no-pheromone controls using Fisher’s Exact Test with the Bonferroni-Holm correction for multiple comparisons. In all strains, sex pheromones from N2 Bristol hermaphrodites elicit attraction in males and avoidance in hermaphrodites.
- B. *C. elegans* hermaphrodites elicit attraction and avoidance behavior in diverse nematode species. For each species, both sexes were tested for both behaviors (Experimental Procedures). Species span the nematode phylogeny

(from Kiontke et al., 2011) and include those that reproduce by obligate male-female mating (♀) and by self-fertilizing hermaphroditism (♂). Blue bars indicate male behavior; light blue indicates males tested for avoidance, and dark blue indicates males tested for attraction. Red bars indicate hermaphrodite or female behavior, depending on species. Light red indicate hermaphrodites or females tested for attraction and dark red indicate hermaphrodites or females tested for avoidance. P values are calculated using Fisher's Exact Test with the Bonferroni-Holm correction for multiple comparison *vs.* the *C. elegans* no-pheromone control for each sex. All species tested display sexually dimorphic pheromone behaviors independent of reproductive mode.

Figure 3: Abolishing attraction reveals avoidance.

- A. Removing attraction sensory neurons reveals avoidance. Hermaphrodites that are de-repressed due to a mutation *daf-7* or ASI ablation are attracted to sex pheromones and do not avoid them (*daf-7* control, *daf-7* mock ablation, and ASI single ablation). Removing the sensory neurons required for attraction in de-repressed hermaphrodites reveals avoidance behavior (*daf-7* mutant with AWA, AWC and ASK neurons ablated; wild-type with ASI, AWA, AWC, and ASK neurons ablated). Animals were scored independently for each behavior. P values for the indicated comparisons were calculated using Fisher's Exact Test with the Bonferroni-Holm correction for multiple comparisons.
- B. Restoring repression reveals avoidance. Hermaphrodites lacking DAF-7/TGF- β (*daf-7*) do not repress attraction behavior (White and Jorgensen, 2012), so *daf-7* mutant hermaphrodites do not avoid pheromones but are instead attracted to them. Ectopic TGF- β expression: DAF-7/TGF- β is normally expressed in the ASI neurons; restoring DAF-7/TGF- β function to *daf-7* mutants, either in sensory neurons including ASI (*Podr-4*) or in sensory neurons other than ASI (*Pceh-36*) restores repression and also avoidance behavior. Genetic TGF- β activation: The *daf-3* mutation activates TGF- β signaling regardless of the presence of DAF-7/TGF- β (Thomas et al., 1993); attraction is repressed in *daf-3* mutant hermaphrodites regardless of *daf-7*, and so both *daf-3* and *daf-7* mutant hermaphrodites avoid sex pheromones. P values were calculated *vs.* *daf-7* mutant hermaphrodites using Fisher's Exact Test with the Bonferroni-Holm correction for multiple comparisons.

Figure 4: ASI and ASJ are avoidance sensory neurons.

- A. The ASI and ASJ sensory neuron pairs are sufficient for avoidance. Restoring TAX-4-dependent activity to the ASI or ASJ neuron pairs, but not the ASK neuron pair, restores avoidance behavior to *tax-4*; *daf-3* double-mutant hermaphrodites. A *tax-4* transgene was expressed from the indicated neuron-selective promotors in *tax-4*; *daf-3* animals. Transgenic hermaphrodites were assayed only for avoidance; controls were assayed for both attraction

and avoidance. P values for the indicated comparisons were calculated using Fisher's Exact Test with the Bonferroni-Holm correction for multiple comparisons.

- B. The ASI and ASJ sensory neuron pairs are required for avoidance. Hermaphrodites lacking both the ASI or ASJ neuron pairs or each pair alone have impaired avoidance. ASI single and combined laser ablations were performed in a *daf-3* mutant background to bypass the function of ASI in establishing repression of attraction behavior. The ASJ neuron pair has no detectable function in establishing repression, so ASJ ablations were performed in a wild-type hermaphrodites. Operated animals were assayed only for avoidance. Ablation of ASI in adult hermaphrodites impairs avoidance, indicating that the ASIs are not required during development to establish avoidance behavior. P values for the indicated comparisons were calculated using Fisher's Exact Test with Bonferroni-Holm correction.

Figure 5: Attraction neurons, not avoidance neurons, must be sexualized for avoidance behavior.

- A. Assay of a male with a feminized nervous system. The photo shows tracks left overnight by a single transgenic male expressing *Prab-3::tra-2ic* in the presence of a source of sex pheromones. The zone of exclusion around the pheromone source indicates the transgender males avoid pheromone.
- B. Computer tracking of males with a feminized nervous system. Four males expressing *Prab-3::tra-2ic* (Experimental Procedures) were recorded for two hours, and their positions analyzed once every second by particle tracking. The color code indicates the amount of time a particular position was occupied. The top and bottom of the circular assay plate are cropped in the image.
- C. Feminization of subsets of neurons in animals that are otherwise male. The indicated neuron-selective promoters were used to feminize different subsets of neurons. Mixes 1, 2, 3, and 4 are combinations of different promoters. Full details of each mix are given in the Experimental Procedures and Supplemental Tables 1-3. Transgenic transgender animals were scored independently for both avoidance and sexual attraction. P values for the indicated comparisons were calculated using Fisher's Exact Test with Bonferroni-Holm correction.

Figure 6: Males possess latent avoidance behavior. The number of independently scored assays for each behavior is given (Avoidance n, Attraction n). P values for the indicated comparisons were calculated using Fisher's Exact Test with Bonferroni-Holm correction.

- A. Elimination of the attraction sensory neurons in males reveals avoidance behavior. The ASK sensory neuron pair was ablated in mutant males lacking CEM, AWA, and AWC neurons (White et al., 2007). Operated mutant males have impaired sexual attraction behavior and exhibit significant avoidance behavior. The mutations do not eliminate all neurons in all males, and the mutant strain contains transgenes that express GFP in the CEM, AWA,

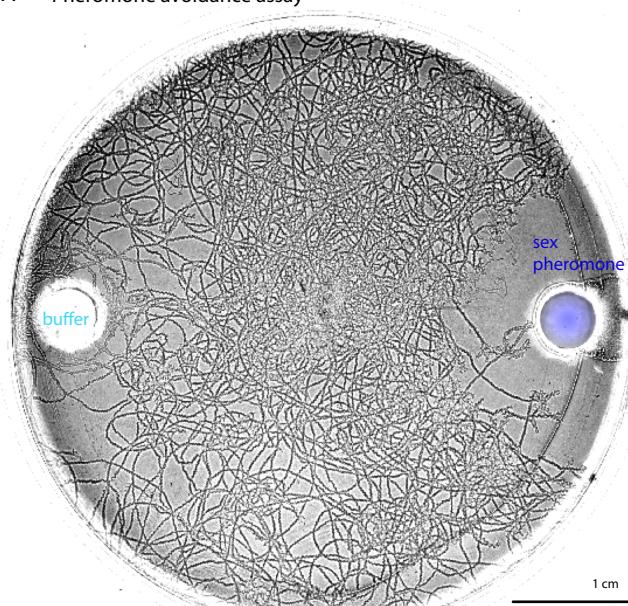
AWC, and ASK neurons, if present. Mock-ablated males were chosen for the presence of GFP, so they have at least some attraction sensory neurons and exhibit attraction behavior.

- B. Mutation of the *pdk-1* gene reveals avoidance behavior. A genetic screen for mutant males with impaired sexual attraction behavior identified the *ox349* allele of *pdk-1*. The *sa680* allele is a strong loss-of function mutation that was isolated previously (Paradis et al., 1999). Males with either *pdk-1* mutation avoid sex pheromones at frequencies comparable to hermaphrodites.

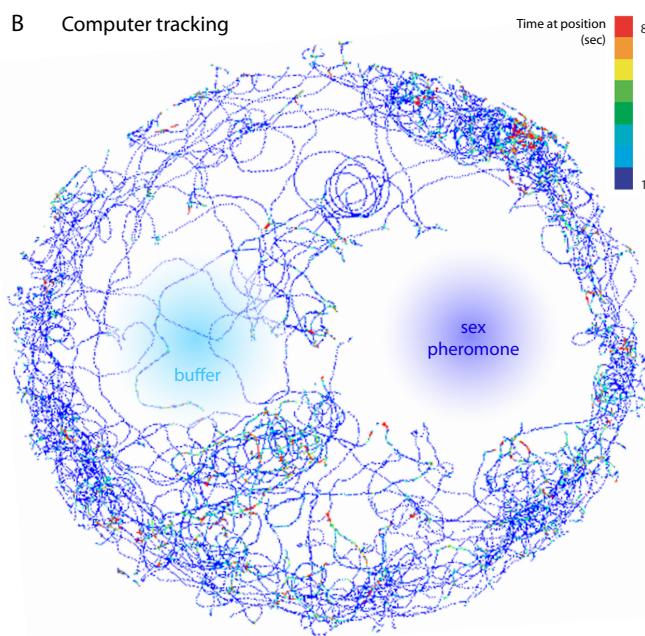
Figure 7: Conflict-resolution model. The thick arrows represent functional connections that define the parallel channels of information for attraction and avoidance. Thin arrows represent the action of TGF- β to establish repression. The thick T-bar represents repression, which functionally disables attraction connections.

- A. In males, the attraction and avoidance sensory channels are both ON, and the attraction interneurons—a degenerate set that includes AIY, AIZ, AIA, and AIB—prioritize attraction over avoidance, so animal are attracted to sex pheromones. The interneurons resolve the conflicting sensory input to prioritize attraction over avoidance.
- B. In hermaphrodites, ASI-dependent, TGF- β mediated repression acts on the female attraction neurons to disable the attraction connections and turn the attraction channel OFF. The avoidance channel is still ON, so the interneurons initiate the default behavior, pheromone avoidance.
- C. In males with feminized attraction sensory neurons, ASI-dependent, TGF- β mediated repression acts on the female neurons to disable the attraction connections, so the attraction channel is off and the interneurons default to avoidance.

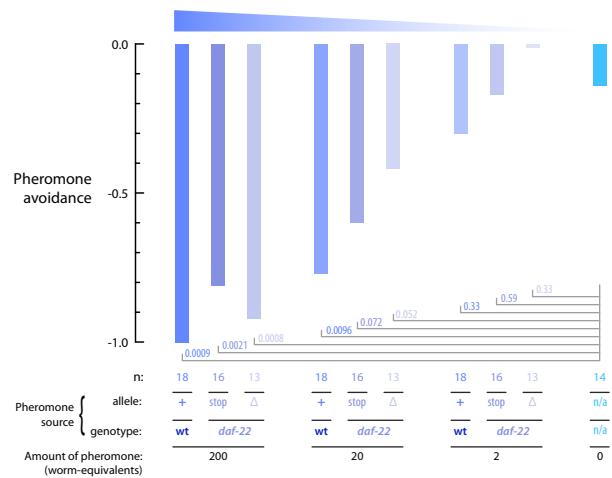
A Pheromone avoidance assay

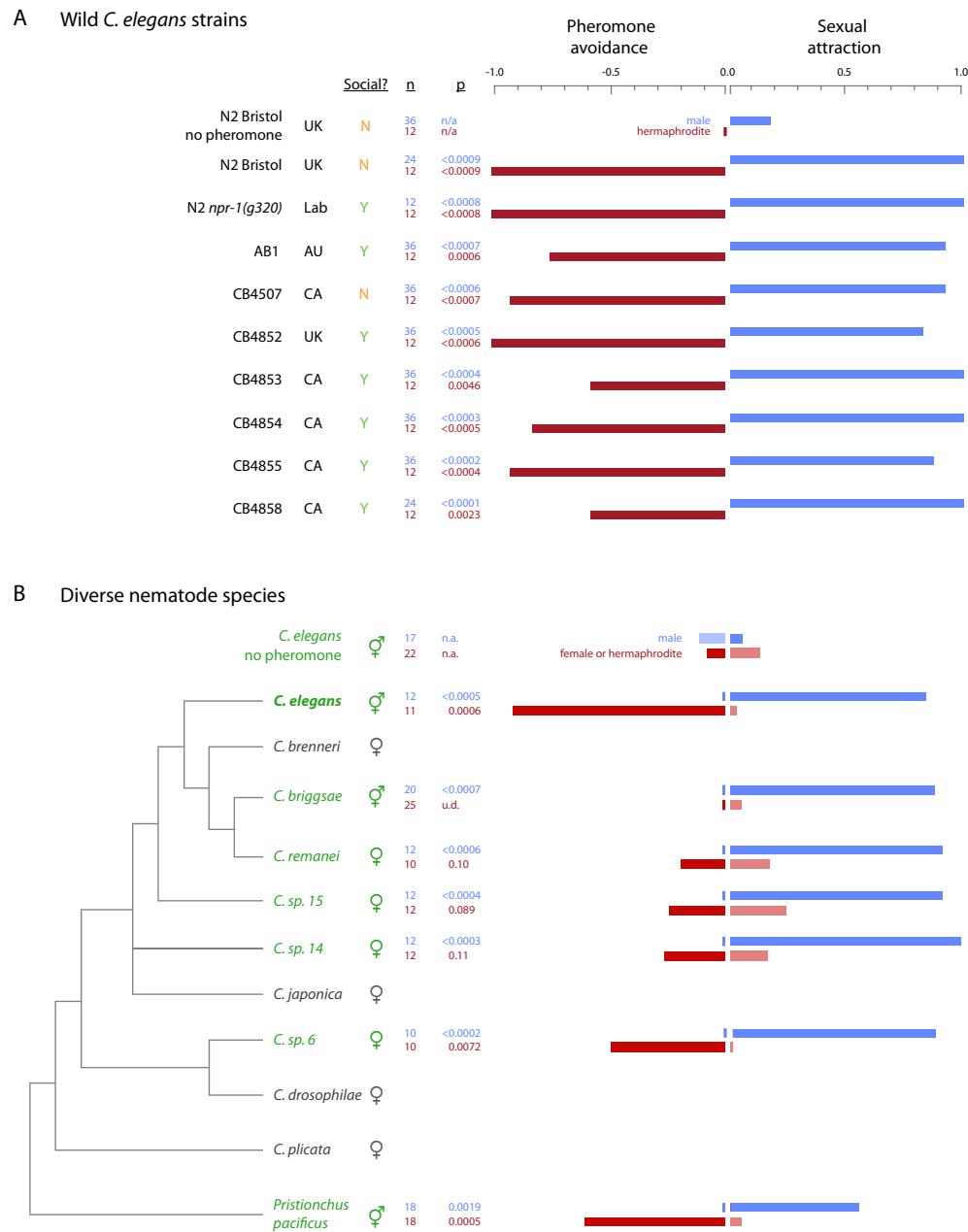


B Computer tracking

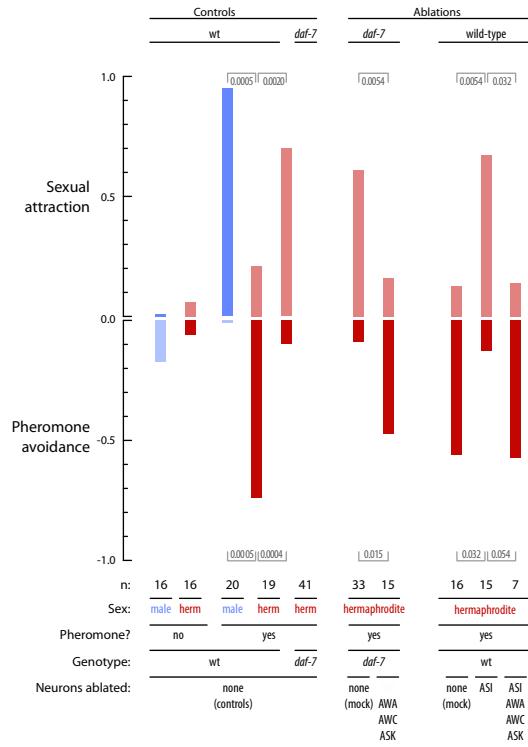


C Avoidance of pheromones from wt and *daf-22* hermaphrodites

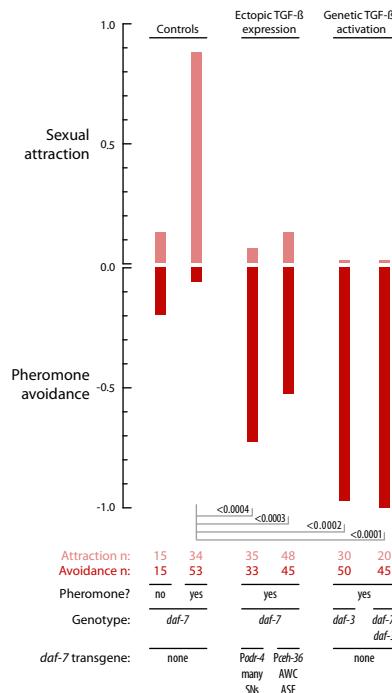




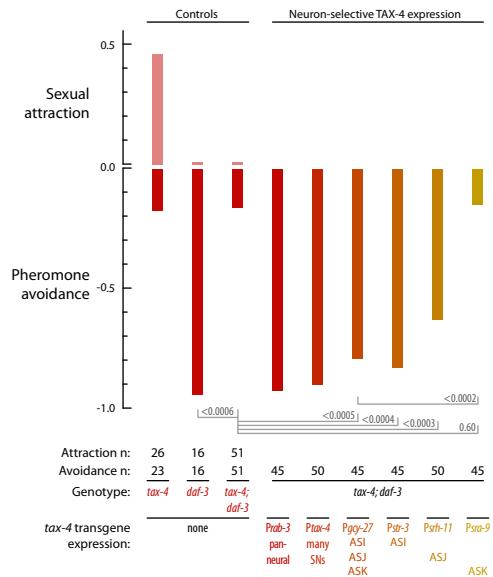
A Attraction sensory neuron ablation



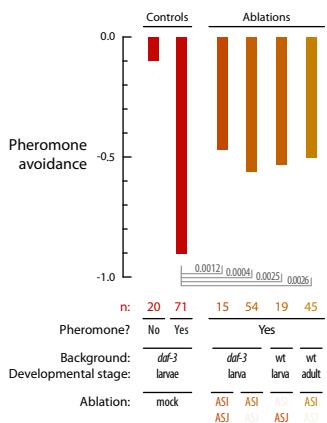
B Restoration of repression



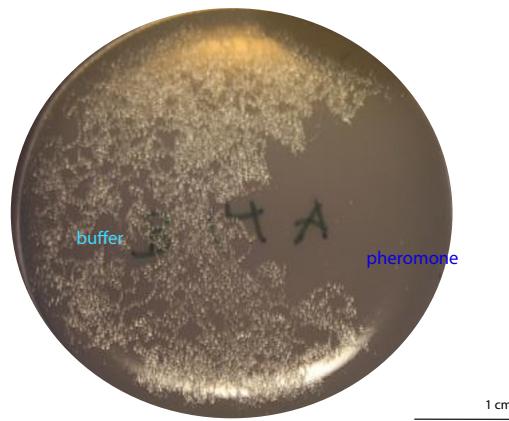
A Restoring ASI and ASJ activity



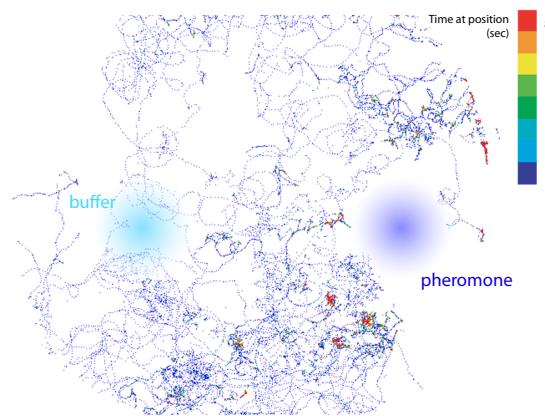
B Ablation of ASI and ASJ



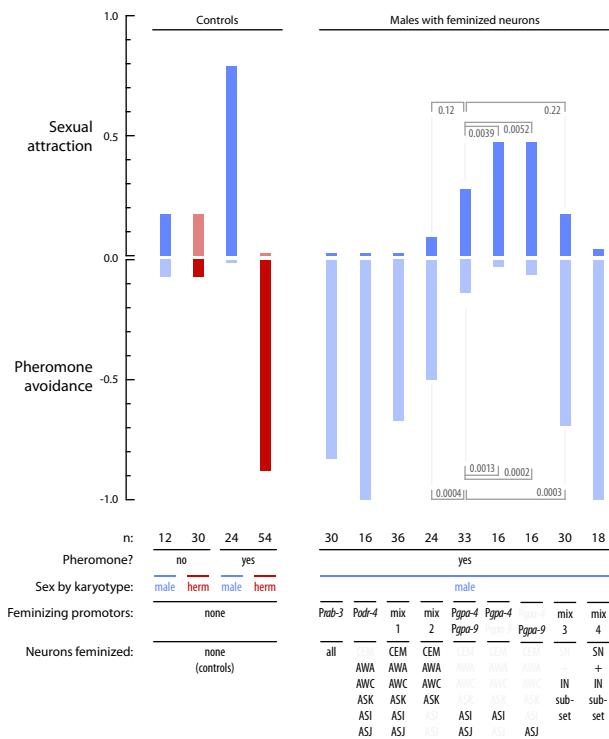
A Assay of males with feminized nervous system

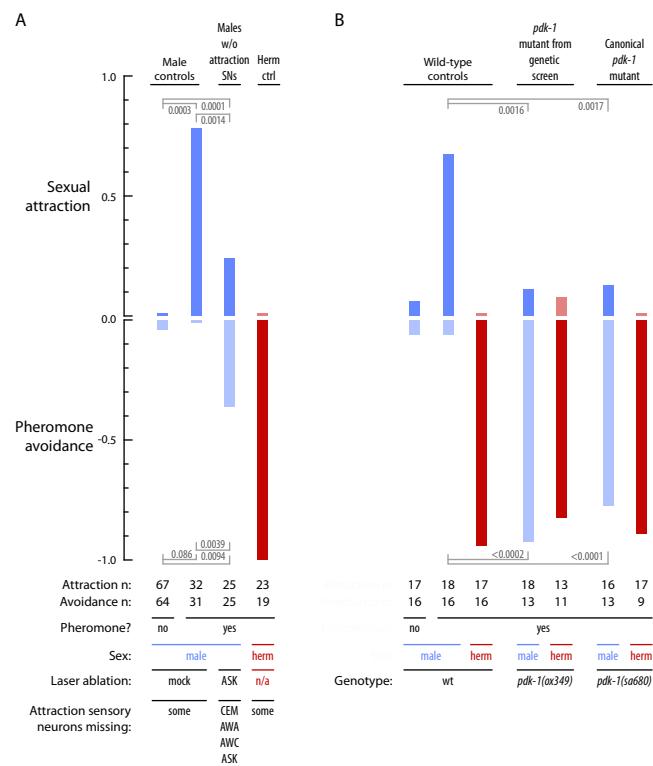


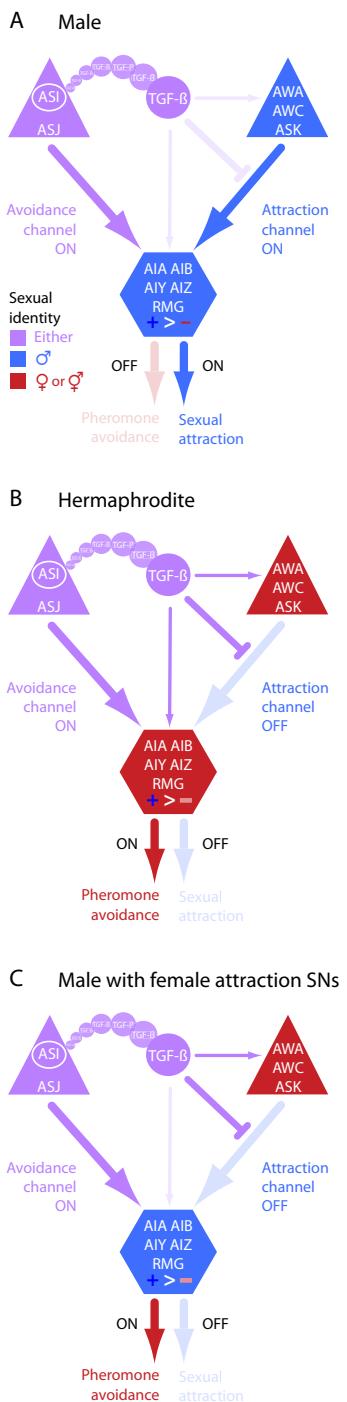
B Computer tracking of males with feminized nervous system



C Feminizing subsets of neurons in males







Supplemental Information

Supplemental Figures 1 and 2 - supports Results

Supplemental Tables 1, 2, and 3 - Supports Experimental Procedures

Supplemental Figures

Supplemental Figure 1: Males are attracted to sex pheromones from an ascaroside-deficient mutant.

- A. Males are attracted to sex pheromones from the ascaroside-deficient mutant. Ten males were recorded for sixty minutes and their positions analyzed by particle tracking once per second. The amount of time a particular position is occupied is indicated by the color code. The tracks are indistinguishable from tracks elicited by wild-type pheromones (White et al., 2007, Figures 1 and S1), and indicate that males locate the pheromone source within a few minutes, and once they find it tend to remain there.
- B. Males are attracted to sex pheromone from both wild-type hermaphrodites and ascaroside-deficient mutants over a range of concentrations. Sex pheromones released from either wild-type or *daf-22* mutant hermaphrodites were assayed as in (A) at different concentrations. The plot shows the observed frequency that wild-type males show the behavior displayed in (A) in blind assays. Pheromones from wild-type and *daf-22* mutants elicit avoidance at comparable frequencies at each concentration. Concentrations were normalized to the density of the pheromone source culture (worm-equivalents). Concentrations below three worm-equivalents did not elicit significant behavior. P values were calculated *vs.* no-pheromone control assays using Fisher's Exact Test with the Bonferroni-Holm correction for multiple comparisons.

Supplemental Figure 2: Hermaphrodite avoidance does not require canonical aversive sensory neurons. The ASH, AWB, ADL aversive neurons (Bargmann, 2006) and the ASK pheromone-detecting neurons (Kim et al., 2009; Srinivasan et al., 2008) were concurrently ablated during development (in L3 larva). Hermaphrodites lacking these four pairs of sensory neurons avoid pheromones, and are comparable to mock ablated controls. The ADF hyperoxia-avoidance neurons were ablated separately during development; hermaphrodites without ADF neurons also avoid pheromones. P values were calculated *vs.* mock-ablated hermaphrodites using Fisher's Exact Test with the Bonferroni-Holm correction for multiple comparisons.

Supplemental Table 1: Strains

Strain	Genotype	Source	Notes	Figure
N2	wild-type	CGC	Laboratory reference strain.	1
DR476	<i>daf-22(m130) II</i>	CGC	Used to prepare media lacking short-chain ascarosides.	1, S1
RB859	<i>daf-22(ok693) II</i>	Butcher et al., 2009	Used to prepare media lacking short-chain ascarosides.	1
EG2717	<i>him-5(e1490) V</i>	White et al., 2007	8x outcrossed against N2 Bristol.	S1, 2
EG2718	<i>him-8(e1489) IV</i>	White et al., 2007	8x outcrossed against N2 Bristol.	2
AB1	<i>Caenorhabditis elegans</i> wild isolate	CGC	Wild isolate 1984 from A Bird, CSIRO, Adelaide, Australia.	2
CB4507	<i>C. elegans</i> wild isolate	CGC	Wild isolate from Palm Canyon, California. Segregated to remove <i>gro-1(e2400)</i> [CB4512].	2
CB4852	<i>C. elegans</i> wild isolate	CGC	Obtained from Rothamsted by Sydney as "Panagrellus redivivus".	2
CB4853	<i>C. elegans</i> wild isolate	CGC	Isolated from Carl Johnson's organic garden in Altadena, California in 1974.	2
CB4854	<i>C. elegans</i> wild isolate	CGC	Also isolated from Carl Johnson's organic garden in Altadena, California in 1974.	2
CB4855	<i>C. elegans</i> wild isolate	CGC	Isolated from compost in Palo Alto, California in 1982.	2
CB4858	<i>C. elegans</i> wild isolate	CGC	Isolated from Caltech flowerbed in the summer of 1971.	2
PS1185	<i>C. briggsae</i> wild isolate	Michael Ailion		2
JT11490	<i>C. remanei</i> wild isolate	Michael Ailion		2
EG5716	<i>C. sp. 14</i> wild isolate	Michael Ailion	Isolated from Moorea, French Polynesia, in rotting chestnut (Kiontke et al., 2011).	2
QG122	<i>C. sp. 15</i> wild isolate	Michael Ailion	Isolated from rotting Hawaiian hibiscus flowers (Kiontke et al., 2011).	2
EG4788	<i>C. sp. 6</i> wild isolate	Michael Ailion	Isolated from Amares, Portugal in rotting apples (Kiontke et al., 2011).	2
EG4168	<i>him-8(e1489) IV; oyls48[P(ceh-36)::gfp, lin-15(+)] V</i>	White et al., 2007	Green AWC and ASE neurons. Routinely used for ablations.	3A
EG6289	<i>daf-7(e1372ts) III; him-5(e1490) V;</i>	(Srinivasan et al., 2008)	For ablations in a <i>daf-7</i> background.	3A
EG6290	<i>oyls48[P(ceh-36)::gfp, lin-15(+)] V</i>			
EG6125	<i>daf-7(e1372ts) III; him-5(e1490) V;</i>	White and Jorgensen, 2012	Rescue of <i>daf-7</i> in most sensory neurons (includes ASI).	3B
EG6126	<i>oxEx1478[Podr-4::egfp_daf-7::unc-54U TR, Punc-17::mCherry]</i>			
EG6127				

Strain	Genotype	Source	Notes	Figure
EG6254	<i>daf-7(e1372ts) III ; him-5(e1490) V;</i>	White and Jorgensen, 2012	<i>daf-7</i> rescue in AWC/ASE.	3B
EG6255	<i>oxEx1496[Pceh-36::egfp_daf-7, Punc-17::mCherry]</i>			
EG6256				
CB1376	<i>daf-3(e1376) X</i>	CGC	<i>daf-3</i> suppresses <i>daf-7</i> .	3B, 4A
PY1671	<i>daf-7(e1372ts) III ; oyIs14[Psra-6::gfp] V ; daf-3(mgDf90) X</i>	Piali Sengupta	GFP in ASH, ASI, and PVQ. Dauer-defective. For ablations in a <i>daf-3</i> background.	3B, 4B
PR678	<i>tax-4(p678) III</i>	CGC	Reference allele. <i>p678</i> allele verified by sequencing.	4A
EG7051	<i>tax-4(p678) III ; daf-3(e1376) X</i>	This study	<i>p678</i> allele verified by sequencing, <i>e1376</i> verified by lack of dauers. Does not avoid sex pheromone.	4A
EG7289	<i>tax-4(p678) III ; daf-3(e1376) X;</i>	This study	Green nervous system, restores TAX-4 function in neurons.	4A
EG7290	<i>oxEx1766[Prab-3::tax-4_GFP(S65C)::unc-54UTR, ccGFP]</i>			
EG7291				
EG7287	<i>tax-4(p678) III ; daf-3(e1376) X;</i>	This study	<i>Ptax-4</i> sensory neurons are green and have TAX-4 function restored.	4A
EG7288	<i>oxEx1764[Ptax-4::tax-4_GFP(S65C)::unc-54UTR, ccGFP]</i>			
EG7289				
EG7293	<i>tax-4(p678) III ; daf-3(e1376) X;</i>	This study	ASI, ASJ, and ASK sensory neurons are green and have TAX-4 function restored. Hermaphrodites avoid sex pheromone.	4A
EG7294	<i>oxEx1769[Pgcy-27::tax-4_GFP(S65C)::unc-54UTR, ccGFP]</i>			
EG7295				
EG7390	<i>tax-4(p678) III ; daf-3(e1376) X;</i>	This study	ASI sensory neurons are green and have TAX-4 function restored (Macosko et al., 2009). Hermaphrodites avoid sex pheromone.	4B
EG7391	<i>oxEx1817[Pstr-3::tax-4_GFP, ccGFP]</i>			
EG7384	<i>tax-4(p678) III ; daf-3(e1376) X;</i>	This study	ASJ sensory neurons are green and have TAX-4 function restored (Macosko et al., 2009).	4B
EG7385	<i>oxEx1813[Psrh-11::tax-4_GFP, ccGFP]</i>		Hermaphrodites avoid sex pheromone.	
EG7386				
EG7387	<i>tax-4(p678) III ; daf-3(e1376) X;</i>	This study	ASK sensory neurons are green and have TAX-4 function restored (Macosko et al., 2009). Hermaphrodites avoid sex pheromone.	4B
EG7388	<i>oxEx1813[Psra-9::tax-4_GFP, ccGFP]</i>			
EG7389				
EG5439	<i>him-5(e1490) V ; oxIs456 [Prab-3::tra-2ic::operon::mCherry::unc-54UTR, Ppkd-2::gfp(S65C), Ptph-1::egfp, lin-15(+)]</i>	This study	Feminized, red nervous system. Males avoid sex pheromone.	5C
EG8030	<i>him-8(e1489) IV ; lin-15(n765ts) X;</i>	This study		
EG8031	<i>oxEx1937[Podr-4i::tra-2ic::mCherry, lin-15(+)]</i>		Feminized, red <i>Podr-4</i> neurons. Males avoid sex pheromone.	5C
EG8032				

Strain	Genotype	Source	Notes	Figure
EG7598	<i>him-8(e1489) IV; lin-15(n765ts) X;</i>	This study		
EG7599	<i>oxEx1940[Podr-10::tra-2ic::_mCherry,</i>			
EG7600	<i>Pceh-26::tra-2ic::_mCherry,</i> <i>Ppkd-2::tra-2ic::_mCherry,</i> <i>Psrg-2::tra-2ic::_mCherry,</i> <i>Psrg-2::tra-2ic::_mCherry,</i> <i>Pgpa-4::tra-2ic::_mCherry,</i> <i>Pgpa-9::tra-2ic::_mCherry, lin-15(+)]</i>		Mix 1 in Figure 5C. Attraction sensory neurons and avoidance sensory neurons are feminized and red (CEM, AWA, AWC, ASK, ASI, and ASJ, plus ASE).	5C
EG7543	<i>him-8(e1489) IV; lin-15(n765ts) X;</i>	This study		
EG7544	<i>oxEx1882[Podr-10::tra-2ic,</i> <i>Pceh-36::tra-2ic,</i> <i>Ppkd-2::tra-2ic, Psrg-2::tra-2ic, Ppkd-2::gfp,</i> <i>lin-15(+)]</i>		Mix 2 in Figure 5C. Attraction sensory neurons only are feminized and red (CEM, AWA, AWC, ASK, plus ASE). Male-specific neurons are green.	5C
EG7501	<i>him-8(e1489) IV; lin-15(n765ts) X;</i>	This study		
EG7502	<i>oxEx1859[Pser-2b::tra-2ic::mCherry,</i>			
EG7503	<i>Pglr-2::tra-2ic::mCherry, Pgtr-5::tra-2ic::mCherry, lin-15(wt)]</i>		Mix 3 in Figure 5C. <i>Pser-2b</i> , <i>Pgtr-2</i> , and <i>Pgtr-5</i> interneurons are red and feminized. Males avoid sex pheromone.	5C
EG8027	<i>him-8(e1489) IV; lin-15(n765ts) X;</i>	This study		
EG8028	<i>oxEx1934[Podr-4i::tra-2ic::mCherry,</i>			
EG8029	<i>Pser-2b::tra-2ic::mCherry,</i> <i>Pgtr-2::tra-2ic::mCherry,</i> <i>Pgtr-5::tra-2ic::mCherry, lin-15(+)]</i>		Mix 4 in Figure 5C. <i>Podr-4</i> sensory neurons and <i>Pser-2b</i> , <i>Pgtr-2</i> , and <i>Pgtr-5</i> interneurons are red and feminized. Males avoid sex pheromone.	5C
EG7545	<i>him-8(e1489) IV; lin-15(n765ts) X;</i> <i>oxEx1882[Podr-10::tra-2ic,</i> <i>Pceh-36::tra-2ic, Psrg-2::tra-2ic,</i> <i>Ppkd-2::gfp, lin-15(+)]</i>	This study	Core attraction sensory neurons only are feminized and red (AWA, AWC, ASK, plus ASE). Male-specific neurons are green.	not shown
EG7601	<i>him-8(e1489) IV; lin-15(n765ts) X;</i>	This study		
EG7602	<i>oxEx1943[Pgpa-4::tra-2ic::_mCherry,</i>			
EG7603	<i>Pgpa-9::tra-2ic::_mCherry, Ppkd-2::gfp]</i>		Avoidance sensory neurons only are feminized and red (ASI and ASJ). Male-specific neurons are green.	5C
EG7604	<i>him-8(e1489) IV; lin-15(n765ts) X;</i>	This study		
EG7605	<i>oxEx1948[Pgpa-4::tra-2ic::_mCherry,</i>		ASI sensory neurons are feminized and red.	5C
EG7606	<i>Ppkd-2::gfp, lin-15(+)]</i>		Male-specific neurons are green.	
EG7607	<i>him-8(e1489) IV; lin-15(n765ts) X;</i>	This study		
EG7608	<i>oxEx1949[Pgpa-9::tra-2ic::_mCherry,</i>			
EG7609	<i>Ppkd-2::gfp, lin-15(+)]</i>		ASJ sensory neurons are feminized and red.	5C
EG7609	<i>Ppkd-2::gfp, lin-15(+)]</i>		Male-specific neurons are green.	
EG4671	<i>bcl-9[P(pkd-2)::gfp] X ceh-30(bc272) X</i>	White et al.,		
EG4672	<i>odr-7(ky4) X ceh-36(ky640) X;</i>	2007		
EG4673	<i>oxEx1022[P(pkd-2)::gfp,</i> <i>P(odr-10)::gfp, P(ceh-36)::gfp,</i> <i>P(unc-17)::mCherry]</i>		The <i>ceh-30 odr-7 ceh-36</i> triple mutant background gives rise to males missing their CEM, AWA, and AWC neurons. GFP on the array labels these neurons if they escape the mutations. Red <i>Punc-17</i> neurons as an injection marker. Mutant for male attraction (Cheo et al., 2004).	6A
EG349	<i>him-5(e1490) V; pkd-1(ox349) X</i>	This study	<i>pkd-1(ox349)</i> males and hermaphrodites avoid pheromone. Animals are long, form dauers on food, and have a dark intestine.	6B

Strain	Genotype	Source	Notes	Figure
EG7460	<i>him-8(e1489) IV; pdk-1(sa680) X</i>	This study	Strong allele of <i>pdk-1</i> (Paradis et al., 1999). <i>pdk-1(sa680)</i> males and hermaphrodites avoid pheromone. Animals are long, form dauers on food, and have a dark intestine.	6B

Supplemental Table 2: Promotors for neuron-selective expression

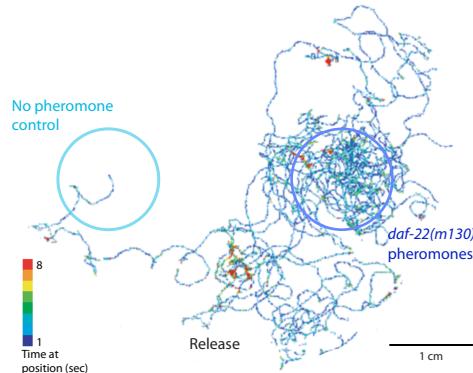
Promotor	Reported expression	Reference	4-1 Entry vector primers	Fig.
<i>Podr-4</i>	AWA, AWB, AWC, ASG, ASI, ASJ, ASK, ADF, ADL, ASH, PHA, PHB	Troemel et al., 1995	F-5'ggggacaacttgtatagaaaagtggatcgaaaccgggtacgacttaccg R-5'ggggactgcttttgtacaaacttgtggattctgtact	3
<i>Pceh-36</i>	AWC, ASE	Lanjuin et al., 2003	F-5'ggggacaacttgtatagaaaagtggATAAGCTTATCCGATAAGGCTGAGCC R-5'GGGGACTGCTTTGTACAAACTTGcatTGTGCATGCCGGGGC	3
<i>Prab-3</i>	pan-neural	Nonet et al., 1997	F-5'ggggacaacttgtatagaaaagtggATCTCAGATGGGAGCAG R-5'ggggactgcttttgtacaaacttgtCATCTGAAAATAGGGCTAC	4A
<i>Ptax-4</i>	AWB, AWC, ASE, ASG, ASI, ASJ, ASK, URX, AFD, BAG	Coburn and Bargmann, 1996b	F-5'ggggacaacttgtatagaaaagtgtcatcttccttgccctc R-5'ggggactgcttttgtacaaacttgtCATcttgaacataattaaatttttag	4A
<i>Pgcy-27</i>	ASI, ASJ, ASK	Ortiz et al., 2006	F-5'ggggacaacttgtatagaaaagtggGATACTTTGGAAAAGAACAAATGaaaaacaagatgg R-5'ggggactgcttttgtacaaacttgtATTTGGTAGAAAATAAAATAG	4A
<i>Pstr-3</i>	ASI	Peckol et al., 2001	Macosko et al., 2009	4A
<i>Psrh-11</i>	ASJ	Macosko et al., 2009	Macosko et al., 2009	4A
<i>Psra-9</i>	ASK	Troemel et al., 1995	(White et al., 1986)	4A
<i>Ppkd-2</i>	Male-specific neurons: CEM, HOB, Ray neurons 1-9	Barr and Sternberg, 1999	F-5'ggggacaacttgtatagaaaagtTGTATACTGTAGATCATGACATTG R-5'ggggactgcttttgtacaaacttgtcAAGACGGCTCGCTGAAACAG	5C
<i>Podr-10</i>	AWA	Sengupta et al., 1996	F-5'ggggacaacttgtatagaaaagtgtCTGATATCTACTTAAATATAGGGACGTGCG R-5'ggggactgcttttgtacaaacttgtATCTCCGACATGGAGCTGTAAGGTATC	5C
<i>Psg-2</i>	ASK	Troemel et al., 1995	F-5'ggggacaacttgtatagaaaagtggTTCACTGATGCTCAAGCAC R-5'ggggactgcttttgtacaaacttgtATTTATTGAITAATATACTTgatattttttattg	5C
<i>Pgpa-4</i>	ASI	Jansen et al., 1999	F-5'ggggacaacttgtatagaaaagtggTGATCATTGGAAATGCGGTTCCC R-5'ggggactgcttttgtacaaacttgtCATtgtggaaaagtgtcacaaaatgaaagtggc	5C
<i>Pgpa-9</i>	ASJ, PHB, PVQ, pharynx muscle, spermatheca	Jansen et al., 1999	F-5'ggggacaacttgtatagaaaagtggGAGTGTGCCCTGTAAATAATCCTGCGTATAAACG R-5'ggggactgcttttgtacaaacttgtATGATTITGCCGATGAAGAAAATGATGC	5C
<i>Pgtr-2</i>	AIA, AIB, AVA, AVD, AVE, DVA, M1, PVC, RIA, RIG, RIR, RMD	Brockie et al., 2001	(White et al., 1986)	5C

Promotor	Reported expression	Reference	4-1 Entry vector primers	Fig.
<i>Pglr-5</i>	AIB (variable), AVA, AVB, AVD, AVE, AVK, DVA (variable), HSN, LUA, PVC, PVQ, RIC, RIF, RIM, RMD, RME, RMG, SAB, SIB, SMD, URA (variable), URB, URY, VC	Brockie et al., 2001	F-5'ggggacaacttgtatagaaagggtggtagcgcc R-5'ggggactgcttttgacaaacttgtCATtttgc当地atttgaggctgccttg	5C
<i>Pser-2b</i>	AIY, AIZ, BDU, DVA, PVT (variable), RID, RME, SIA	Tsalik and Hobert, 2003		SC

Supplemental Table 3: Promotor mixes for neuron-selective feminization

	Mix 1	Mix 2	Mix 3	Mix 4
Category	Avoidance and attraction sensory neurons	Attraction sensory neurons only	Attraction interneurons only	Attraction sensory- and interneurons
Promotors	<i>Ppkd-2</i> <i>Podr-10</i> <i>Pceh-36</i> <i>Psrg-2</i> <i>Pgpa-4</i> <i>Pgpa-9</i>	<i>Ppkd-2</i> <i>Podr-10</i> <i>Pceh-36</i> <i>Psrg-2</i>	<i>Pglr-2</i> <i>Pglr-5</i> <i>Pser-2b</i>	<i>Podr-4</i> <i>Pglr-2</i> <i>Pglr-5</i> <i>Pser-2b</i>

A Male attraction to pheromones from *daf-22* hermaphrodites



B Attraction to pheromones from wt and *daf-22* hermaphrodites

