# PDF-1 neuropeptide signaling modulates a neural circuit for mate-searching behavior in *C. elegans*

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**Appetitive behaviors require complex decision making that involves the integration of environmental stimuli and physiological needs. *C. elegans* mate searching is a male-specific exploratory behavior regulated by two competing needs: food and reproductive appetite. We found that the pigment dispersing factor receptor (PDFR-1) modulates the circuit that encodes the male reproductive drive that promotes male exploration following mate deprivation. PDFR-1 and its ligand, PDF-1, stimulated mate searching in the male, but not in the hermaphrodite. *pdf-1* was required in the gender-shared interneuron AIM, and the receptor acted in internal and external environment-sensing neurons of the shared nervous system (URY, PQR and PHA) to produce mate-searching behavior. Thus, the *pdf-1* and *pdfr-1* pathway functions in non–sex-specific neurons to produce a male-specific, goal-oriented exploratory behavior. Our results indicate that secretin neuropeptidergic signaling is involved in regulating motivational internal states.**



Biological drives are internal physiological states that produce goal oriented behaviors that are critical for survival and reproduction[1]. Drives shape behavioral decisions according to the animal's physiological needs to maintain homeostasis. In turn, the physiological needs of the animal are determined by ecological niche[2,3], gender[4] and genetic make-up[5]. Often, animals need to choose between competing needs and prioritize one drive over another. Thus, when faced with identical stimuli, animals may respond differently on the basis of previous experience and physiological states. The cellular and molecular basis of behavioral choice under competing needs is only beginning to be elucidated.

*C. elegans* mate-searching behavior is an example of such a goal-orientated behavior that is shaped by at least two competing internal physiological needs: feeding and reproduction[6,7]. Well-fed isolated males leave a plentiful source of food to explore their environment. In contrast, when mates are present on the food, male exploratory behavior is suppressed and males remain at the food source. A period of starvation also suppresses mate-searching behavior in the absence of mates. Internal signals that indicate the nutritional and reproductive status of the male are conveyed through an insulin-like signaling pathway and a steroid nuclear hormone receptor, respectively[6,8]. External environmental cues, that is, food and mates, are sensed through two independent circuits that include the core chemosensory amphid neurons in the head and the male-specific ray neurons in the tail, respectively. In the absence of mates, ray neurons promote exploration away from food and food-sensing amphid neurons inhibit exploration away from food[7]. At the mechanistic level, the switch from exploration to retention with a mate is achieved by regulating the characteristics of locomotion, such as the frequency of reversals and high angle turns, after exiting the edge of the food lawn[7]. The molecules and circuitry controlling mate-searching behavior are only partially known.

Neuropeptides are molecular indicators of internal states and important modulators of mood and appetitive behaviors in all animals. We found that PDFR-1, a member of the secretin family of G protein–coupled receptors, and its neuropeptide ligand PDF-1 (refs. 9,10) are major regulators of mate-searching behavior in *C. elegans*. PDF-1 and PDFR-1 signaling modulated the circuit that conveyed the male reproductive drive to explore. The lack of exploratory behavior by *pdfr-1* mutant males reflected an imbalance in the relative contribution of the circuits regulating a distributed neural network for exploration. Moreover, the PDF-1 and PDFR-1 pathway functioned in a discrete non–sex-specific neuronal circuit in a sexually dimorphic neural network for navigation.

## RESULTS

### PDF-1 neuropeptide signaling stimulates mate searching

To identify molecular pathways that regulate male mate-searching behavior, we screened for leaving assay–defective (Las) mutant males that remain on food in the absence of mates. The Las mutation *bx142* was mapped to the *pdfr-1* locus on chromosome III using single-nucleotide polymorphisms[11], two-point genetic mapping and genetic complementation tests with a series of chromosomal deficiencies[12,13] (**Supplementary Fig. 1a**). *bx142* introduces a missense mutation, which results in a G298D substitution in the fourth trans-membrane domain of the PDFR-1 G protein–coupled receptor (**Fig. 1a**). A *pdfr-1* deletion allele, *tm4457*, displayed the same Las phenotype and failed to complement *bx142* (**Fig. 1b**). The *bx142* phenotype was rescued with a genomic fragment containing the *pdfr-1* locus (**Fig. 1b**). Thus, *bx142* is a loss-of-function allele of *pdfr-1*.

Two genes have been identified in *C. elegans* that encode neuropeptide ligands for PDFR-1, *pdf-1* and *pdf-2* (ref. 10). A *pdf-1* null allele, *tm1996*, was Las, while *pdf-2*(*tm4393*) males displayed wild-type

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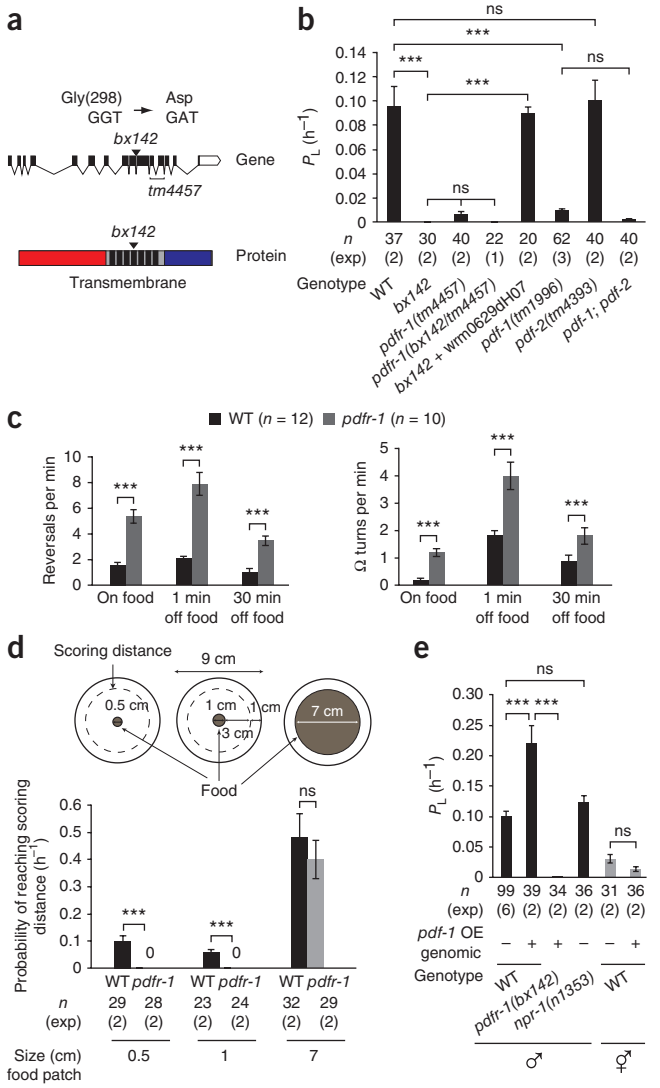


**Figure 1** PDF-1 neuropeptide signaling stimulates mate searching. (**a**) Genomic and protein domain structures of *pdfr-1*. Top, exons (black boxes) and introns (lines) of the *pdfr-1* locus. Bottom, extracellular (red), seven trans-membrane domains (black) and intracellular (blue) regions are shown. The changes in codon and amino acid produced by the *bx142* mutation are indicated. (**b**) Graph shows $P_L$ (probability of leaving food) values of wild-type (WT), *pdfr-1* (*bx142*) and *pdfr-1*(*tm4457*) mutants, and *pdf-1*(*tm1996*) and *pdf-2*(*tm4393*) mutants. The *pdfr-1*(*tm4457*) allele failed to complement *bx142*, indicating that the two mutations affect the same locus. The fosmid wrm0629dH07 contains the *pdfr-1* locus. Error bars indicate s.e.m. *n* indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Maximum likelihood statistical analysis was used to compare $P_L$ values, $^{***}P < 0.001$; ns, no statistically significant difference ($P \geq 0.05$). (**c**) Graphs show the frequency of reversals and high angle turns (Ω turns) performed by wild-type and *pdfr-1*(*bx142*) mutant males over 5 min. Locomotion was observed on food and at two different time points off food: 1 min after removal from food and 30 min after removal from food. *n* indicates total number of animals tested. Error bars indicate s.e.m. Statistical analysis was performed using Mann-Whitney test, $^{***}P < 0.001$. (**d**) Graphs show the rate at which wild-type and *pdfr-1*(*bx142*) males traveled 3.5 cm from the starting point at the center of the plate in a food patch to the scoring distance 1 cm away from the edge of the assay plate. This was calculated as in the leaving assay (probability of reaching the scoring distance). Error bars indicate s.e.m. *n* indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Maximum likelihood statistical analysis was used to compare probability of reaching the scoring distance. $^{***}P < 0.001$; ns, no statistically significant difference ($P \geq 0.05$). (**e**) Graph shows $P_L$ values of wild-type and *pdfr-1*(*bx142*) mutant males and wild-type hermaphrodites with or without the *pdf-1* genomic overexpression (*pdf-1* OE) transgene. *npr-1*(*n1353*) mutant males displayed rates of mate-searching behavior similar to those of wild-type males. Error bars indicate s.e.m. *n* indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Maximum likelihood statistical analysis was used to compare $P_L$ values. $^{***}P < 0.001$; ns, no statistically significant difference ($P \geq 0.05$).

leaving behavior (**Fig. 1b**). *pdfr-1* and *pdf-1* mutant males had no drive to explore away from a food source (**Supplementary Fig. 1b**). Consistent with a suppression of mate-searching behavior (that is, male exploration away from food), *pdfr-1* mutants produced an increase in the frequency of both reversals and high-angle turns (omega turns) compared with wild-type males (**Fig. 1c**). In addition, *pdfr-1* mutant males displayed slow movement on food as a result of a decrease in the frequency of body bends (propagation of the sinusoidal wave; **Supplementary Fig. 1c**). The slow movement phenotype could be dissociated genetically from the defects in mate-searching behavior, indicating that the slow movement phenotype is not responsible for the mate-searching defect (**Supplementary Fig. 1c**,**d**).

The lack of mate-searching behavior by *pdfr-1* mutant males is not a result of a general locomotion defect. *pdfr-1* mutant males traveled as far as the food edge, regardless of the size of the food lawn. If the food area was restricted to 1- or 0.5-cm diameter, *pdfr-1* mutant males, unlike wild-type males, only traveled 1 or 0.5 cm, even by 24 h (**Fig. 1d**). In contrast, *pdfr-1* mutant males were able to travel the scoring distance of the leaving assay plate (3.5-cm radius) at a rate similar to that of wild-type males if the area was covered with food (**Fig. 1d**). Thus, *pdfr-1* mutants display a specific defect in their ability to leave food, resulting in a loss of mate-searching behavior.

*pdf-1* and *pdfr-1* mutants were able to successfully mate, although they did not respond as avidly as wild-type males to mate contact (**Supplementary Fig. 1e**). Thus, PDFR-1 regulates appetitive, but not consummatory, reproductive behaviors in *C. elegans* males.

PDF-1 acts in a sexually dimorphic manner. Mate-searching behavior is a male-specific exploratory behavior[6]. However, *pdf-1* mutant hermaphrodites also display similar locomotion defects, such as increased frequency of reversals and slow locomotion[10]. We asked whether overexpression of PDF-1 from a transgene under the control of the endogenous promoter in wild-type hermaphrodites could cause them to behave similar to wild-type males in the leaving assay. In wild-type males, PDF-1 overexpression resulted in a twofold increase in the rate of leaving behavior that was dependent on the receptor, PDFR-1 (**Fig. 1e**). In contrast, PDF-1 overexpression did not cause wild-type hermaphrodites to produce leaving behavior (**Fig. 1e**), despite rescuing the locomotion defects of *pdf-1*(*tm1996*) mutant hermaphrodites (data not shown). Male-specific PDF-1–expressing neurons were not required for the production of male mate-searching behavior (see below). This indicates that PDF-1 acts in gender-shared neurons through a sexually dimorphic circuit for navigation to produce mate-searching behavior in males.

Hermaphrodites of some wild isolates of *C. elegans* explore away from food, albeit at much shorter distances than males[14]. Hermaphrodite exploration away from food is a food-searching strategy when animal density is high and food resources are depleting[15]. Hermaphrodite food-leaving behavior is associated with polymorphisms in the *npr-1* and *tyra-3* genes, which

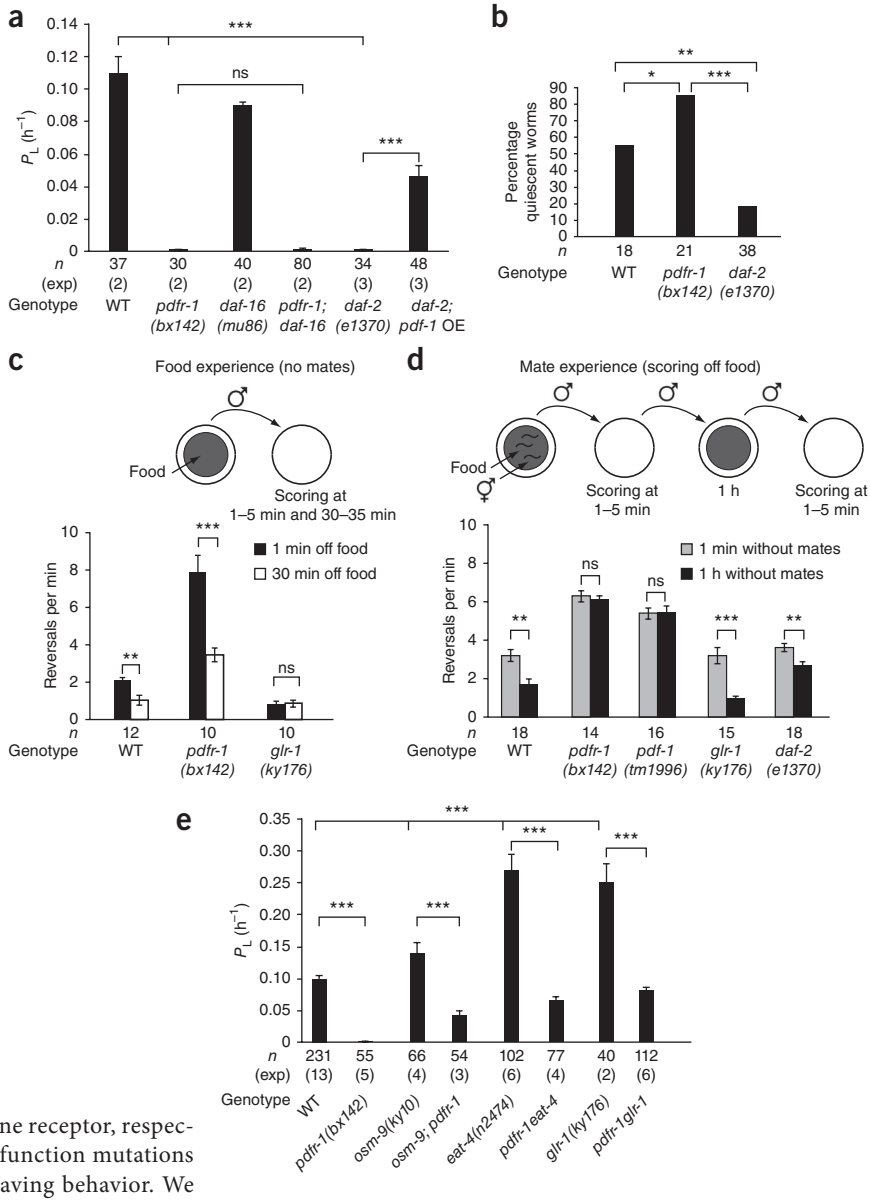


**Figure 2** The PDF-1 and PDFR-1 pathway stimulates dispersal following mate deprivation. (**a**) $P_L$ values are shown for mutants in the insulin pathway. *daf-16* encodes a forkhead transcription factor inhibited by insulin signaling and *daf-2* encodes an insulin receptor. Error bars indicate s.e.m. *n* indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Maximum likelihood statistical analysis was used to compare $P_L$ values, $^{***}P < 0.001$; ns, no statistically significant difference ($P \geq 0.05$). (**b**) Percentage of quiescent worms in nutritionally high food (*E. coli* HB101). *n* indicates number of animals tested. $\chi^2$ test was used for statistical analysis. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. (**c**) Frequency of reversals (during 5 min) was measured 1 min and 30 min after removal from food. (**d**) Frequency of reversals (during 5 min) was measured off food 1 min and 1 h after removal from mates. Note that values in the first column of **c** and the second column of **d** are equivalent. Variability exists as a result of experiments being performed in different days for **c** and **d**. In **c** and **d**, *n* indicates number of animals tested, error bars indicate s.e.m., and Mann-Whitney test was used for statistical analysis. $^{***}P < 0.001$, $^{**}P < 0.01$; ns, no statistically significant difference ($P \geq 0.05$). (**e**) $P_L$ values are shown for mutants in the food-sensing pathway. *osm-9* encodes a TRPV channel expressed in amphid chemosensory neurons; *eat-4* encodes a glutamate vesicular transporter expressed in amphid neurons; *glr-1* encodes an AMPA glutamate receptor expressed in amphid interneurons. Error bars indicate s.e.m. *n* indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Maximum likelihood statistical analysis was used to compare $P_L$ values. $^{***}P < 0.001$.



encode a neuropeptide Y receptor and a tyramine receptor, respectively[14,16]. Hermaphrodites with reduction-of-function mutations in the *npr-1* gene display high rates of food-leaving behavior. We asked whether PDF-1 overexpression could increase the levels of exploration in wild-type hermaphrodites in the hermaphrodite food-leaving assay[14]. In contrast with *npr-1* mutant hermaphrodites, hermaphrodites overexpressing PDF-1 did not significantly increase exploration levels ($P \geq 0.05$; **Supplementary Fig. 1f**). This indicates that the PDF-1 and PDFR-1 pathway functions instructively specifically in males to produce exploration away from food. Furthermore, although reduction-in-function mutations in *npr-1* had marked effects on hermaphrodite food-leaving behavior, they had no significant effects on male mate-searching behavior ($P \geq 0.05$; **Fig. 1e**). Taken together, these data are consistent with the idea that food-leaving behavior in males and hermaphrodites are two distinct exploratory behaviors with different genetic contributions.

### The PDFR-1 pathway does not signal nutritional status

The decision to explore away from food depends on two competing needs, feeding and reproduction. A period of starvation suppresses mate-searching behavior[6]. Thus, the lack of mate-searching behavior in *pdfr-1* mutant males may reflect defects in a pathway that signals the nutritional status of the animal or a pathway that signals reproductive drive. In vertebrates, orthologs of PDFR-1 have an important role regulating energy homeostasis and insulin secretion[17]. Furthermore, mate-searching behavior is regulated by internal signals indicating the nutritional status of the animal and mediated in part through insulin signaling[6]. DAF-2 is the main insulin receptor in *C. elegans*[18] and like *pdfr-1* mutants, *daf-2*(*e1370*) mutant males are severely defective in mate searching behavior[6] (**Fig. 2a**). DAF-2 acts via inactivation of the forkhead transcription factor DAF-16 (ref. 19). Consistent with this, the mate-searching defects of *daf-2* mutant males are suppressed by *daf-16* loss-of-function mutations[6]. If *pdfr-1* exerts its effect on mate-searching behavior through the regulation of the DAF-2/DAF-16 insulin pathway, then we would expect the *daf-16* (*mu86*) null mutation to suppress the phenotype of *pdfr-1*(*bx142*) mutants. However, *pdfr-1*(*bx142*); *daf-16*(*mu86*) double mutants displayed the same mate-searching defects as *pdfr-1*(*bx142*) single mutants (**Fig. 2a**). Moreover, overexpression of the ligand PDF-1 in *daf-2*(*e1370*) mutants can rescue the mate searching defects of *daf-2* mutants (**Fig. 2a**). These results indicate that the PDF-1 and PDFR-1 pathway acts downstream or independently of the DAF-2 and DAF-16 insulin pathway to regulate mate-searching behavior.

Two pieces of evidence support a model in which PDFR-1 and DAF-2 act in different pathways (reproductive drive and nutritional status, respectively) to regulate mate-searching behavior. First, unlike *daf-2* mutants, *pdfr-1* mutants underwent behavioral quiescence in nutritionally high food (**Fig. 2b**), a behavior that is associated with nutritional satiety and is characterized by the cessation of both movement and pharyngeal pumping[20]. Thus, unlike *daf-2* mutants, *pdfr-1* mutants reached satiety and did not appear to be nutritionally deprived. Second, *daf-2* mutant males, similar to wild-type males, modulated their patterns of locomotion on the basis of previous experience with a mate (see below). In contrast, *pdfr-1* mutant males did not modulate their patterns of locomotion after mate deprivation (see below). Thus, although nutritional status affects the levels of reproductive drive, our data does not support a role for PDFR-1 in the signaling of overall nutritional state. Instead, our results are most consistent with a role for PDFR-1 in the signaling of reproductive drive.

### PDF-1 signaling limits local search following mate deprivation

*C. elegans* explores its environment in an optimal manner by modulating locomotion patterns in response to prior sensory experience. Hermaphrodites respond to loss of contact with a food source by increasing reversal frequency and turns, a behavior known as ARS (area-restricted search)[21–23]. ARS behavior tends to bring them back to the food. In hermaphrodites, ARS behavior depends on glutamate signaling from food-sensing chemosensory neurons and the glutamate receptor GLR-1 (refs. 21,24). After approximately 30 min, frequency of reversals and turns decreases, allowing dispersal in search of a new food source[23].

Previously, we found that the presence of mates affects the behavior of males at the food edge, inhibiting them from fully exiting the lawn. This modulation of exploration is an important component of the behavioral effect on males that is induced by contact experience with a mate[7]. We examined whether loss of a food source with or without mates induced the expression of ARS behavior in males. We also asked whether the PDF-1 and PDFR-1 pathway modulated the response to loss of a source of food or loss of a source of mates.

Following removal from a food source without mates, males expressed ARS behavior initially and began to disperse after 30 min off food (**Fig. 2c**). In contrast, *glr-1* mutant males did not display ARS behavior following removal from food and dispersed immediately, indicating that the response of males to the loss of a food source is regulated similarly to that of hermaphrodites[21]. Although *pdfr-1* mutant males displayed higher absolute levels of reversals than wild-type males, reversals decreased twofold by 30 min after the last food exposure, as in wild-type males (**Fig. 2c**). This indicates that PDFR-1 does not function in the pathway that modulates reversal frequency in response to food experience and that *pdfr-1* mutants can reduce reversals.

Following removal of a male from a food lawn with mates, the frequency of reversals was enhanced twofold compared with removal from a lawn without mates (**Fig. 2d**). Thus, contact-experience with a mate enhanced ARS. The frequency of reversals decreased 1 h after last experience with a mate, and this decrease required the PDF-1 and PDFR-1 pathway (**Fig. 2d**). In contrast, reduction of ARS behavior following mate deprivation did not require either the glutamate pathway (*glr-1* background) or the insulin signaling pathway (*daf-2* background). Thus, PDF-1 signaling functioned specifically to modulate ARS in response to mate experience, whereas components of the food-response pathway were not required for this modulation.

We conclude that PDFR-1 and GLR-1 act in parallel pathways to regulate ARS and dispersal in response to sensory experience: GLR-1 promotes reversals following recent food experience and PDFR-1 suppresses reversals following mate deprivation. The relative contribution of each pathway to a distributed navigation circuit will determine the ability of males to leave food and explore in search of mates. Consistent with this interpretation, males with mutations in the food-searching pathway had a higher tendency to explore away from food than did wild-type males, and their levels of exploration were dependent on the function of PDFR-1 (**Fig. 2e**).

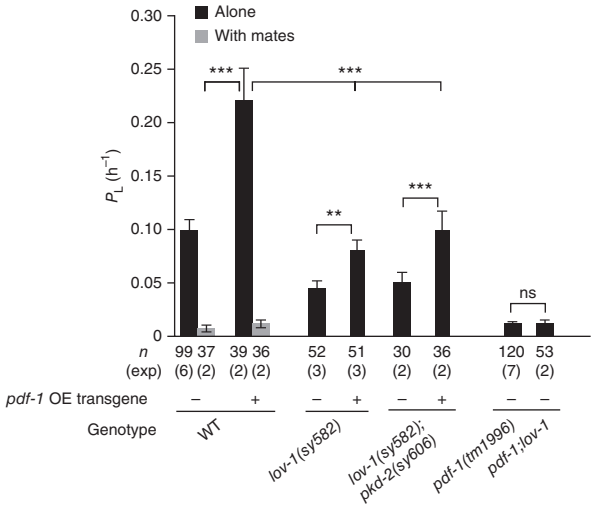


**Figure 3** Mate sensation and ray activity modulate the effects of PDF-1 signaling. The effects on mate-searching behavior ($P_L$ values) of *pdf-1* overexpression in wild-type males with and without mates and in mutants with defects in B-type ray neuron function are shown. *lov-1* and *pkd-2* encode TRP polycystins required for ray neuron function. Error bars indicate s.e.m. *n* indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Maximum likelihood statistical analysis was used to compare $P_L$ values. $^{***}P < 0.001$, $^{**}P < 0.01$; ns, no statistically significant difference ($P \geq 0.05$).

### Mate-sensing neurons modulate the effects of PDF signaling

Wild-type males that have recently been in contact with a mate do not display mate-searching behavior[6,7] (**Fig. 3**). Introduction of a *pdf-1* transgene into wild-type males resulted in a twofold increase in mate-searching activity that was completely suppressed by the presence of mates (**Fig. 3**). This indicates that previous experience with a mate completely inhibits the effects of PDF-1 signaling and further supports the idea that PDF-1 acts in the circuit that signals reproductive drive after mate deprivation.

Mate-sensing male ray neurons stimulate mate-searching behavior by reducing reversals on exiting food after mate deprivation[7]. We asked whether ray neurons modulate the effects of PDF-1 signaling on mate-searching behavior. We first tested whether ray neurons were required for increased levels of mate-searching behavior produced by PDF-1 overexpression. To disrupt the function of a subset of ray neurons (B-type ray neurons), we used mutations in the TRP polycystin genes *lov-1* and *pkd-2* (refs. 25,26). Disruption of B-type ray neurons reduced the levels of mate-searching behavior produced by the *pdf-1* transgene, although not to the levels of *lov-1* and *lov-1; pkd-2* mutants (**Fig. 3**). Thus, PDF-1 signaling has B-type ray neuron–dependent and B-type ray neuron–independent effects on mate-searching behavior.

Reducing ray neuron function in *pdf-1* mutants by introducing the *lov-1* mutation did not enhance the defects in mate-searching behavior (**Fig. 3**). Together, these results are consistent with the interpretation that PDF-1 and PDFR-1 signaling sets the level of reproductive

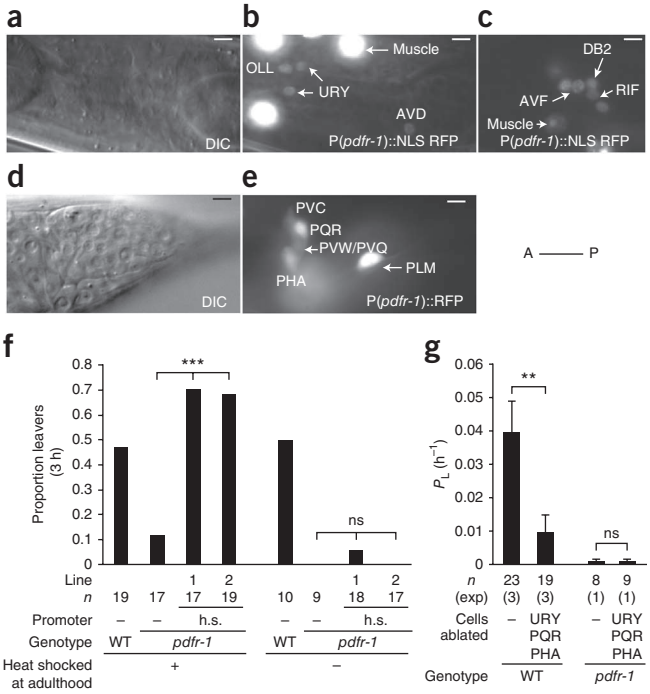


**Figure 4** *pdfr-1* is required in sensory neurons URY, PQR and PHA to produce mate-searching behavior. (**a**–**e**) Expression of P(*pdfr-1*)::*RFP* in males at the L4 stage. Differential interference contrast (DIC) images (**a**,**d**) and fluorescence images (**b**,**c**,**e**) are shown. Neurons in the head (**a**,**b**) and the retro-vesicular ganglion (**c**) were labeled with a *pdfr-1*::NLS RFP reporter. Neurons in the tail were labeled with a *pdfr-1*::RFP reporter (**d**,**e**). Anterior is to the left in all images. Lateral views are shown in **a**,**b**,**d** and **e** and a ventral view is shown in **c**. Scale bars represent 5 µm. A, anterior; P, posterior. (**f**) Rescue of mate-searching behavior in *pdfr-1*(*bx142*) mutants with a heat shock–inducible promoter driving isoforms b and d. Lines indicate independent arrays of the transgene. Graphs show the proportion of males that left food after 3 h of leaving assay. *n* indicates number of animals tested; $\chi^2$ test was used for statistical analysis. $^{***}P < 0.001$; ns, no statistically significant difference ($P \geq 0.05$). (**g**) Effects of laser ablation of URY, PQR and PHA on mate-searching behavior in wild-type and *pdfr-1*(*bx142*) males. The rate of leaving in non-ablated control wild-type males was slightly reduced by the presence of the transgenes used to identify the target neurons. Error bars indicate s.e.m. *n* indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Maximum likelihood statistical analysis was used to compare $P_L$ values. $^{**}P < 0.01$; ns, no statistically significant difference ($P \geq 0.05$).

drive for mate-searching behavior, which is modulated by the activity of ray neurons and mate sensation. In a context in which the drive to explore is low, such as in *pdf-1* or *pdfr-1* mutants, ray function becomes irrelevant.

## PDFR-1 acts in gender-shared sensory neurons

To determine the site of action of PDFR-1, we generated a variety of transcriptional reporters and rescue constructs. Transcriptional reporters containing 3 kb of the endogenous promoter upstream of the translational start site were expressed in neurons and body wall muscle (**Fig. 4a**–**e**) and were similarly expressed in both males and hermaphrodites. Colocalization with other reporters and anatomical criteria enabled identification of the expressing neurons as the ciliated sensory neurons OLL, PHA and PQR, the nonciliated sensory neurons URY and URX, the touch receptor neurons ALM, PLM, AVM and PVM, the interneurons in the retro-vesicular ganglion RIF and AVF, the command interneurons AVD and PVC, the ring motor neurons RMED and RMEV, and two other neurons tentatively identified as either PVQ or PVW and DB2 (**Fig. 4a**–**e**). No expression was observed in amphid or male-specific neurons.

The defects in mate-searching behavior were fully rescued with constructs that express *pdfr-1* cDNAs under the control of the 3-kb *pdfr-1* promoter. Three different isoforms (a, b and c) encoded by the *pdfr-1* locus have been identified[9,10]. From a mixed stage and mixed gender mRNA pool we isolated five isoforms: a, b and c, and two new isoforms, d and e. The five isoforms differed in their extracellular and intracellular domains (**Supplementary Fig. 2a**). Simultaneous expression of both isoforms b and d under the 3-kb *pdfr-1* promoter fully rescued the defects in mate-searching behavior of *pdfr-1* mutants (**Table 1** and **Supplementary Fig. 2b**). Having obtained full rescue

**Table 1 Rescue and expression of transgenes driving *pdfr-1* isoforms b and d under different promoters**

| | *pdfr-1*–positive cells | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Promoter | Muscle | OLL | URY | RMED/V | URX | AVD | TRN | RIF | AVF | DB2 | PVC | PQR | PHA | PVW/PVQ | Other neurons | Rescue | Lines |
| *pdfr-1* (3 kb) | + | + | + | + | + | + | + | + | + | + | + | + | + | + | – | +++ | 4/4 |
| *rab-3* | – | + | + | + | + | + | + | + | + | + | + | + | + | + | + | +++ | 2/4 |
| *pdfr-1* (2 kb proximal) | + | – | + | ± | ± | – | + | – | + | – | – | ± | ± | ± | – | ++ | 2/7 |
| *glr-4* | – | – | + | – | – | – | – | – | – | ? | – | + | – | – | + | +++ | 4/4 |
| *npr-1* | – | – | – | – | + | – | – | – | – | – | – | + | + | – | + | +++ | 3/4 |
| *pdfr-1* (1 kb prox) + *osm-6* | + | + | + | – | – | – | – | – | – | – | – | + | + | – | + | ++ | 2/2 |
| *osm-6* | – | + | – | – | – | – | – | – | – | – | – | + | + | – | + | + | 1/5 |
| *pdfr-1* (1 kb proximal) | + | – | + | – | – | – | – | – | – | – | – | – | – | – | – | – | 5/5 |
| *pdfr-1* (1 kb medial) | – | + | – | + | ± | + | + | – | + | – | + | – | – | + | – | – | 4/4 |
| *pdfr-1* (1 kb distal) | – | + | – | – | + | + | + | + | – | ± | – | – | – | – | – | – | 5/5 |
| *gcy-32* | – | – | – | – | + | – | – | – | – | – | – | + | – | – | + | – | 5/5 |
| *pdfr-1* (1 kb prox) + *gcy-32* | + | – | + | – | + | – | – | – | – | – | – | + | – | – | + | – | 4/4 |
| *glr-1* | – | – | + | – | – | + | – | – | – | – | + | – | – | ? | + | – | 4/4 |
| *eat-4* | – | + | – | – | – | – | + | – | – | – | – | – | – | ? | + | – | 4/4 |
| *tol-1* | – | – | + | – | – | – | + | ? | ? | – | – | – | – | – | ? | – | 3/3 |
| *mec-17* | – | – | – | – | – | – | + | – | – | – | – | – | – | – | – | – | 4/4 |
| *unc-7* + *unc-4* | – | – | – | – | – | – | – | + | + | ? | – | – | – | – | + | – | 4/4 |

+ and – indicate positive or negative expression in that cell, respectively; ± indicates variable expression. The number of + symbols indicates the levels of rescue: +++ indicates mate-searching/probability of leaving ($P_L$) not significantly different from wild type ($P_L = 0.1$, $P \geq 0.05$); ++ indicates $0.1 > P_L > 0.045$; + indicates $P_L = 0.025$; – indicates $P_L < 0.025$. The proportion of lines that displayed the levels of $P_L$ assigned is indicated. Expression pattern of heterologous reporters was observed in males as reported: *rab-3* (ref. 38), *glr-4* (see Online Methods for promoter), *npr-1* (ref. 39), *osm-6* (ref. 40), *gcy-32* (ref. 41), *glr-1* (ref. 42), *eat-4* (ref. 43), *tol-1* (ref. 44), *mec-17* (ref. 45), *unc-7b* (ref. 46), *unc-4* (ref. 47).

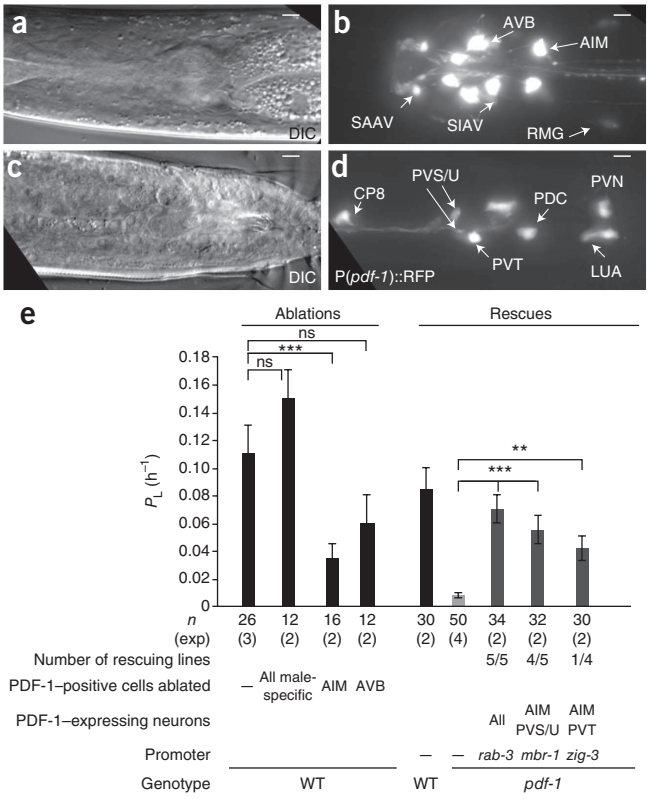


**Figure 5** The interneuron AIM is a source of PDF-1 for mate-searching behavior. (**a**–**d**) Expression of P(*pdf-1*)::RFP in adult males. DIC images (**a**,**c**) and fluorescence images (**b**,**d**) are shown of neurons in the head (**b**) and the tail and posterior ventral cord (**d**). All images show a ventral view, anterior to the left. Scale bars represent 5 µm. (**e**) Effects on mate-searching behavior ($P_L$ values) of ablation of all PDF-1–expressing male-specific neurons, AVB or AIM in wild-type males. Rescue of mate-searching behavior in *pdf-1*(*tm1996*) mutants by expression of a *pdf-1* cDNA in AIM. Error bars indicate s.e.m. *n* indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Maximum likelihood statistical analysis was used to compare $P_L$ values. $^{***}P < 0.001$, $^{**}P < 0.01$; ns, no statistically significant difference ($P \geq 0.05$).

with isoforms b and d together, we did not test any other isoform combinations, and all subsequent rescue experiments were performed with both the b and d isoforms of *pdfr-1*.

To test whether PDFR-1 is required during development or in adulthood, we drove expression of *pdfr-1* cDNA under a heat shock–inducible promoter[27]. Induction of the heat-shock promoter at adulthood fully rescued the mate-searching defects in both of the lines that we tested, indicating that PDFR-1 is not required during development for male exploratory behavior (**Fig. 4f**). The rescuing effects produced by heat-shock induction were extinguished after 5 h, indicating that PDFR-1 needs to be regularly produced in the adult to stimulate mate-searching behavior.

Expression of *pdfr-1* pan-neuronally (with a *rab-3* promoter) restored mate-searching behavior in *pdfr-1* mutants (**Table 1** and **Supplementary Fig. 2b**). In contrast, expression in muscle (with a 1-kb proximal *pdfr-1* promoter region) did not (**Table 1** and **Supplementary Fig. 2b**). Together, these results indicate that PDFR-1 functions in neurons in the adult male to produce mate-searching behavior.

To determine which of the PDFR-1–expressing neurons are responsible for the production of mate searching, we performed a series of rescue experiments with smaller regions of the *pdfr-1* promoter and with heterologous promoters (**Table 1** and **Supplementary Fig. 2b**). We found that *pdfr-1* was required in three classes of neurons, URY (four neurons), PQR (one neuron) and PHA (two neurons), to elicit reproductive drive (**Table 1** and **Supplementary Fig. 2b**). To confirm this hypothesis, we restored PDFR-1 expression in all three classes of neurons by crossing two arrays that individually produced a minimal rescue of mate-searching behavior: the *pdfr-1* 1-kb minimal promoter (URY) and the *osm-6* promoter (PQR, PHA, OLL and other *pdfr-1*–negative ciliated neurons). Mate-searching behavior was significantly rescued in *pdfr-1* mutants ($P < 0.01$; **Table 1** and **Supplementary Fig. 2b**). These results implicate the sensory neurons URY, PQR (which sense the internal environment of the animal) and PHA (which is exposed to the outside) as part of the circuit in which PDFR-1 functions for the production of mate-searching behavior.

Laser ablation of all three classes of neurons, URY, PQR and PHA, significantly decreased mate-searching behavior in wild-type males ($P < 0.01$; **Fig. 4g**). These results indicate that URY, PQR and PHA neurons are necessary for the production of wild-type levels of mate-searching behavior and suggest that PDFR-1 stimulates mate-searching behavior through positive regulation of the activity of these neurons.

### The interneuron pair AIM is a source of PDF-1

To determine the relevant source of the ligand PDF-1 for mate searching, we expressed a *pdf-1* genomic fragment or a *pdf-1* cDNA under an endogenous promoter containing the 3.4 kb upstream of the translational start site. Both of these constructs fully rescued the mate-searching defects of *pdf-1* mutants (**Supplementary Fig. 3**). This 3.4-kb promoter region drove expression in both sexes in the head interneurons SAAV, SAAD, SIAV, AVB, AIM, RMG and two other unidentified ciliated neurons (possibly ASK and AFD; **Fig. 5a**,**b**). In the tail, *pdf-1* was consistently expressed in interneurons PVN, LUA, PVT, and the pair PVS and PVU (referred to as PVP left and right in the hermaphrodite; **Fig. 5c**,**d**). We also observed expression in male-specific neurons (ventral cord neurons CP7 and CP8, and PDC) and in one unidentified interneuron, possibly PVX, in the pre-anal ganglion (**Fig. 5c**,**d**).

PDF-1 acts instructively in males, but not in hermaphrodites, to produce mate-searching behavior (**Fig. 1e**). Ablation of all *pdf-1*–positive male-specific neurons in wild-type males with a laser micro-beam did not cause any significant reduction in mate-searching behavior ($P \geq 0.05$; **Fig. 5e**). Thus, male-specific neurons are not a necessary source of neuropeptide secretion for the stimulation of mate-searching behavior. In contrast, ablation of the AIM pair resulted in a significant reduction in mate searching, indicating that AIM interneurons are a component of the mate-searching circuit ($P < 0.001$; **Fig. 5e**). Expression of *pdf-1* in AIM and PVS/U (and other *pdf-1*–negative neurons) with the *mbr-1* promoter[28] or in AIM and PVT (and other *pdf-1*–negative neurons) with the *zig-3* promoter[29] restored mate-searching behavior in *pdf-1* mutants (**Fig. 5e** and **Supplementary Fig. 3**). These results indicate that AIM is an important source of PDF-1 release for the stimulation of mate-searching behavior.

Taken together, our results indicate that the PDF-1 and PDFR-1 pathway acts in a sexually dimorphic manner, yet functions in neurons that are present in both males and hermaphrodites. This indicates that the PDF-1 and PDFR-1 circuit is dimorphic in the functional properties of the neurons and/or in their connectivity.

## DISCUSSION

Our results reveal an evolutionarily conserved neuropeptide system as a major regulator of reproductive drive in *C. elegans*. The risky

decision to explore away from a food source in search of mates depends on the balance of two competing drives, feeding and reproduction. Mates and food are sensed through two independent sensory circuits with input to a distributed network for exploration. Our results indicate that the lack of mate-searching behavior in *pdfr-1* mutants reflects a reduction in the reproductive drive and corresponding increase in the relative contribution of the food-searching circuit to the network for exploration. *pdfr-1* did not appear to function in the food-searching circuit. First, *pdfr-1* mutants did not act as though they were nutritionally deprived and, similar to wild-type animals, reached satiety in nutritionally high food (**Fig. 2b**). Second, *pdfr-1* mutants modulated their locomotion in response to previous food experience (**Fig. 2c**). Third, *pdfr-1* expression in the food-sensing circuit with the *glr-1* or *osm-6* promoters was not sufficient to restore mate-searching behavior (**Table 1**). *pdfr-1* was required in sensory neurons exposed to the internal environment of the animal, URY and PQR, and a ciliated sensory neuron exposed to the outside, PHA. Thus, the PDF-1 and PDFR-1 system appears to modulate a circuit that senses the internal state of the male and antagonizes the food-sensing circuit. This is consistent with a role for neuropeptides as molecular indicators of internal states and modulators of circuit properties and contributions[30].

Gender may be considered another dimension of internal state that shapes behavioral decision making. Unlike their hermaphrodite counterparts, *C. elegans* males must find a mate to reproduce. As with other physiological needs, reproduction is subject to homeostatic regulation, with the drive to reproduce increasing after deprivation and decreasing after experience[31]. The PDF-1 and PDFR-1 pathway is an important component of the drive for mate-searching behavior after mate deprivation. *pdf-1* and *pdfr-1* mutants did not produce mate-searching behavior and were unable to modulate their locomotion to disperse after mate deprivation (**Figs. 1b** and **2d**). The effects of PDF-1 signaling were regulated by the activity of mate-sensing ray neurons and were completely suppressed by previous experience with a mate (**Fig. 3**). Our results indicate that the neural circuit for mate-searching behavior that conveys the reproductive drive to explore is regulated by internal state-sensing neurons, modulated by the PDF-1 and PDFR-1 pathway, and mate-sensing ray neurons. Mate sensation may block the effects of PDF-1 signaling by regulating the secretion of the neuropeptide ligand or by modulating the activity of the receptor-expressing neurons or downstream targets. Determining the exact mechanism awaits further studies.

The PDF-1 and PDFR-1 pathway acts in non–sex-specific neurons to produce a male-specific behavior. The sexual dimorphism may lie in the intrinsic functional characteristics of the *pdf-1*– and *pdfr-1*–expressing neurons or in their connectivity. Indeed, there is evidence for dimorphism in both molecular expression and connectivity in circuits shared by both males and hermaphrodites[32,33]. The wiring diagram of connectivity of the *C. elegans* male indicates that dimorphic synaptic connections are extensive throughout the shared nervous system and suggests possible sites for integration of the *pdfr-1* circuit in the navigation network[33]. Both PHA and PQR synapse directly to AVA, the command interneuron responsible for backward movement. Another possible site of integration are the male-specific EF interneurons, which are postsynaptic to PQR, PHA and ray neurons, and are required for the suppression of mate-searching behavior by the presence of mates[7]. The connectivity of URY and AIM in the male is not known.

Our results open new avenues for the study of the secretin family of neuropeptides in circuits regulating appetitive reproductive behaviors. We found that the PDF-1 and PDFR-1 pathway is an important component of the reproductive drive for mate searching. A role for PDF-1 in the regulation of internal drives appears to be phylogenetically conserved. PDF regulates male sex drive in *Drosophila*[34]. In the monarch butterfly, the circadian pacemaker system, which is regulated by PDF, is involved in the induction of the migratory state[3]. In humans, members of the secretin family of neuropeptide receptors, orthologs of *pdfr-1* (ref. 35), have been associated with disorders affecting motivation such as bipolar disorder[36] and post-traumatic stress syndrome[37]. Determining how the PDF-1 and PDFR-1 system modulates neuronal physiology and circuit properties will reveal the mechanisms by which neuropeptides shape drives and behavioral decision making.

## METHODS

Methods and any associated references are available in the online version of the paper.

*Note: Supplementary information is available in the online version of the paper.*


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### AUTHOR CONTRIBUTIONS

A.B. designed and performed the experiments and co-wrote the manuscript. R.G. performed the genetic screen. C.F. contributed to mapping process. S.W.E. and M.M.B. co-wrote and discussed the manuscript with A.B.

### COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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## ONLINE METHODS

**Strains used.** CB1490, *him-5(e1490)*, was used as the wild-type strain for males. Bristol N2 was used for wild-type hermaphrodites and *unc-51(e369)* hermaphrodites were used for retention experiments. EM938, *pdfr-1 (bx142);him-5(e1490)*; PT2248, *pdf-1(tm1996);him-5(e1490)*; PT2159, *pdf-1 (tm1996);pdf-2(tm4393);him-5(1490)*; PT2177, *pdf-2(tm4393);him-5(e1490)*; PT2547 *pdfr-1(tm4457);him-5(e1490)*; PT2367,*myEx721*[*P(pdf-1)3kb ::pdf-1genomic + P(unc-122) ::gfp*]; PT2422, *myEx721*[*P(pdf-1)3kb ::pdf-1genomic + P(unc-122) ::gfp*];*him-5(e1490)*; PT2364, *npr-1(n1353);him-5(e1490)*; DA508, *npr-1(n1353)*; PT2247, *glr-1(ky176);him-5(e1490)*; PT2176, *dpy-17 (e164) pdfr-1(bx142); him-5(e1490)*; PT2246, *dpy-17(e164)pdfr-1(bx142)glr-1 (ky176);him-5(e1490)*; PT2260, *him-5(e1490);akIs9*[*Pglr-1:glr-1*(A687T)]; PT2548, *daf-16(mu86);him-5(e1490)*; PT2549, *daf-16(mu86);pdfr-1(bx142)*; EM814, *daf-2(e1370);him-5(e1490)*; PT2501, *daf-2(e1370);myEx721*[*P(pdf-1) 3kb ::pdf-1genomic + P(unc-122) ::gfp*];*him-5(e1490)*; PT2350, *egl-4 (n479);him-5(e1490)*; PT839, *osm-9(ky10);him-5(e1490)*; PT2300, *osm-9 (ky10);pdfr-1(bx142);him-5(e1490)*; PT2193, *eat-4(n2474);him-5(e1490)*; PT2195, *dpy-17(e164)eat-4(n2474)pdfr-1(bx142);him-5(e1490)*; PT2479, *pkd-2(sy606);lov-1(sy582);him-5(e1490);myEx721*[*P(pdf-1)3kb ::pdf-1genomic + P(unc-122) ::gfp*]; PT2480, *pdf-1(tm1996);lov-1(sy582);him-5(e1490)*; PT2481, *lov-1(sy582);him-5(e1490);myEx721*[*P(pdf-1)3kb ::pdf-1genomic + P(unc-122) ::gfp*]; PT2417, *pdfr-1(bx142);him-5(e1490);myEx768*[*P(hsp-16.2) ::pdfr-1cDNA isof b+P(hsp-16.2) ::pdfr-1cDNA isof d + unc-122 :: gfp*]; PT2315, *him-5(e1490); myEx731*[*P(pdfr-1)3kb ::rfp + P(unc-122) :: gfp*]; PT2351, *him-5(e1490);myEx741*[*P(pdfr-1)3kb ::nls rfp + P(unc-122) ::gfp*]; PT2294, *pdf-1(tm1996);him-5(e1490);myEx721*[*P(pdf-1)3kb ::pdf-1genomic + P(unc-122) ::gfp*]; PT2249, *him-5(e1490);myEx696*[*P(pdf-1)3kb ::rfp + P(unc-122) ::gfp*]; PT2565, *IqIs3(osm-6 ::GFP); him-5(e1490)*; PT2493, *pdf-1 (tm1996);him-5(e1490);myEx783*[*P(flp-10) ::pdf-1cDNA) + P(unc-122) ::rfp*]; PT2504, *pdf-1(tm1996);him-5(e1490);myEx786*[*P(pdf-1)3kb ::pdf-1cDNA) + P(unc-122) ::rfp*]; PT2282, *pdfr-1(bx142);him-5(e1490);myEx709*[*P(pdfr-1) 3kb ::pdfr-1 cDNA isof b + P(pdfr-1)3kb ::pdfr-1 cDNA isof d + P(unc-122) ::gfp*]; PT2427, *pdfr-1(bx142)*;him-5(e1490);*myEx769*[*P(pdfr-1)2kb ::pdfr-1 cDNA isof b + P(pdfr-1)2kb ::pdfr-1 cDNA isof d +P(unc-122) ::gfp*]; PT2459, *pdfr-1 (bx142);him-5(e1490);myEx774*[*P(pdfr-1)1kb distal ::pdfr-1cDNA isof b + P(pdfr-1)1kb distal ::pdfr-1cDNA isof d + unc-122 ::gfp*]; PT2460, *him-5 (e1490);myEx775*[*P(pdfr-1)1kb distal ::nls rfp tag + unc-122 ::gfp*]; PT2471, *pdfr-1(bx142);him-5(e1490);myEx776*[*P(pdfr-1)1kb medial ::pdfr-1cDNA isof b + P(pdfr-1)1kb medial ::pdfr-1cDNA isof d + unc-122 ::gfp*]; PT2472, *him-5(e1490);myEx777*[*P(pdfr-1)1kb medial ::nls rfp + unc-122 ::gfp*]; PT2473, *pdfr-1(bx142);him-5(e1490);myEx778*[*P(pdfr-1)1kb proximal ::pdfr-1cDNA isof b + P(pdfr-1)1kb proximal ::pdfr-1cDNA isof d + unc-122 ::rfp*]; PT2474, *him-5(e1490);myEx779*[*P(pdfr-1)1kb proximal ::nls rfp + unc-122 ::gfp*]; PT2356, *pdfr-1(bx142);him-5(e1490);myEx745*[*P(osm-6) ::pdfr-1cDNA isof b + P(osm-6) ::pdfr-1cDNA isof d + unc-122 ::gfp*]; PT2141, *pdfr-1(bx142); him-5 (e1490) V;myEx694*[*P(rab-3) ::pdfr-1cDNA isof b + P(rab-3) ::pdfr-1cDNA isof d + P(unc-122) ::gfp*]; PT2346, *pdfr-1(bx142);him-5(e1490);myEx739*[*P(mec-17) ::pdfr-1cDNA isof b + P(mec-17) ::pdfr-1cDNA isof d + P(unc-122) ::gfp*]; PT2358, *pdfr-1(bx142);him-5(e1490);myEx747*[*P(eat-4) ::pdfr-1cDNA isof b + P(eat-4) ::pdfr-1cDNA isof d + P(unc-122) ::gfp*]; PT2373, *pdfr-1 (bx142);him-5(e1490);myEx751*[*P(glr-1) ::pdfr-1 cDNA isof b + Pglr-1 ::isof d + P(unc-122) ::gfp*]; PT2416, *pdfr-1(bx142);him-5(e1490);myEx767*[*P(unc-4) :: pdfr-1cDNA isof b + P(unc-4) ::pdfr-1cDNA isof d + P(unc-7b) ::pdfr-1cDNA b + P(unc-7b) ::pdfr-1cDNA isof d + P(unc-122) ::gfp*]; PT2475, *pdfr-1 (bx142);him-5(e1490);myEx780*[*P(gcy-32) ::pdfr-1cDNA isof b + P(gcy-32) :: pdfr-1cDNA isof d + P(unc-122) ::gfp*]; PT2494, *pdfr-1(bx142);him-5 (e1490);myEx785*[*P(tol-1)5kb ::pdfr-1cDNA isof b + P(tol-1)5kb ::pdfr-1cDNA isof d + P(unc-122) ::rfp*].

**Mapping.** The *bx142* mutation was placed on chromosome III between *dpy-17* and *unc-32* using single-nucleotide polymorphisms[11] and two-point genetic mapping. The recessive *bx142* mutation was crossed to deficiency strains covering the region between *dpy-17* and *unc-32*. The F1 offspring were tested in the leaving assay. The proportion of animals with the Las(*bx142*) phenotype was scored. A 0.5 proportion of Las animals in the F1 offspring indicated no complementation. For deficiency strains, we used BC4638, *dpy-17(e164) sDF127(s2428)unc-32(e189)III;sDp3(III,f)*; BC4634, *dpy-17(e164) sDF125(s2424)unc-32(e189)III;sDp3(III,f)*; CX2914, *nDf16/ dpy-17(e164)unc-32(e189)III*.

A pool of fosmids containing the genomic region between the two genes with known physical positions deleted in *sDF125* (left limit, *F23F12.3*) and *nDf16* (right limit, *odr-4*) was injected into *bx142* mutants (three of five lines rescued). Complementation tests with available mutants of genes in that region (*acr-5*, *oig-1*, *acr-21*) and sequencing (*srxa-5*, *srh-40*, *rbf-1*, *pdfr-1*) revealed a mutation in the coding region of *pdfr-1*. The *bx142* was subsequently rescued by injection of the genomic fragment containing the *pdfr-1* locus.

**DNA constructs.** cDNAs were isolated by reverse transcription PCR from a mixed gender and mixed stage mRNA pool and gene-specific primers. For *pdfr-1* cDNAs, we used 5′ ctgactcatatcatggcggatg 3′ and 5′ tgacacggtgggtttgacaac 3′ or 5′ ttatggagattttgtggagcgattgg 3′. The 3′ UTR of the *unc-54* gene was used in the *pdfr-1* cDNA constructs. For *pdf-1* cDNA, we used 5′ atgaacagattcatcatttc 3′ and 5′ aacatttgttatgctgaac 3′. The *pdf-1* endogenous 3′ UTR was used in the constructs. We used a starting coding site 279 nucleotides downstream from the one previously annotated in Wormbase.

The *pdf-1* genomic region was amplified with primers, 5′ aaaaggttgctttaccgagacgtg 3′ and 5′ gccacttttcacgcttcagca 3′. Rescuing and expression constructs were constructed by PCR fusion[48] and injected at 10 or 15 ng $\mu l^{-1}$, except for *P(mbr-1) ::pdf-1* and *P(zig-3) ::pdf-1*, which were injected at 50 ng $\mu l^{-1}$. The *pdfr-1* distal and medial promoter regions (which do not include the proximal region of the *pdfr-1* promoter) were built to include the minimal promoter found in pPD95.75 (indicated as capital letters in the primers): 3 kb, 5′ cactactttcagcgacct ttacttgctc 3′ and 5′ gatatgagtcagtgatgaa 3′; 2 kb, 5′ caccctccaaatgtcttgaaactga 3′ and 5′ gatatgagtcagtgatgaa 3′; 1 kb distal, 5′ cactactttcagcgacctttacttgctc 3′ and 5′TTTGGGTCCTTTGGCCAATCtcagtttcaagacatttgga 3′; 1 kb medial, 5′ ctaattacttc ggacgggtctagacgtt 3′, and 5′ TTTGGGTCCTTTGGCCAATCaccagctttattgcgtgac 3′; 1 kb proximal, 5′ cacgcaataaagctggttagggaag 3′ and 5′ gatatgagtcagtgatgaa 3′. Primers for heterologous promoters driving the *pdfr-1* cDNAs, amplified from a fosmid library, were made for *rab-3* (5′ gcgagttttgactggctttc 3′, 5′ cctgatttca ggcggcggaa 3′), *unc-4* (5′ cgacgtcgtccgagattaaagattc 3′, 5′ tttcacttttttggaagaag 3′), *eat-4* (5′ ggagttcgaaactgcagcaatga 3′, 5′ ggtttctgaaaatgatgatg 3′), *glr-4* (5′ caaaaaggc acacttgagctg 3′, 5′ gctgtgtaaaagtttagctc 3′), *npr-1* (5′ acgatctgtgtcgcgttttgatttt 3′, 5′ ttggcctatgtctgaaattt 3′), *gcy-32* (5′ gccatggtgttaatacgtcaagcaa 3′, 5′ tctataatac aatcgtgat 3′), *glr-1* (5′ actacgtggagtcgagggctca 3′, 5′ tgtgaatgtgtcagattgg 3′), *osm-6* (5′ cgcagttttcattacatttcatgggtatt 3′, 5′ agatgtatactaatgaagg 3′), *unc-7b* (5′ aagaagg gggaaagaaagcactgaa 3′, 5′ cggttcaggattgctggag 3′), *tol-1* (5′ tatgtaggttgcctgtgtgcctgt 3′, 5′ tttcgttgtgtcatgtgatc 3′), *mec-17* (5′ cttgagaggtccggcaacttgtttg 3′, 5′ cggtgaa gcagcttctc 3′) and *heat shock* (from pPD.49.78; 5′ gctatgaccatgattacgccaagc 3′, 5′ cggggatccagtgagatg 3′). Primers to amplify the *pdf-1* promoter for expression constructs were reporter 1 (5′ aaaaggttgctttaccgagacgtg 3′,5′ caacgccgagctta tcaac 3′), reporter 2 (5′ aaaaggttgctttaccgagacgtg 3′, 5′ ctcattaagattatcccacta 3′), reporter 3 (5′ aaaaggttgctttaccgagacgtg 3′, 5′ cttcactcaacgtcaacatg 3′) and reporter 4 (5′ gtgacgtcgacatgattcagaca 3′, 5′ cttcactcaacgtcaacatg 3′). Primers to amplify heterologous promoters driving *pdf-1* cDNA were *pdf-1prom2* (5′ aaaaggttgctttacc gagacgtg 3′, 5′ cttcactcaacgtcaacatg 3′), *rab-3* (5′ gcgagttttgactggctttc 3′, 5′ cctgat ttcaggcggcggaa 3′), *mbr-1* (5′ aataattgcgaaaagggcgattagc 3′, 5′ attgtattttcgtagg taca 3′) and *zig-3* (5′ gtatggatgtccaacgatgagcttg 3′, 5′ ctatattttgcatttggaaaa 3′).

**Leaving assay.** Assay was performed as described[6,7]. A population of between 10 and 20 worms (picked the night before at the L4 stage) was assayed by placing each worm individually in a Petri plate (9 cm in diameter and 10 ml of agar) in the center of a 20-µl (10-mm diameter) patch of food (*E. coli* OP50 seeded the night before). To assay retention, each individual test male was placed together with two paralyzed *unc-51(e369)* hermaphrodites. Plates were kept at 20 °C during the 24 h assay, and the proportion of males that had left the food was scored blindly at four time points. A worm was considered a leaver and left censored if at the scoring time point it had reached a distance 1 cm away from the edge of the plate (3 cm away from the food edge).

**Hermaphrodite leaving assay.** Assay was performed as described[14], with some modifications. We picked 30 L4 hermaphrodites the night before the assay onto a fresh plate. Conditioning plates (with a 70-µl food lawn) and assay plates (with a 10-µl food lawn) were seeded with a fresh culture of *E. coli* OP50 diluted in LB to

$A_{600}$ = 2.0. We placed ten young adult hermaphrodites in the conditioning plate for 30 min 90 min after seeding the plates. Seven hermaphrodites were then transferred from the conditioning plate to the assay plate and left for 1 h before recording the assay for 20 min. Leaving events were scored manually by examining the video recordings. A leaving event was scored if the whole body of the worm was outside of the bacterial lawn and did not reverse immediately to return to the food. The rate of leaving events was calculated by dividing the number of leaving events per worm per minute of assay. Experiments of each genotype were performed at least five times. Statistical analysis was performed with Kruskal Wallis test.

**Locomotion assays.** Single worm assays were performed on 5-cm plates with or without food. Worms at the L4 stage were isolated from mates the night before on a plate with food. Body bends on food were scored by eye during 3-min observation periods under a dissecting microscope.

For locomotion in response to food, reversals and omega turns for each worm were scored during 5 min on food. The worm was then transferred to a transition plate without food and immediately to a scoring plate without food where it was left for 1 min to settle. Reversals and omega turns were scored for 5 min and again 30 min later.

For locomotion in response to mates, ten males (picked at the L4 stage the night before) were placed in a plate with 20 mates on a 1-cm patch of food for 3 h. A male that was observed to have initiated the response and backing steps of mating was transferred to a transition plate without food and immediately to a scoring plate without food. The male was left to settle for 1 min and reversals were scored for 5 min. The male was subsequently transferred to a plate with food in isolation for 1 h and then transferred to a transition and scoring plate with no food, where reversals were scored again for 5 min.

Assays for response to food experience and mate experience were performed on different days. Wild-type and mutant worms were assayed in parallel. Mann-Whitney test was used for statistical analysis.

**Quiescence assay.** Assay was performed as previously described[20] with some modifications. Worms were grown in *E. coli* HB101 for several generations. Prior to the assay, about 20 worms (picked at the L4 stage the night before) were transferred to a fresh plate for 5 h before scoring lack of movement and pharyngeal pumping under a dissecting microscope. $\chi^2$ test was used for statistical analysis.

**Response efficiency assays.** Males were picked at the L4 stage the night before. Single males were then placed with 30 mates (hermaphrodites) in a 20-mm food lawn. The number of contacts with a mate until the male responded was scored for a maximum of 3 min. A good response was scored if the male placed its tail ventral down on the mate body and backed to make a turn. A bad response was scored if the male lost contact during backing to the opposite end of the mate body. Mann-Whitney test was used for statistical analysis.

**Statistical analysis.** As previously demonstrated[6], male leaving behavior was modeled with the exponential function $N(t)/N(0) = exp(-\lambda t)$. $N(0)$ is equal to the number of worms at time zero, $N(t)$ is the number of worms at time $t$ (in hours). $\lambda$ is the $P_L$ value per worm per hour. $P_L$ values for each genotype and condition were calculated using R (http://www.r-project.org/) to fit the censored data with an exponential parametric survival model, using maximum likelihood. The hazard values obtained were reported as the $P_L$ values. To estimate the $P_L$ values, s.e.m. and the 95% confidence intervals, worms from each experimental treatment were then pooled across replicas and contrasted against controls using maximum likelihood.

Non-parametric statistical tests were used for locomotion and hermaphrodite leaving assays because the data did not always follow a normal distribution. Kruskal-Wallis test was used to compare multiple groups of independent data (genotypes) and Mann-Whitney test was used to compare two groups of independent data (conditions) against each other.

**Laser ablations.** The standard protocol was used[49]. L4 or adult animals were mounted on a glass slide on a 5% agarose pad with 20 mM sodium azide as anesthetic. Animals were left to recover for 1 d and then assayed. The *IqIs3*(*osm-6::GFP*) and *myEx741*[*P*(*pdfr-1*)*3kb::nls rfp+P*(*unc-122*)*::gfp*] transgenes were used to identify URY, PQR and PHA neurons. The *myEx696*[*P*(*pdf-1*)*3kb::rfp*] transgene was used to identify AIM, AVB and male-specific *pdf-1* positive cells.

**Heat-shock induction.** Control and test worms were heat-shocked for 45 min at 37 °C, left to recover for 90 min at 20 °C and then tested in the leaving assay. Proportion of leavers was scored 3 h later.

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