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

Arantza Barrios[*1,2], Rajarshi Ghosh[3,4], Chunhui Fang[3], Scott W. Emmons[3], Maureen M. Barr[1]

[1] Dept. Genetics, Rutgers University, Piscataway, NJ08854, US
[2] Current address: University College London, Gower St, London WC1E 6BT, UK
[3] Dept. Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY10461, US
[4] Current address: Car Icahn Laboratory, Princeton University, Princeton, NJ08544, US
[*] Corresponding author: a.barrios@ucl.ac.uk; arantzabarrios@gmail.com

Abstract

Appetitive behaviors require complex decision-making, involving the integration of environmental stimuli and physiological needs. *C. elegans* mate searching is a male-specific exploratory behavior regulated by two competing needs: food versus reproductive appetite. Here we show that the Pigment Dispersing Factor Receptor (PDFR-1) modulates the circuit that encodes the male reproductive drive promoting male exploration upon mate-deprivation. PDFR-1 and its ligand PDF-1 stimulate mate searching in the male but not in the hermaphrodite. *pdf-1* is required in the gender-shared interneuron AIM and the receptor acts 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/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 plays an ancient role in regulating motivational internal states.

Introduction

Biological drives are internal physiological states that produce goal oriented behaviors which 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. Therefore, when faced with identical stimuli, animals may respond differently based on 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, i.e. 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, upon

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. Here we show that pigment dispersing factor receptor (PDFR-1), a member of the secretin family of G protein-coupled receptors, and its neuropeptide ligand PDF-1 [9,10] are major regulators of mate-searching behavior in *C. elegans*. PDF-1/PDFR-1 signaling modulates the circuit that conveys the male reproductive drive to explore. The lack of exploratory behavior by *pdfr-1* mutant males reflects an imbalance in the relative contribution of the circuits regulating a distributed neural network for exploration. Moreover, the PDF-1/PDFR-1 pathway functions in a discrete non sex-specific neuronal circuit within a sexually dimorphic neural network for navigation.

Results

PDF-1 neuropeptide signaling stimulates mate searching.

In order 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 within 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* [10]. A *pdf-1* null allele, *tm1996,* was Las, while *pdf-2(tm4393)* males displayed wild-type leaving behavior (Fig.1b).

pdfr-1 and *pdf-1* mutant males have lost their drive to explore away from a food source (Supplementary Fig. 1b). Consistent with a suppression of mate-searching behavior (i.e. male exploration away from food), *pdfr-1* mutants produce an increase in the frequency of both reversals and high angle turns (omega turns) compared to wild-type males (Fig.1c). In addition, *pdfr-1* mutant males display slow movement on food due to a decrease in the frequency of body bends (propagation of the sinusoidal wave) (Supplementary Fig.1c). The slow movement phenotype can 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 and d).

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

pdf-1 and *pdfr-1* mutants can successfully mate, although they do 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* hermaphrodites also display similar locomotion defects, such as increased frequency of reversals and slow locomotion [10]. We asked whether over-expression of *pdf-1* from a transgene under the control of the endogenous promoter (*pdf-1* OE) in wild-type

hermaphrodites could cause them to behave like wild-type males in the leaving assay. In wild-type males, *pdf-1* OE caused a two-fold increase in the rate of leaving behavior that was dependent on the receptor, *pdfr-1* (Fig.1e). In contrast, *pdf-1* OE 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 are not required for the production of male mate-searching behavior (see below Fig. 5e). 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 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* OE could increase the levels of exploration in wild-type hermaphrodites in the hermaphrodite food-leaving assay [14]. In contrast to *npr-1* mutant hermaphrodites, hermaphrodites over-expressing *pdf-1* did not significantly increase exploration levels (Supplementary Fig.1f). This indicates that the *pdf-1/pdfr-1* pathway functions instructively specifically in males to produce exploration away from food. Furthermore, although reduction-in-function mutations in *npr-1* have dramatic effects on hermaphrodite food-leaving behavior they have no significant effects on male mate-searching behavior (Fig.1e). Combined, 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* 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 [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/PDFR-1 pathway acts downstream or independently of the DAF-2/DAF-16 insulin pathway to regulate mate-searching behavior.

Two pieces of evidence support a model in which PDF-1/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 associated with nutritional satiety and 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 nutritionally deprived. Second, *daf-2* males, like wild-type males, modulated their patterns of locomotion based on previous experience with a mate (see below) (Fig. 2d). In contrast, *pdfr-1* males did not modulate their patterns of locomotion after mate deprivation (see below) (Fig. 2d). Thus, although nutritional status impacts the levels of

reproductive drive, our data does not support a role for *pdfr-1* in the signaling of overall nutritional state. Rather, our results are most consistent with a role for *pdfr-1* in the signaling of reproductive drive.

PDF-1 signaling limits local search upon 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* [21,24]. After approximately 30 minutes, frequency of reversals and turns decreases, allowing dispersal in search of a new food source [23].

We have shown previously that 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 induced by contact experience with a mate [7]. Here we examined whether loss of a source of food with or without mates induced the expression of ARS behavior in males. Second, we asked whether the *pdf-1/pdfr-1* pathway modulated the response to loss of a source of food or loss of a source of mates.

Upon removal from a food source without mates, males expressed ARS behavior initially and began to disperse after 30 minutes off food (Fig. 2c). In contrast, *glr-1* mutant males did not display ARS behavior upon removal from food and dispersed immediately showing that the response of males to loss of a food source is regulated similarly to that of hermaphrodites [21]. Although *pdfr-1* males displayed higher absolute levels of reversals compared to wild-type males, reversals were decreased two-fold after 30 minutes since 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.

Upon removal of a male from a food lawn with mates, the frequency of reversals was enhanced two-fold compared to removal from a lawn without mates (Fig 2d). Hence contact-experience with a mate enhanced ARS. The frequency of reversals decreased one hour after last experience with a mate and this decrease required the PDF-1/PDFR-1 pathway (Fig 2d). In contrast, reduction of ARS behavior upon mate-deprivation did not require neither the glutamate pathway (*glr-1* background) nor 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 *pdf-1/pdfr-1* and *glr-1* act in parallel pathways to regulate ARS and dispersal in response to sensory experience: *glr-1* promotes reversals upon recent food experience and *pdfr-1* suppresses reversals upon 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 *wild-type* males and their levels of exploration were dependent on the function of *pdfr-1* (Fig.2e).

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 two-fold 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 within the circuit that signals reproductive drive after mate deprivation.

Mate-sensing male ray neurons stimulate mate-searching behavior by reducing reversals upon 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 sub-set of ray neurons (B-type ray neurons) we used mutations in the TRP polycystin genes *lov-1* and *pkd-2* [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 both, B-type ray neuron-dependent and 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/PDFR-1 signaling sets the level of reproductive drive for mate-searching behavior, which is modulated by the activity of ray neurons and mate-sensation. In a context where 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 3kb of the endogenous promoter upstream of the translational start site show expression in neurons and body wall muscle (Fig. 4a-e) and show similar expression in both males and hermaphrodites. Co-localization with other reporters and anatomical criteria enabled identification of the expressing neurons as the ciliated sensory neurons OLL, PHA and PQR; the non-ciliated sensory neurons URY and URX; the touch receptor neurons (ALM, PLM, AVM and PVM); 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 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 3kb *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, c and two new isoforms d and e. The five isoforms differ in their extracellular and intracellular domains (Supplementary Fig.2a). Simultaneous expression of both isoforms b and d under the 3kb *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 with isoforms b and d together, we did not test any other isoform combinations and all subsequent rescue experiments were performed with both *pdfr-1* b and d isoforms.

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 lines 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 five hours, 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 1kb 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). This analysis suggested that *pdfr-1* is required in three classes of neurons, URY (4 neurons), PQR (1 neuron) and PHA (2 neurons) to elicit reproductive drive (Table 1 and Supplementary Fig.2b). To confirm this hypothesis, we restored *pdfr-1* expression in all 3 classes of neurons by crossing together two arrays that individually produced a minimal rescue of

mate-searching behavior: the *pdfr-1* 1kb minimal promoter (URY) and the *osm-6* promoter (PQR, PHA, OLL and other *pdfr-1*-negative ciliated neurons). This experiment significantly rescued mate-searching behavior in *pdfr-1* mutants (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 where *pdfr-1* functions for the production of mate-searching behavior.

Laser ablation of all three classes of neurons, URY, PQR and PHA, together, significantly decreased mate-searching behavior in wild-type males (Fig. 4g). These results indicate that URY, PQR and PHA 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 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 drives 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 (called PVP left and right in the hermaphrodite) (Fig.5c-d). We also saw expression in male specific neurons of the ventral cord CP7, CP8, in 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 (Fig. 5e). Therefore, 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 caused a significant reduction in mate searching, indicating that AIM interneurons are a component of the mate-searching circuit (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 demonstrate that the PDF-1/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/PDFR-1 circuit is dimorphic in the functional properties of the neurons and/or in their connectivity.

Discussion

Our work reveals 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 for 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* does not appear to function within the food-searching circuit. First, *pdfr-1* mutants do not act as though they are nutritionally deprived, and, like wild-type animals, reach satiety in nutritionally high food (Figure 2b). Second, *pdfr-1* mutants modulate their locomotion in response to previous food experience (Figure 2c). Third, *pdfr-1* expression in the food-sensing circuit with the *glr-1* or *osm-6* promoters is not sufficient to restore mate-searching behavior (Table 1). *pdfr-1* is 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/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 in order to reproduce. Like other physiological needs, reproduction is subject to homeostatic regulation, the drive to reproduce increasing after deprivation and decreasing after experience [31]. The *pdf-1/pdfr-1* pathway is an important component of the drive for mate-searching behavior after mate deprivation. *pdf-1* and *pdfr-1* mutants do not produce mate-searching behavior and are unable to modulate their locomotion to disperse after mate-deprivation (Figure 1b and 2d). The effects of PDF-1 signaling are regulated by the activity of mate-sensing ray neurons and are completely suppressed by previous experience with a mate (Figure 3). Our results indicate that the neural circuit for mate-searching behavior that conveys the reproductive drive to explore is under the regulation of internal state-sensing neurons, modulated by the *pdf-1/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/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/pdfr-1* neurons or in their connectivity. Indeed, there is evidence for dimorphism in both, molecular expression and connectivity within 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 within 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 post-synaptic 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 work opens new avenues for the study of the secretin family of neuropeptides in circuits regulating appetitive reproductive behaviors. We have shown that the PDF-1/PDFR-1 pathway is an important component of the reproductive drive for mate searching. A role for pigment dispersing factor 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* [35], have been associated with disorders affecting motivation such as bipolar disorder [36] and post-traumatic stress syndrome [37]. Determining how the PDF-1/PDFR-1 system modulates neuronal physiology and circuit properties will reveal the mechanisms by which neuropeptides shape drives and behavioral decision-making.

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Figure legends

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), 7 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 (*tm4457)* mutants and mutants in the PDFR-1 ligands *pdf-1(tm1996)* and *pdf-2(tm4393)*. The *pdfr-1(tm4457)* allele fails to complement *bx142* indicating that the two mutations affect the same locus. The fosmid wrm0629dH07 contains the *pdfr-1* locus. Error bars indicate SEM. 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≥0.05).

(c) Graphs show the frequency of reversals and high angle turns (Ω turns) performed by wt and *pdfr-1(bx142)* mutant males during 5 minutes. Locomotion was observed on food and at two different time points off food: 1 minute after removal from food and 30 minutes after removal from food. n indicates total number of animals tested. Error bars indicate SEM. Statistical analysis was performed using Mann-Whitney test, ***p<0.001.

(d) Graphs show the rate at which wild-type (wt) and *pdfr-1(bx142)* males travel 3.5 cm, from the starting point at the center of the plate within 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, as P_L values (probability of reaching the scoring distance). Error bars indicate SEM. 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 (probability of reaching the scoring distance), ***p<0.001, ns: no statistically significant difference (p≥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 display rates of mate-searching behavior similar to wild-type males. Error bars indicate SEM; 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≥0.05).

Figure 2. The PDF-1/PDFR-1 pathway stimulates dispersal upon 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 SEM. 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≥0.05).

(b) Percentage of quiescent worms in nutritionally high food (*E. coli* HB101). n indicates number of animals tested. χ^2 test was used for statistical analysis, ***p<0.001, **p<0.01, *p<0.05.

(c) Frequency of reversals (during 5 minutes) was measured 1 minute and 30 minutes after removal from food. (**d**) Frequency of reversals (during 5 minutes) was measured off food 1 minute and 1 hour after removal from mates. Note that values in the first column of (c) and the second column of (d) are equivalent. Variability exists due to experiments being performed in different days for (c) and (d). In (c) and (d), n indicates number of animals tested, error bars indicate SEM and Mann-Whitney test was used for statistical analysis, ***p<0.001, **p<0.01, ns: no statistically significant difference (p≥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 SEM; 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.

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 (*pdf-1* OE) 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 SEM; 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≥0.05).

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. (a and d) DIC images, (b, c and e) fluorescence images. Neurons in the head (a, b) and the retro-vesicular ganglion (c) labeled with a *pdfr-1*::NLS RFP reporter. (d and e), neurons in the tail labeled with a *pdfr-1*::RFP reporter. Anterior is to the left in all images. (a, b, d and e) lateral views; (c) ventral view. Scale bars, 5 μm

(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 hours of leaving assay. n indicates number of animals tested; χ^2 test was used for statistical analysis, ***p<0.001, ns: no statistically significant difference (p≥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 SEM; 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≥0.05).

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. (a and c) DIC images, (b and d) fluorescence images 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, 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 SEM; 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≥0.05).

Materials and methods

Strains used:

CB1490, *him-5(e1490)* was used as the wild-type strain for males; Bristol N2 was used as 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, *lqIs3(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 1/2 proportion of Las animals in the F1 offspring indicated no complementation.
Deficiency strains 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 (3 of 5 lines rescued). Complementation tests with available mutants of genes within 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 RT-PCR from a mixed gender and mixed stage mRNA pool and gene-specific primers:
pdfr-1 cDNAs: 5’ ctgactcatatcatggcggatg 3’ and 5’ tgacacggtgggtttgacaac 3’ or 5’ ttatggagattttgtggagcgattgg3’. The 3’UTR of the *unc-54* gene was used in the *pdfr-1* cDNA constructs.
pdf-1 cDNA*:* 5’ atgaacagattcatcatttc3’ 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/μl except for *P(mbr-1)*::*pdf-1* and *P(zig-3)::pdf-1*, which were injected at 50 ng/μl.

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).

3kb: 5' cactactttcagcgacctttacttgctc 3' and 5' gatatgagtcagtgatgaa 3'

2kb: 5' caccctccaaatgtcttgaaactga 3' and 5' gatatgagtcagtgatgaa 3'

1kb distal: 5' cactactttcagcgacctttacttgctc 3' and

5' TTTGGGTCCTTTGGCCAATCtcagtttcaagacatttgga 3"

1kb medial: 5' ctaattacttcggacgggtctagacgtt 3', and 5'

TTTGGGTCCTTTGGCCAATCaccagctttattgcgtgac 3'

1kb proximal: 5' cacgcaataaagctggttagggaag 3' and 5' gatatgagtcagtgatgaa 3'

Primers for heterologous promoters driving the *pdfr-1* cDNAs, amplified from a fosmid library:

rab-3: 5' gcgagttttgactggctttc 3', 5' cctgatttcaggcggcggaa 3'

unc-4: 5' cgacgtcgtccgagattaaagattc 3', 5' tttcacttttggaagaag 3'

eat-4: 5' ggagttcgaaactgcagcaatga 3' , 5' ggtttctgaaaatgatgatg 3'

glr-4 : 5' caaaaaggcacacttgagctg 3', 5' gctgtgtaaaagtttagctc 3'

npr-1: 5' acgatctgtgtcgcgttttgattt 3', 5' ttggcctatgtctgaaattt 3'

gcy-32: 5' gccatggtgttaatacgtcaagcaa 3', 5' tctataatacaatcgtgat 3'

glr-1: 5' actacgtggagtcgagggctca 3', 5' tgtgaatgtgtcagattgg 3'

osm-6: 5' cgcagttttcattacatttcatgggtatt 3', 5' agatgtatactaatgaagg 3'

unc-7b: 5' aagaaggggaaagaaagcactgaa 3', 5' cggttcaggattgctggag 3'

tol-1: 5' tatgtaggttgcctgtgtgcctgt 3', 5' tttcgttgtgtcatgtgatc 3'

mec-17: 5' cttgagaggtccggcaacttgtttg 3', 5' cggtgaagcagcttctc 3'

heat-shock (from pPD.49.78): 5' gctatgaccatgattacgccaagc 3', 5'

cggggatccagtgagatg 3'

Primers to amplify the *pdf-1* promoter for expression constructs:

Reporter 1: 5' aaaaggttgctttaccgagacgtg 3',5' caacgccgagcttatcaac 3'

Reporter 2: 5' aaaaggttgctttaccgagacgtg 3', 5' ctcattaagattatcccacta 3'

Reporter 3: 5' aaaaggttgctttaccgagacgtg 3', 5' cttcactcaacgtcaacatg 3'
Reporter 4: 5' gtgacgtcgacatgattcagaca 3', 5' cttcactcaacgtcaacatg 3'
Primers to amplify heterologous promoters driving *pdf-1* cDNA:
pdf-1prom2: 5' aaaaggttgctttaccgagacgtg 3', 5' cttcactcaacgtcaacatg 3'
rab-3: 5' gcgagttttgactggctttc 3', 5' cctgatttcaggcggcggaa 3'
mbr-1: 5' aataattgcgaaaagggcgattagc 3', 5' attgtattttcgtaggtaca 3'
zig-3: 5' gtatggatgtccaacgatgagcttg 3', 5' ctatattttgcatttggaaaa 3'

Behavioral assays

Leaving assay: As previously described in [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 diameter and 10 ml 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 2 paralyzed *unc-51(e369)* hermaphrodites. Plates were kept at 20^{o}C during the 24 hrs assay and at 4 time points the proportion of males that had left the food was scored blindly. 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: As previously described in [14], with some modifications. 30 L4 hermaphrodites were picked the night before of 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. 90 minutes after seeding the plates, 10 young adult hermaphrodites were placed in the conditioning plate for 30 minutes. 7 hermaphrodites were then transferred from the conditioning plate to the assay plate and left for 1 hour before recording the assay for 20 minutes. 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 5 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 minutes observation periods under a dissecting microscope.

Locomotion in response to food: reversals and omega turns for each worm were scored during 5 minutes 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 minute to settle. Reversals and omega turns were scored for 5 minutes and again 30 minutes later.

Locomotion in response to mates: 10 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 hours. 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 minute and reversals were scored for 5 minutes. The male was subsequently transferred to a plate with food in isolation for 1 hour and then, transferred to a transition and scoring plate with no food where reversals were scored again for 5 minutes.

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

Quiescence assay:

As previously described in [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 hours before scoring lack of movement and pharyngeal pumping under a dissecting microscope. χ^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 minutes. 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:

Calculation of leaving rates and statistical analysis: As previously demonstrated in [6] male leaving behavior was modeled with the exponential function: *N(t)/N(0)=exp(-□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). □ is the P_L value or probability of leaving, 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.

Values in graphs represent means and error bars represent standard error of the mean (SEM). 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 2 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 20mM NaAzide as anesthetic. Animals were left to recover for 1 day and then assayed. The *lqIs3(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 minutes at 37 ^{o}C, left to recover for 90 minutes at 20 ^{o}C and then tested in the leaving assay. Proportion of leavers was scored 3 hours later.

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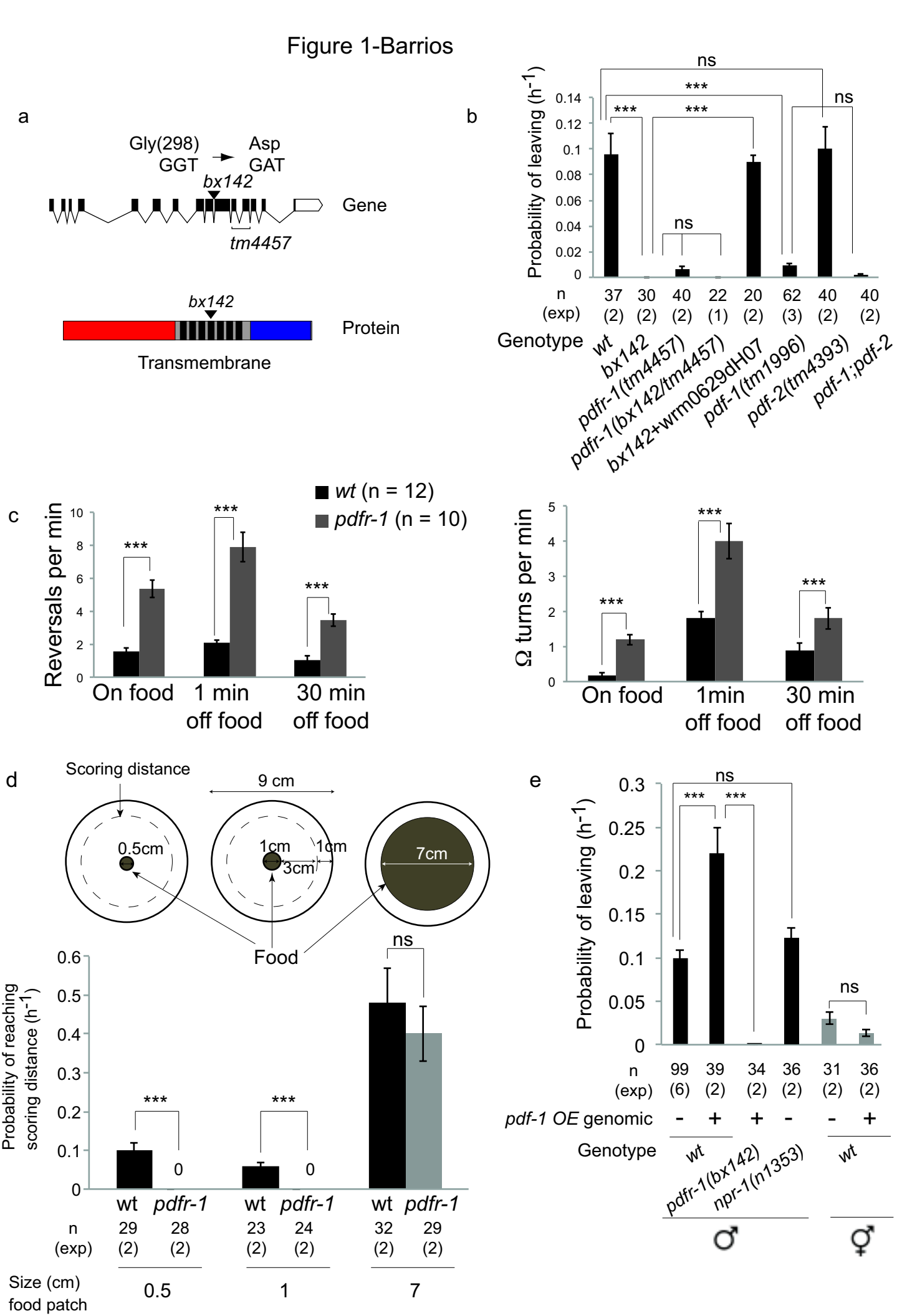
Figure 1-Barrios



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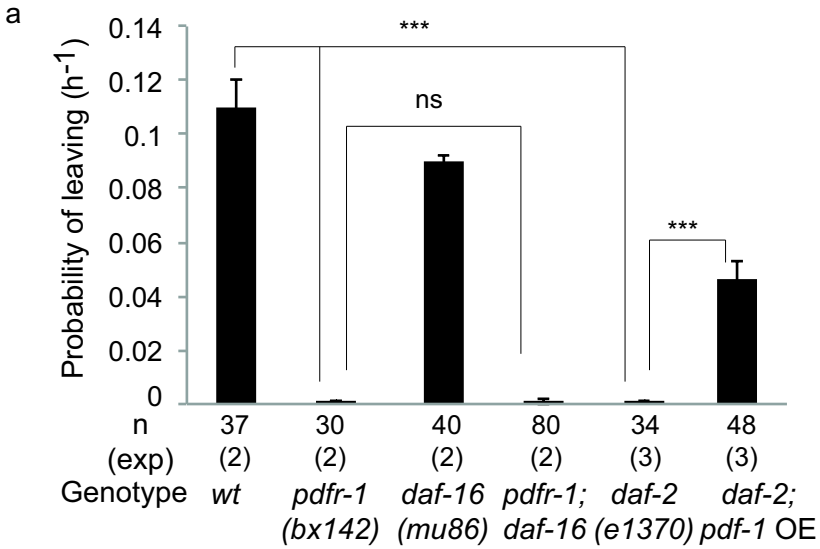

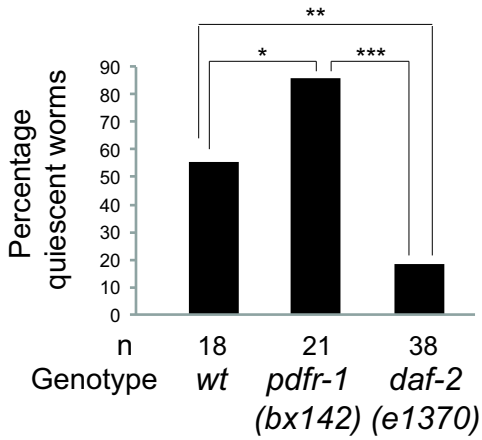

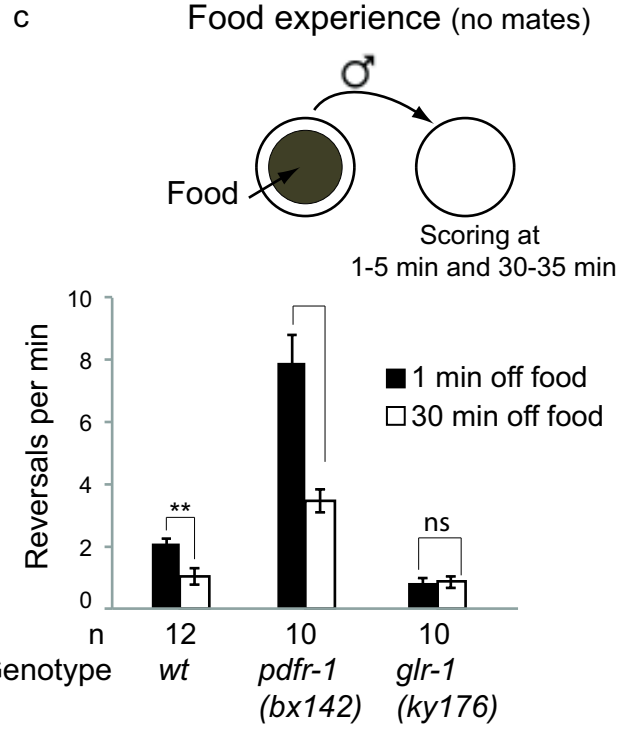

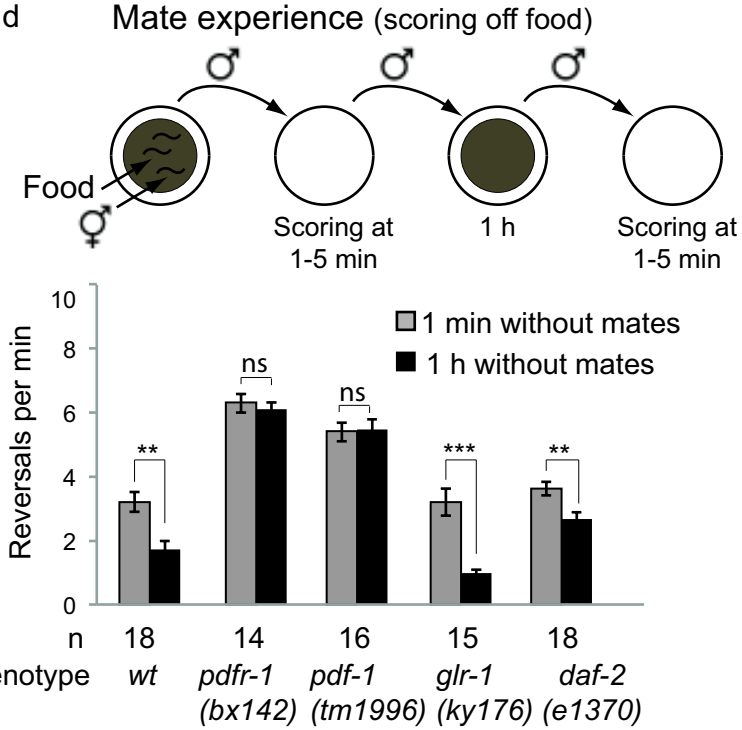

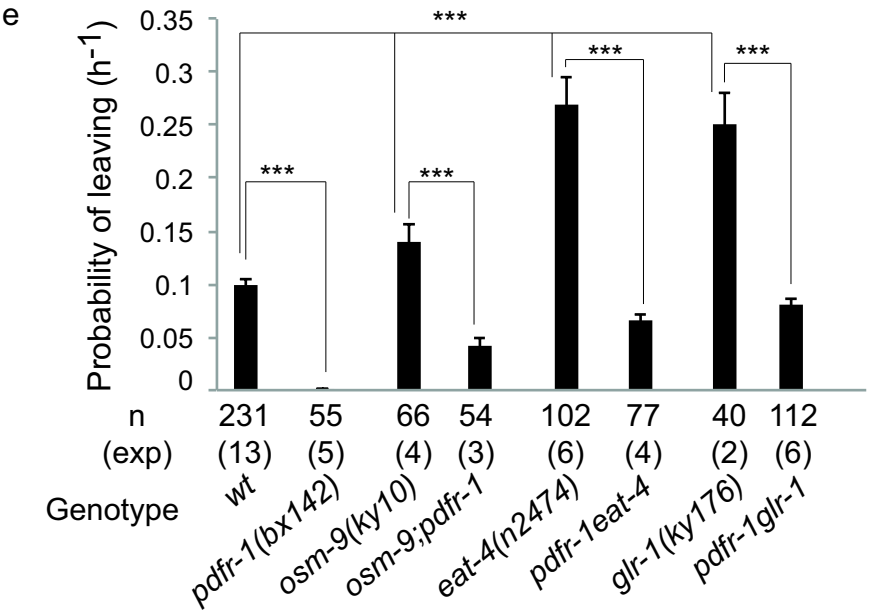

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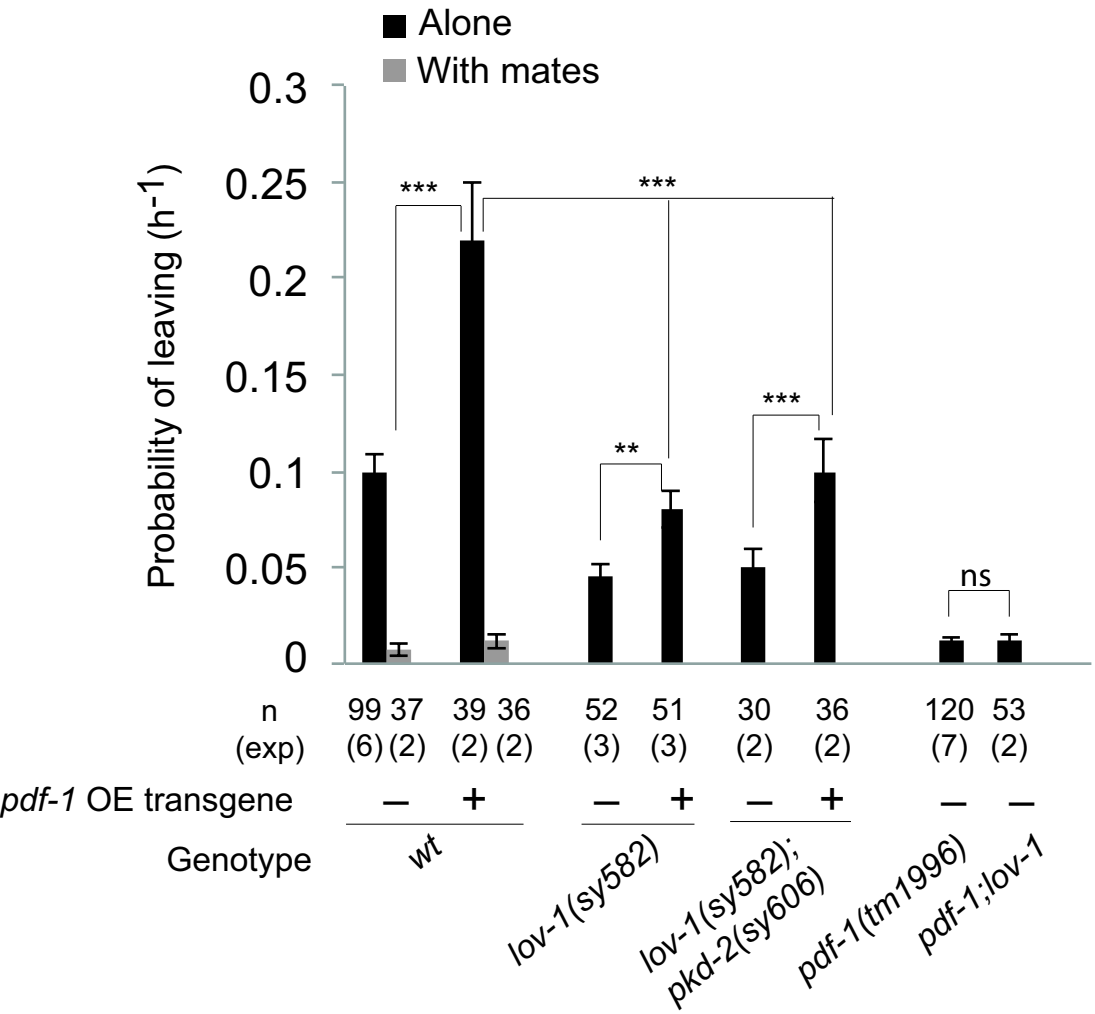

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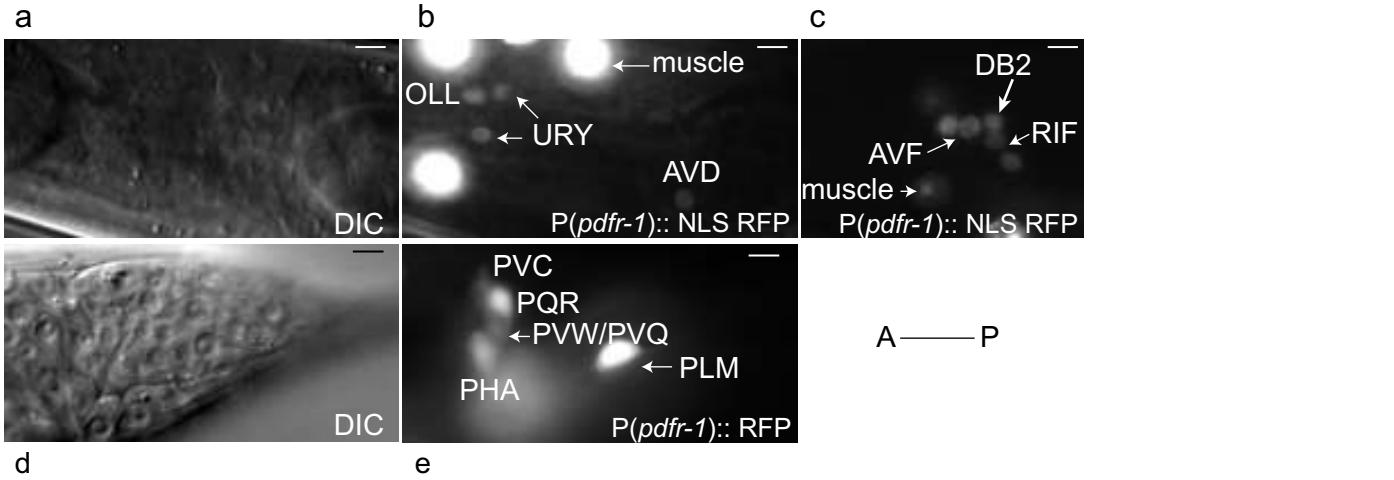
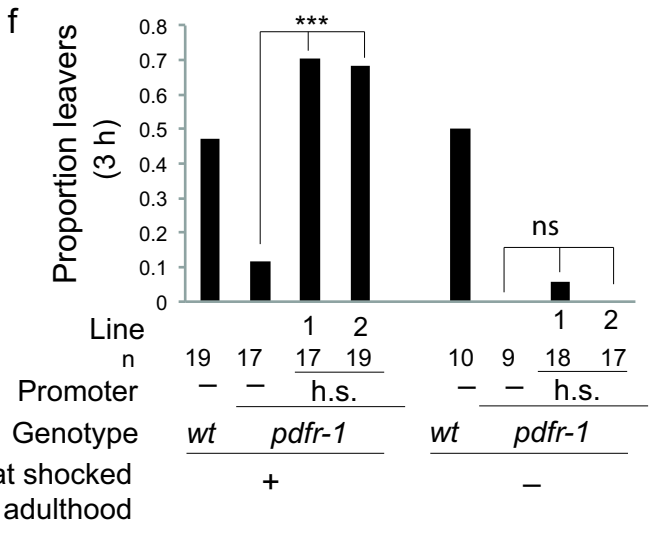
Figure 4-Barrios





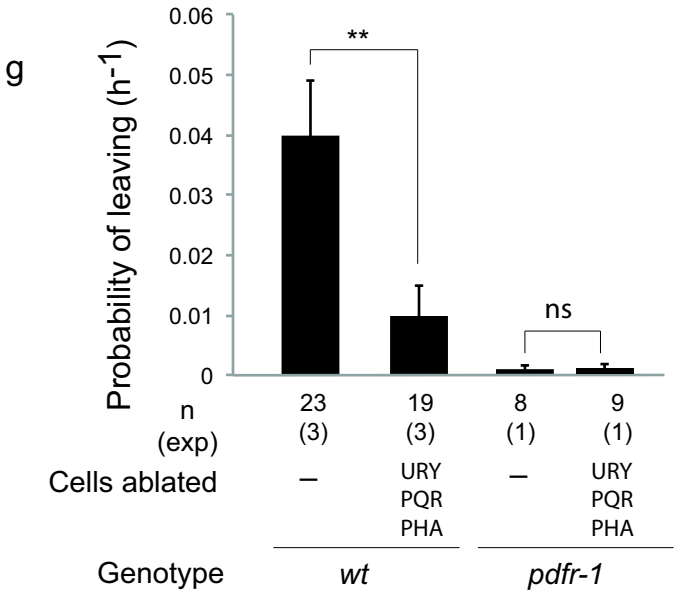


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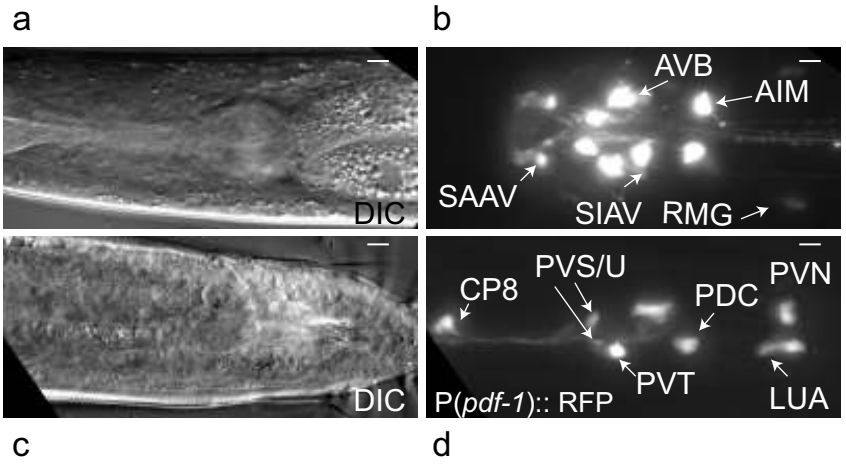
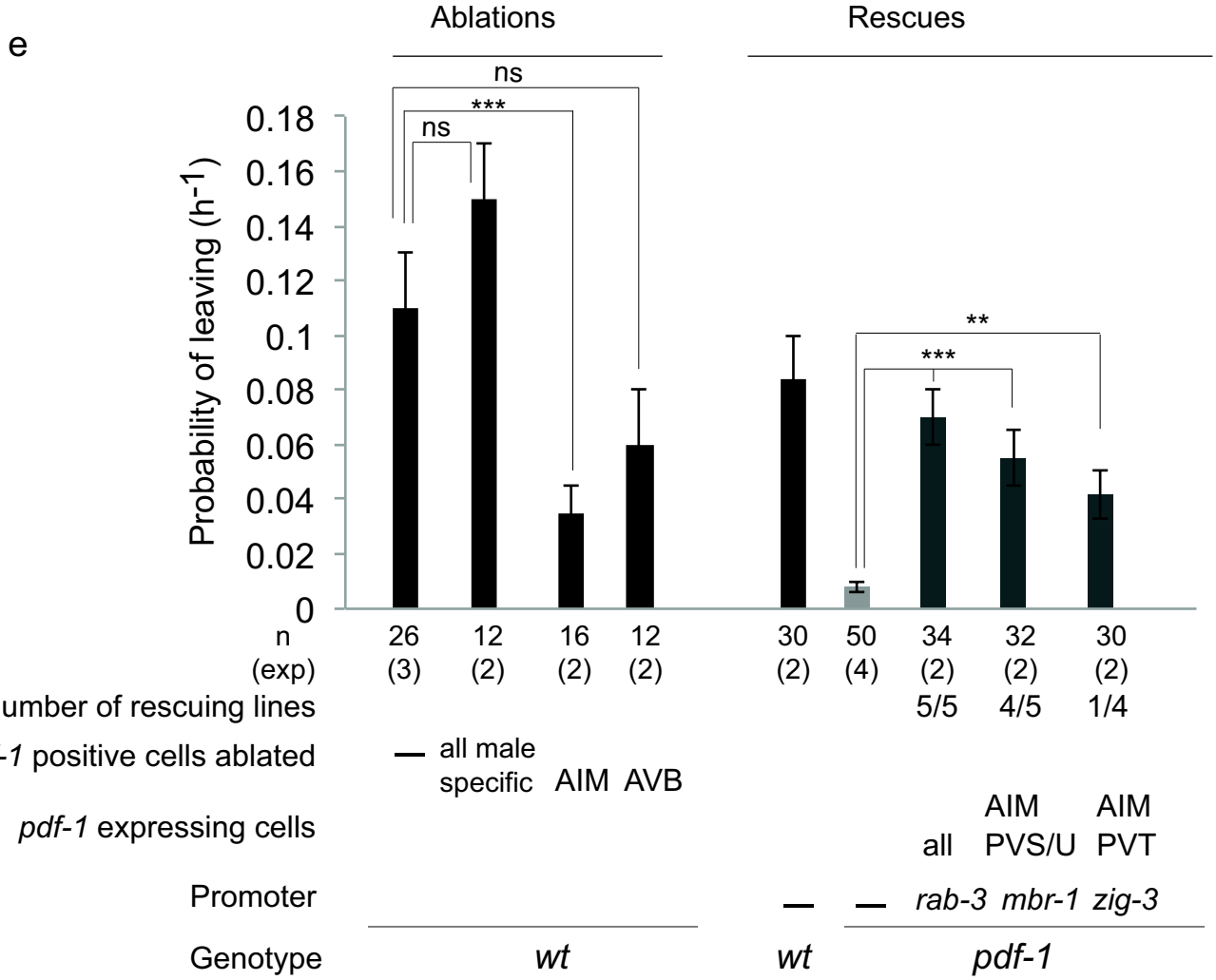
Figure 5-Barrios





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Promoter	*pdfr-1* positive cells																
	muscle	OLL	URY	RMED/V	URX	AVD	TRN	RIF	AVF	DB2	PVC	PQR	PHA	PVW/ PVQ	other neurons	Rescue	Lines
pdfr-1 (3kb)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	○	+++	4/4
rab-3	○	●	●	●	●	●	●	●	●	●	●	●	●	●	●	+++	2/4
pdfr-1 (2kb proximal)	●	○	●	◑	◑	○	●	○	●	○	○	◑	◑	◑	○	++	2/7
glr-4	○	○	●	○	○	○	○	○	○	?	○	●	○	○	●	+++	4/4
npr-1	○	○	○	○	●	○	○	○	○	○	○	●	●	○	●	+++	3/4
pdfr-1 (1kb prox) + *osm-6*	●	●	●	○	○	○	○	○	○	○	○	●	●	○	●	++	2/2
osm-6	○	●	○	○	○	○	○	○	○	○	○	●	●	○	●	+	1/5
pdfr-1 (1kb proximal)	●	○	●	○	○	○	○	○	○	○	○	○	○	○	○	–	5/5
pdfr-1 (1kb medial)	○	●	○	●	◑	●	●	○	●	○	●	○	○	●	○	–	4/4
pdfr-1 (1kb distal)	○	●	○	○	●	●	●	●	○	◑	○	○	○	○	○	–	5/5
gcy-32	○	○	○	○	●	○	○	○	○	○	○	●	○	○	●	–	5/5
pdfr-1 (1kb 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

Table 1. Rescue and expression of transgenes driving *pdfr-1* isoforms b and d under different promoters. Black and white circles indicate positive or negative expression in that cell respectively; a mixed circle indicates variable expression. The number of + indicates the levels of rescue: +++ indicates mate-searching/probability of leaving (P_L) not significantly different from *wild type* (P_L=0.1 and $p \geq 0.05$); ++ 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,* [38], *glr-4* (see Material and Methods for promoter), *npr-1* [39], *osm-6* [40], *gcy-32* [41], *glr-1* [42], *eat-4* [43], *tol-1* [44]*, mec-17* [45], *unc-7b* [46], *unc-4* [47].

The NIHMS has received the file 'supp_info_1.pdf' as supplementary data. The file will not appear in this PDF Receipt, but it will be linked to the web version of your manuscript.