Sex-specific, *pdfr-1*-dependent modulation of pheromone avoidance by food abundance enables flexibility in *C. elegans* foraging behavior

**Highlights**
- Abundant food blocks hermaphrodite avoidance of a key population density pheromone
- Food abundance is encoded by the conserved PDF/PDFR-1 neuropeptide system
- PDFR-1 acts in interneurons to modulate the salience of the aversive pheromone cue
- Food-pheromone integration allows value assessment and promotes adaptive foraging

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**In brief**
To forage optimally, animals must predict the future value of an existing food resource. Here, Luo and Portman show that *C. elegans* can assess this by weighing food abundance against population density. The authors find that food abundance engages a conserved neuromodulatory pathway to modulate avoidance of a key population density pheromone.
Article

Sex-specific, pdfr-1-dependent modulation of pheromone avoidance by food abundance enables flexibility in C. elegans foraging behavior

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SUMMARY

To make adaptive feeding and foraging decisions, animals must integrate diverse sensory streams with multiple dimensions of internal state. In C. elegans, foraging and dispersal behaviors are influenced by food abundance, population density, and biological sex, but the neural and genetic mechanisms that integrate these signals are poorly understood. Here, by systematically varying food abundance, we find that chronic avoidance of the population-density pheromone ascr#3 is modulated by food thickness, such that hermaphrodites avoid ascr#3 only when food is scarce. The integration of food and pheromone signals requires the conserved neuropeptide receptor PDFR-1, as pdfr-1 mutant hermaphrodites display strong ascr#3 avoidance, even when food is abundant. Conversely, increasing PDFR-1 signaling inhibits ascr#3 aversion when food is sparse, indicating that this signal encodes information about food abundance. In both wild-type and pdfr-1 hermaphrodites, chronic ascr#3 avoidance requires the ASI sensory neurons. In contrast, PDFR-1 acts in interneurons, suggesting that it modulates processing of the ascr#3 signal. Although a sex-shared mechanism mediates ascr#3 avoidance, food thickness modulates this behavior only in hermaphrodites, indicating that PDFR-1 signaling has distinct functions in the two sexes. Supporting the idea that this mechanism modulates foraging behavior, ascr#3 promotes ASI-dependent dispersal of hermaphrodites from food, an effect that is markedly enhanced when food is scarce. Together, these findings identify a neurogenetic mechanism that sex-specifically integrates population and food abundance, two important dimensions of environmental quality, to optimize foraging decisions. Further, they suggest that modulation of attention to sensory signals could be an ancient, conserved function of pdfr-1.

INTRODUCTION

Feeding and foraging provide ideal opportunities to understand the neural and genetic mechanisms underlying flexibility in innate behavior. Despite wide variation in the mechanics of foraging, the logic that guides decisions about exploiting or abandoning food resources is remarkably conserved. In animals as diverse as bees, birds, and fish, foraging decisions weigh the current benefit of feeding against the costs of exploration and the potential benefits of new food sources.1,2 When assessing the value of an existing food resource, optimal foraging requires that animals assess local food abundance and quality as well as population density, nutritional needs, and the risk of predation. Experimental studies in multiple systems have provided important insights into the neural mechanisms underlying these calculations and the genetic mechanisms that specify and optimize them.3

The nematode C. elegans feeds on microbes that colonize decaying vegetation4 and modulates its feeding and exploratory behaviors in ways that are consistent with optimal foraging theory. Foraging and dispersal in adults vary with food abundance,5-7 nutritional value,7,8 pathogenicity and toxicity,9,10 and experience.11,12 Foraging behavior also exhibits substantial natural variation, owing in part to altered sensory responses.6,13-17 Furthermore, foraging and dispersal are sexually dimorphic and sensitive to nutritional state; solitary males will abandon a food source in search of mates, but only if well fed.18-22

In many species, foraging is sensitive to population density. Conspecifics can be competitors that reduce the future value of a crowded food source, but in other contexts, collective foraging can be advantageous.23 In C. elegans, worms will leave a food source more often if it is crowded,17,24 but some pheromones inhibit dispersal and exploration.6,15,16 Further, many natural isolates feed in groups,13 and collective foraging may be favored in patchy food environments.25,26 Thus, the role of population density in C. elegans foraging and dispersal may not be fixed. Indeed, in the wild, balancing selection can simultaneously maintain alleles that promote both strategies.16,17

Because of its “boom-and-bust” life cycle, in which food availability and population density can vary dramatically,27...
C. elegans actively monitors both of these variables when feeding. Population density in C. elegans is mainly signaled by ascaroside pheromones, derivatives of the dideoxy sugar ascarylose that serve as a modular chemical language. A prominent member of this group is ascr#3, which, together with other ascarosides, signals population density to young larvae, guiding the decision to enter the stress-induced dauer stage. ascr#3 is also a sex pheromone; it is produced predominantly by adult hermaphrodites and elicits strong male-specific attraction. Adult hermaphrodite behavioral responses to ascr#3 are typically aversive, though this depends on the context of ascr#3 presentation as well as animals’ internal state and previous experience.

In earlier studies on sexual dimorphism, we encountered variability in hermaphrodite ascr#3 avoidance responses, leading us to wonder whether flexible responses to this population density signal might have adaptive value. Here, we find that this is the case, identifying a neuronal and genetic mechanism whereby food abundance modulates ascr#3 aversion specifically in hermaphrodites to adaptively modulate feeding and foraging decisions.

RESULTS

Hermaphrodite pheromone avoidance is modulated by food abundance

To explore the relationship between food and pheromones, we used a quadrant-format assay (Figure 1A). This assay allows robust quantitation of medium-term (1 to 2 h) behavioral responses to non-volatile chemical stimuli. To control the abundance of food, we prepared standardized lawns of E. coli OP50 of three densities (Figure 1B). “T3” is a smooth, uniform lawn providing ample but not excessive food, similar to standard culture conditions. “T2” is a thin, slightly patchy lawn. “T1” is a very thin lawn with many food-free patches, providing a scarce-food environment. Notably, other factors (e.g., bacterial metabolites and environmental gases) also likely differ between these conditions.

In hermaphrodites assayed with thin-food conditions (Figures 1C and S1), we observed modest but consistent avoidance of 1–5 μM ascr#3 but no response to lower concentrations (0.1 and 0.01 μM). In males, 1–5 μM ascr#3 elicited robust attraction, but lower concentrations (0.01 μM and 0.1 μM) had little or no effect on behavior (Figure 1C). Thus, as expected, hermaphrodites and males on sparse food exhibit concentration-dependent, qualitatively distinct responses to ascr#3.

Surprisingly, when we assayed hermaphrodite behavior in the presence of thicker T3 food, we found that ascr#3 avoidance was absent (Figure 1D). Adult males, in contrast, displayed

Figure 1. Hermaphrodite ascr#3 avoidance depends on food abundance

(A) The quadrant-format behavioral assay. Images of representative bacterial lawns. T3, a thick lawn; T2, intermediate thickness; T1, a sparse, patchy lawn.

(B) Quadrant chemotaxis index (QCI) values for both sexes in response to a range of ascr#3 concentrations in the presence of thin food (T1).

(C) Quadrant chemotaxis index (QCI) values for both sexes in response to 1.0 μM ascr#3 in three food conditions (T1, T2, and T3).

(D) QCIs for adult hermaphrodites to 1.0 and 5.0 μM ascr#3 on thick food (T3). For all quadrant assay data shown in this and subsequent figures, data points are colored by sex (hermaphrodites, red; males, blue). Each point represents a single assay containing 10 worms. Violin plots are shaded according to food thickness (T1, lighter; T3, darker). The median and interquartile intervals are indicated by thick and thin lines, respectively. Statistical comparisons are indicated with black brackets and asterisks (*p ≤ 0.05; **p ≤ 0.005; ***p ≤ 0.001) or dotted gray brackets (p > 0.05). Open circles above each bar show the results of one-sample t tests, indicating whether the observed QCI differs significantly from zero (p ≤ 0.05; **p ≤ 0.005; ***p ≤ 0.001).

See also Figure S1.
robust ascR#3 attraction, regardless of food thickness (Figure 1D). This modulation of ascR#3 aversion by food abundance could reflect active integration of food and pheromone signals by the hermaphrodite nervous system; alternatively, thick food might simply block ascR#3 detection or response. We found that more concentrated ascR#3 (5 μM) was sufficient to elicit avoidance on thick food (Figure 1E), suggesting that the nervous system actively integrates these signals.

**PDF neuropeptide signaling modulates pheromone avoidance**

Because the conserved neuropeptide receptor PDFR-1 is associated with both food-dependent and sex-specific behavioral states,19,41 we examined ascR#3 responses in pdfr-1 null mutants. Interestingly, on thin food, pdfr-1 hermaphrodites displayed markedly enhanced ascR#3 avoidance (Figures 2A and S2A). Single null mutations in pdf-1 and pdf-2, which encode ligands for PDFR-1,40 caused little change in ascR#3 avoidance. However, pdf-1; pdf-2 double mutants showed strong avoidance (Figure 2A), suggesting functional redundancy. We detected no significant difference in ascR#3 avoidance between pdfr-1 and pdf-1; pdf-2 mutants but cannot rule out possible signaling by additional PDFR-1 ligands or ligand-independent basal PDFR-1 signaling.

Because larval exposure to ascR#3 can alter adult ascR#3 responses,38 our results might point to a role for pdfr-1 in experience-dependent plasticity. However, ascR#3 avoidance measured by the quadrant assay was independent of larval exposure to endogenously produced ascR#3, as the loss of daf-22, an enzyme necessary for synthesis of ascR#3 and other short-chain ascarosides,42 had no effect on aversion to exogenous ascR#3 in this context (Figure S2B).
PDFR-1 signaling intensity likely encodes food thickness information

Interestingly, we found that the asc#3 responses of pdfr-1 mutants were insensitive to food thickness. Regardless of food abundance, pdfr-1 hermaphrodites showed strong aversion to asc#3 (Figure 2B). This indicates that pdfr-1 function is necessary for the integration of food-thickness information into hermaphrodite behavioral responses to asc#3.

Because the neuropeptide receptor npr-1 also modulates asc#3 responses, we asked whether it might also have a role in this integration. On thin food, loss of npr-1 had no apparent effect on asc#3 avoidance. Moreover, npr-1 mutants remained sensitive to food abundance, as asc#3 avoidance was significantly blunted in the presence of thick food (Figure 2B). However, asc#3 avoidance was not completely absent in npr-1 mutants on thick food, suggesting that npr-1 might have a role in the suppression of asc#3 avoidance. Compared to pdfr-1, however, this role is subtle.

Consistent with the requirement for pdfr-1, asc#3 avoidance was also insensitive to food abundance in pdf-1; pdf-2 double mutants (Figure 2C). Surprisingly, food dependence was also absent in pdf-1 single mutants but remained intact in pdf-2 mutants (Figures 2D and 2E). Thus, although ligands encoded by both genes appear to have roles in the modulation of asc#3 aversion, pdf-1 has the primary role in linking food thickness to the strength of the aversive response.

We noted that the inhibitory function of pdfr-1 appeared to be stronger on thick food than on thin food (Figure 2B), suggesting that the intensity of PDFR-1 signaling might encode information about food thickness. To test this, we asked whether artificially increasing pdfr-1 signaling would block asc#3 avoidance on thin food. To bring this about, we used transgenes carrying extra copies of wild-type pdf-1 or pdf-2. Consistent with our hypothesis, pdf-1 overexpression reduced asc#3 avoidance on thin food (Figures 2F and 2G). However, overexpression of pdf-2 had no apparent effect (Figure 2F), again suggesting that pdf-1, but likely not pdf-2, conveys food thickness information. In agreement with this, overexpression of pdf-1 had no apparent effect in the presence of thick food (Figure 2G). As expected, pdfr-1 was required for the effect of pdf-1 overexpression (Figure 2G). These results indicate that the intensity of PDFR-1 signaling, driven primarily by pdf-1 activity, provides an instructive internal representation of food thickness that modulates asc#3 avoidance.

The ASI sensory neurons are an essential component of the asc#3 avoidance circuit

Next, we sought to characterize the neuronal basis for asc#3 avoidance. In the absence of food, acute aversive and attractive responses to asc#3 are mediated by the sensory neurons ADL and ASK, respectively. However, genetic ablation of these neurons had no apparent effect on asc#3 avoidance in the quadrant assay, either in wild-type or pdfr-1 hermaphrodites (Figures 3A and 3B). Thus, the longer term, on-food asc#3 responses examined here are likely mediated by mechanisms distinct from those previously described.

We also considered the ASI neurons, which detect ascarcosides in other contexts, including the dauer-entry decision and the response to the exploration-suppressing pheromone icas#9. We found that asc#3 avoidance was abolished upon ASI ablation, both in wild-type and pdfr-1 backgrounds (Figure 3C), regardless of food thickness (Figures 3D and 3E). The requirement for ASI in both wild-type and pdfr-1 mutants indicates that the strong asc#3 avoidance of pdfr-1 mutants likely results from increased activity of the same mechanism that generates avoidance in wild-type hermaphrodites, rather than the activation of a parallel mechanism.

These results suggested that ASI might directly detect asc#3. However, using validated GCaMP transgenes and a range of pheromone concentrations, others have detected no evidence of asc#3-evoked calcium responses in ASI (E. DiLoreto and J. Srinivasan, personal communication; K. Kim and P. Sengupta, personal communication). This is consistent with previous findings that ascarcosides can engage signaling mechanisms over longer timescales without triggering calcium transients. The simplest interpretation of our results is that ASI directly detects asc#3; however, it is also possible that ASI is required downstream of asc#3 detection or in parallel with it.

We also considered the possibility that ASI detects or implements the thin-food state, because ASI-ablated hermaphrodites mimic thick-food behavior (i.e., indifference to asc#3) on thin food (Figures 3C and 3D). However, this appeared not to be the case, as ASI ablation in pdfr-1 mutants did not recapitulate the moderate asc#3 repulsion typical of pdfr-1 hermaphrodites on thick food (Figures 3C and 3E).

Because it is produced primarily by ASI, we also examined the transforming growth factor β (TGF-β)-superfamily ligand DAF-7. Like ASI-ablated hermaphrodites, daf-7 hermaphrodites exhibited no net response to asc#3 under any conditions (Figure 3F). We also tested pdfr-1; daf-7 double mutants but observed exceptionally high variability in asc#3 responses, suggesting a dysregulation of behavior in these animals. These results, which should be interpreted cautiously, could suggest a general requirement for daf-7 in hermaphrodite asc#3 avoidance. If so, daf-7 might have a role in asc#3 detection or might signal the presence or abundance of food (see Discussion).

PDFR-1 functions in multiple interneurons to modulate asc#3 avoidance

pdf-1 is expressed in multiple head and tail neurons, as well as peripheral tissues, including body-wall muscle. Using an established intersectional strategy, we tested the ability of a conditionally activatable Ppdf-1::inv[pdr-1::SL2::gfp] transgene, together with neuron-specific nCre constructs, to rescue the enhanced asc#3 avoidance of pdfr-1 hermaphrodites. This transgene produces functional pdf-1 and GFP only if the Pdf-1 promoter is active and nCre-mediated recombination has taken place (Figure 4A).

Pan-neural nCre expression completely rescued the pdf-1 defect, but muscle expression had no effect (Figures 4B and 4C). Next, we tested a three-transgene combination ("tmg::nCre," driven by Ptdc-1, Pmod-1, and Pfg-9) previously shown to partially rescue the persistent dwelling of pdfr-1 mutants. However, we observed no rescue of asc#3 avoidance with this transgene (Figure 4D). To ask whether pdf-1 functions in sensory neurons, we used Posm-6::nCre but again saw no rescuing activity (Figure 4E). This suggests that pdfr-1 is unlikely...
to function in ASI. Consistent with this, no GFP was detectable in animals carrying the ASI/AWA-specific *Pgpa-4::nCre* and the conditional *pdfr-1* transgene (not shown).

We generated and tested several other nCre constructs in combination with the conditional *pdfr-1* transgene. These strains exhibited no detectable GFP (*Pmec-10*, touch neurons) or failed to rescue the ascr#3 avoidance phenotype (*Pmod-1 + Posm-6*, GFP in several sensory neurons and interneurons; *Ptmd-1 + Pglr-3 + Pnmr-1*, GFP in many interneurons; and *Pgcy-28.d*, GFP in AIA interneurons; data not shown; note that we cannot rule out the possibility of weak, undetected rescue in these experiments). However, with another combination, *Pgcy-28.d::nCre + Pnmr-1::nCre*, we observed a small but statistically significant reduction of ascr#3 avoidance in two independent transgenic lines (Figure 4F). To determine whether these effects were biologically meaningful, we assayed these lines with thick food, again observing significant rescuing activity (Figure 4G). In these animals, GFP was detected in several interneurons, including AIA, PVC, and one or more *nmr-1*-expressing head interneurons (AVA, AVD, AVE, AVG, and RIM). This partial rescue indicates that *PDFR-1* likely has additional important sites of action outside this subset of neurons. From these results, we conclude that PDFR-1 has a distributed role in which it modulates multiple interneuronal components of the ascr#3 avoidance circuit.

The modulatory effects of food thickness are sex specific

In males, sex-specific detection of ascr#3 by the ADF neurons promotes attraction in the quadrant assay. Loss of *pdfr-1* or...
Figure 4. *pdfr-1* acts in a distributed set of interneurons to repress *ascaridoid* avoidance

(A) The strategy for Cre-based conditional *pdfr-1* rescue.41

(B–G) QCs for hermaphrodites carrying a conditional *pdfr-1* transgene with the indicated *pdfr-1* genotypes (+, wild type; −, mutant) without or with nCre transgenes expressed pan-neuronally ("Neur::nCre"; B), in muscle ("Musc::nCre"; C), in a set of interneurons where *pdfr-1* regulates motor state ("tmg::nCre"; D),41 in sensory neurons ("Sens::nCre"; E), and in AIA and neurons expressing *nmr-1* ("AIA+nmr-1::nCre"; F and G) on T1 (B–F) or T3 (G) food.
ASI had no effect on this behavior (Figures 5A and 5B), indicating that they are dispensable for male ascr#3 attraction.

When ADF is genetically feminized or ablated, males display hermaphrodite-like aversive responses to ascr#3, indicating that an avoidance mechanism is unmasked (Figure 5C). Simultaneous ablation of ADF and ASI ablation eliminated all response to ascr#3 in males (Figure 5C), suggesting that avoidance is mediated by a mechanism common to both sexes. Supporting this, loss of pdfr-1 enhanced the ascr#3 avoidance of ADF-ablated males, and this enhanced avoidance also required ASI (Figure 5D).

However, the behavior of ADF-ablated males was not completely equivalent to that of hermaphrodites. In particular, even in the presence of thick food, ADF-ablated males continued to manifest clear ascr#3 aversion (Figure 5E). We observed no effect of food thickness on ascr#3 attraction in pdfr-1 males or on ascr#3 aversion in ADF-ablated pdfr-1 males (Figures 5F and 5G). Thus, under these conditions, the modulation of ascr#3 avoidance by food thickness is a hermaphrodite-specific feature of the nervous system.

To investigate this sex difference, we genetically masculinized the hermaphrodite nervous system by pan-neuronal expression of the male sexual regulator fem-3. This has been shown to functionally sex reverse many behaviors mediated by shared circuits, including chemosensation and locomotion. Simultaneously, we genetically ablated ADF to prevent it from driving ascr#3 attraction. Interestingly, the modulatory effects of food thickness remained intact: thick food eliminated ascr#3 aversion in masculinized hermaphrodites, just as in wild type (WT) (Figure 5H). Thus, sex-specific neurons and/or non-neuronal tissues, rather than sex-shared neurons, appear to be responsible for the hermaphrodite specificity of food-pheromone integration.
Foraging decisions that integrate food abundance and population density cues require ASI

Finally, we wondered whether plasticity in ascr#3 avoidance might allow hermaphrodites to incorporate population density into their assessment of the value of an existing food resource. To explore this, we examined the dispersal of WT and ASI-ablated hermaphrodites from food sources of variable quality. In this assay, a thin (T1) or thick (T3) food source, supplemented with vehicle control or 1 μM ascr#3, lies at the center of a 10-cm agar plate (Figure 6A). Four peripheral patches of thick food without ascr#3, providing favorable alternative environments, lie 1.8 cm from the source patch. At the beginning of the assay, 50 young adult hermaphrodites (25 WT and 25 ASI ablated, distinguishable by a fluorescent marker) were deposited on the

Figure 6. Modulation of ascr#3 aversion by food thickness enables flexibility in C. elegans foraging decisions

(A) Experimental design.

(B) Foraging rates, shown as the frequency of animals dispersing to the outer food sources, of WT and ASI-ablated hermaphrodites placed on a source patch of thick (T3) or thin (T1) food, without (−) or with (+) ascr#3. Each data point represents a single assay containing 25 worms per genotype. Filled and open circles indicate control and ASI-ablated animals, respectively. Violin plots are shaded according to food thickness at the source patch (T1, lighter; T3, darker). The median and interquartile intervals are indicated by thick and thin black lines, respectively. Statistical comparisons between groups are indicated with black brackets and asterisks (*p ≤ 0.05; **p ≤ 0.005; ***p ≤ 0.001) or dotted gray brackets (p > 0.05).

(C) A neural circuit model showing the parallel inputs of food abundance, ascr#3 via ASI, and ascr#3 via ADF. Hermaphrodite- and male-specific aspects are shown in red and blue, respectively.

(D) A proposed model illustrating the adaptive value of context-dependent flexibility in C. elegans foraging behavior driven by modulation of ascr#3 avoidance.
source patch. After 3 h, we scored the number of animals of each genotype that had migrated to the peripheral lawns.

When the source was a patch of thin food, relatively few animals migrated away, regardless of ASI ablation (Figure 6B). Supplementing the source patch with ascr#3 markedly increased dispersal rate, with nearly 50% of animals migrating to the outer patches (Figure 6B). When ASI was ablated, however, addition of ascr#3 did not increase dispersal rates above baseline (Figure 6B). Thus, consistent with our findings in the quadrant assay, ascr#3 serves as a potent driver of dispersal when food is scarce, and this effect requires ASI.

With a thick-food source, we again observed relatively few animals dispersing (Figure 6B). Adding ascr#3 caused an increase in dispersal, but the magnitude of this effect was far smaller than with thin food (Figure 6B). Again, ascr#3-mediated dispersal was dependent on ASI. Thus, even on thick food, ascr#3 can have aversive effects; however, its ability to promote dispersal is markedly blunted.

Together, these results show that flexibility in ascr#3 aversion allows hermaphrodites to modulate foraging decisions according to population density and food abundance. The ASI dependence of this plasticity strongly suggests that it arises through the same mechanism that modulates ascr#3 aversion in the quadrant assay. Because it provides a means for animals to optimize foraging decisions, this mechanism likely has adaptive value in the wild.

DISCUSSION

In making foraging decisions, animals weigh the future value of an existing food resource against the potential risks and benefits of abandoning it. A food resource’s value depends not only on its quality and size but also its projected rate of depletion, a primary determinant of which is local population density. How these variables are internally represented and used by neural circuits to calculate behavioral outcomes is poorly understood. Here, we show that C. elegans hermaphrodites dynamically assign a weight to the aversive population density signal ascr#3 based on food abundance, such that the salience of population density increases as food abundance diminishes (Figure 6C). This flexibility could allow hermaphrodites to disperse from a crowded, rapidly depleting food source before it is completely exhausted, avoiding potential starvation. When food is abundant, however, suppression of the population density signal might allow animals to continue to exploit an existing resource (Figure 6D). By identifying neural and genetic mediators of this process, our results provide insight into the neural mechanisms that implement behavioral plasticity and the genetically specified rules by which these mechanisms operate.

Our work builds on several studies showing that C. elegans hermaphrodites actively modulate their dispersal from an existing food resource. Interestingly, recent work found that adult C. elegans hermaphrodites tend to increase food-avoiding behavior as their self-progeny accumulate and that this is likely a response to daf-22-dependent ascaroside pheromones produced by L1 larvae. Our results indicate that the depletion of food might enhance this pheromone aversion, such that the larval-adult social interaction identified by these investigators could reflect a synergistic effect of abundant pheromone and scarce food. Another recent study provided evidence that dynamic modulation of the valence of ascaroside cues (i.e., repulsion versus attraction) can optimize the foraging strategies of a population in a patchy food environment. The relationship of these processes to the ascr#3 avoidance plasticity we report here will be an interesting area for future work.

Our findings indicate that hermaphrodites use two distinct sensory streams to carry pheromone and food information (Figure 6C). For the former, the ASI sensory neuron pair plays a key role. Although there is no evidence that ASI exhibits a calcium response to ascr#3 stimulation, we favor the possibility that ASI directly detects ascr#3 in the behavioral paradigm employed here. Indeed, previous work indicates that several classes of C. elegans sensory neurons, including ASI, can detect ascaroside pheromones directly without displaying a calcium transient. Interestingly, these responses seem to act over a longer timescale than typical calcium-based neuronal signals, suggesting that they mediate “primer” effects of pheromones. It is also possible that ASI function is required for the effects of an ascr#3 signal from other sensory neuron(s); these possibilities are not mutually exclusive. Our results also suggest that ASI’s function depends on the TGF-β superfamily signal DAF-7 (see below). Interestingly, ascr#3 detection mechanisms appear to be highly context dependent, as the behaviors elicited by ascr#3 in other settings (e.g., the acute drop test) have distinct neuronal substrates.

In our model, food abundance is signaled via a separate, parallel sensory stream (Figure 6C). Though the means by which food abundance is detected remain unclear, our findings strongly suggest that this variable is internally represented by the activity level of the neuropeptide receptor PDFR-1. Multiple lines of evidence support this. Animals lacking pdf-1 or the two ligand genes pdf-1 and pdf-2 display constitutively strong ascr#3 aversion that requires ASI but is completely insensitive to food thickness. Because this aversion is stronger than that seen in WT animals under thin-food (T1) conditions, we infer that low levels of PDRF-1 signaling are active when animals are on thin food, slightly blunting ascr#3 aversion. On thick food (T3), pdf-1 and pdf-2; pdf-2 hermaphrodites still strongly avoid ascr#3, even though WT hermaphrodites are essentially indifferent to it. Here, high levels of PDFR-1 signaling completely inhibit ascr#3 aversion. Our results are most consistent with the idea that pdf-1 and pdf-2 have partially redundant functions but that pdf-1-derived ligand(s) have a more important role in signaling food thickness. Such partial redundancy also exists in other contexts, but several studies have shown that pdf-2 can also antagonize pdf-1, indicating that the dynamics of this system are complex. Importantly, overexpressing pdf-1 in animals feeding on thin food is sufficient to cause them to behave as though they were on thick food—that is, they are now indifferent to ascr#3—but overexpressing pdf-1 in animals feeding on thick food has no effect. Recently, pdf-2 has been implicated in signaling nutritional status information from the intestine, raising the intriguing possibility that pdf-1 and pdf-2 communicate external and internal information about nutrition, respectively, to pdf-1.

How might food abundance regulate pdf-1 signaling? We propose that the secretion of pdf-1-encoded neuropeptide(s) is regulated by a food thickness signal. Because sensory neurons
are not a major site of pdf-1 expression. Sensory signals might be linked to pdf-1 indirectly. One possibility is that daf-7 conveys food abundance information to pdf-1. daf-7 expression is responsive to food, and it can link food abundance to behavior, aging, and germline progenitor proliferation. However, daf-7 activity is expected to be low under thin-food conditions, where we see its strongest requirement (Figure 3F). On thick food, where daf-7 activity should be stronger, daf-7 is not required for ascr#3 inhibition (Figure 3F). Thus, it is unlikely that daf-7 conveys food thickness information to the ascr#3 avoidance circuit. Instead, daf-7 could have a permissive role, simply signaling the presence of food to enable pheromone avoidance. Alternatively, because daf-7 is regulated by ascarosides in the dauer entry decision, daf-7 might signal ascr#3 detection by ASI. However, daf-7 is downregulated by ascaroside exposure, which would be problematic for such a model. Understanding the role of daf-7 in ascr#3 avoidance will be an important area for future work.

At least four other previously identified mechanisms might couple food abundance to pdf-1 activity. The physical presence of bacteria can be detected by the deirid sensory neuron ADE; secretion. Bacterial food is also a rich source of chemical cues, the detection of these by amphid chemosensory neurons could indirectly regulate pdf-1. Further, in bacteria-rich environments, local concentrations of O₂ and CO₂ are decreased and increased, respectively, allowing worms to use the activity of gas-sensing neurons as proxies for food abundance pdf-1 signaling could be downstream of such a signal. A final possibility is related to the physical structure of the sparse-food T1 environment, where bacterial patches are interspersed with many small gaps. As animals navigate this environment, the frequency with which they cross food boundaries is markedly higher than in an abundant-food environment. Recent work has shown that worms can use this information to assess the physical structure of food patches; an intriguing possibility is that this might occur at least in part via modulation of PDF-1 release and/or PDFR-1 signaling. Downstream of its regulation by food abundance, we find that PDFR-1 signaling represses ascr#3 aversion by modulating a distributed set of interneurons. pdfr-1 is known to modulate the balance between roaming and dwelling states, another behavior affected by food quantity. However, restoring pdfr-1 function to neurons that mediate these effects was unable to rescue the increased ascr#3 aversion of pdfr-1 mutants, suggesting these are distinct functions. Future work will be required to understand how pdfr-1 modulates the ascr#3 aversion circuit downstream of ASI. Interestingly, biological sex regulates the processes we describe here in at least two ways. First, as shown previously, male-specific ascr#3 detection by ADF overrides a latent aversion drive. Here, we show that this aversion appears to be mediated by a sex-shared mechanism, as it requires ASI and is repressed by pdfr-1 in both sexes. However, the aversion driven by this mechanism is insensitive to food thickness: even in the presence of thick food, ADF-ablated males displayed ASI-dependent avoidance of ascr#3. Thus, some aspect of the food thickness signal—either its existence or its ability to modulate the aversive signal—is hermaphrodite specific. Interestingly, this hermaphrodite-specific feature was not eliminated by genetic masculinization of the hermaphrodite nervous system. This suggests that the sex specificity of food-pheromone integration depends on sex-specific neurons or sex-specific signals from peripheral tissues. Further, why males lack this sensory integration is unclear. One possibility is that, rather than encoding food thickness, pdfr-1 signaling conveys some other dimension of information in males. The importance of pdfr-1 in promoting male mate-searching behavior strongly supports this idea.

Because of these differences, the scenarios of Figure 6D apply only to hermaphrodites. In males, ascr#3 would likely retain males under most of the conditions shown. This helps tune behavior to the distinct goals of adult males, in which mate searching is typically favored over feeding. Nevertheless, it seems unlikely that pheromone attraction in males is completely independent of food signals. In the absence of food, males copulate infrequently and poorly; both detection and ingestion of food are important for promoting efficient mating. How males might integrate food and pheromone cues will be an interesting area for future research.

Neuromodulation is an ancient, widespread feature allowing the dynamic reconfiguration of fixed neural circuits. In simple nervous systems, myriad neuromodulatory signals exist: the C. elegans genome, for example, contains over 100 neuropeptide genes, each of which can produce multiple ligands. Although important progress has been made in identifying the effects of these signals on physiology and behavior, understanding the functional significance of the modulation they bring about can be challenging. Here, we provide evidence that PDF-1/PDFR-1 signaling is an internal representation of food abundance that regulates the behavioral response to stimulation by ascr#3. Interestingly, in several systems, PDF-family neuropeptides and/or the neurons that release them can modulate arousal and sensitivity to external stimuli. Our results suggest that modulation of attention to sensory stimuli like ascr#3 could be an ancient function of this family of neuropeptides.
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AUTHOR CONTRIBUTIONS

Conceptualization, J.L. and D.S.P.; investigation, J.L.; writing – original draft, J.L.; writing – review & editing, D.S.P.; supervision, D.S.P.; funding acquisition, D.S.P.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and virus strains** |
| E. coli OP50 | CGC | OP50 |
| **Chemicals, peptides, and recombinant proteins** |
| ascaroside #3 (ascr#3, also known as C9 or asc-ΔC9) | Synthesized by F. Schroeder laboratory, Boyce Thomson Institute, Ithaca, NY | N/A |
| Dil (1,1’-Dioctadecyl-3,3’3’-tetramethylindocarbocyanine perchlorate) | Sigma-Aldrich | Cat # 42364 |
| Phusion HF DNA Polymerase | New England Biolabs | Cat # M0530L |
| **Critical commercial assays** |
| MultiSite Gateway Three-Fragment Vector Construction Kit | Thermo Fisher Scientific | Cat # 12537-023 |
| **Experimental models: Organisms/strains** |
| him-5(e1490) V | CGC | DR466 |
| C. elegans wild type | CGC | N2 |
| npr-1(ky13) X | CGC | CX4148 |
| pdfr-1(ok3425) III | CGC | VC2609 |
| pdfr-1(ok3425) III; him-5(e1490) V | This work (from DR466 and VC2609) | UR930 |
| pdf-1(tm1996) III; him-5(e1490) V | This work (from DR466 and LSC2785) | UR954 |
| pdfr-1(bx142) III; him-5(e1490) V | Barrios et al.19 | EM938 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx596[rab-3p::Cre + vha-6p::mCherry #1] | This work and Flavell et al.41 | UR1363 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx596[rab-3p::Cre + vha-6p::mCherry #2] | This work and Flavell et al.41 | UR1364 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx597[mmyo-3p::Cre + vha-6p::mCherry #1] | This work and Flavell et al.41 | UR1365 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx598[mmyo-3p::Cre + vha-6p::mCherry #2] | This work and Flavell et al.41 | UR1366 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx599[nmr-1p::Cre + gcy-28d p::Cre + vha-6p::mCherry #1] | This work and Flavell et al.41 | UR1367 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx600[nmr-1p::Cre + gcy-28d p::Cre + vha-6p::mCherry #2] | This work and Flavell et al.41 | UR1368 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx601[mod-1p::Cre +tdc-1p::Cre + glr-3::Cre + vha-6p::mCherry #1] | This work and Flavell et al.41 | UR1369 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx602[mod-1p::Cre +tdc-1p::Cre + gcy-28d p::Cre + vha-6p::mCherry #2] | This work and Flavell et al.41 | UR1370 |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
|                       | This work and Flavell et al. | UR1371 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv [pdfr-1.d-sl2-GFP]]; fsEx603[osm-6p::Cre + vha-6p::mCherry #1] | | |
|                       | This work and Flavell et al. | UR1372 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv [pdfr-1.d-sl2-GFP]]; fsEx604[osm-6p::Cre + vha-6p::mCherry #2] | | |
|                       | This work and Flavell et al. | UR1373 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv [pdfr-1.d-sl2-GFP]]; fsEx605[osm-6p::Cre+ mod-1::Cre + vha-6p::mCherry #1] | | |
|                       | This work and Flavell et al. | UR1374 |
| pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv [pdfr-1.d-sl2-GFP]]; fsEx606[osm-6p::Cre+ mod-1::Cre + vha-6p::mCherry #2] | | |
|                       | This work and Beverly et al. | UR1110 |
| him-5(e1490) V; oyls84[Pgpa-4::TU813 + Pgcy-27::TU814 + Pgcy-27::GFP + Punc-122::dsRed] | | |
|                       | This work (from UR1110 and UR930) | UR1375 |
| pdfr-1(ok3425) III; him-5(e1490) V; oyls84[Pgpa-4::TU813 + Pgcy-27::TU814 + Pgcy-27::GFP + Punc-122::dsRed] | | |
|                       | This work and T. Hirotsu | UR913 |
| daf-7(e1372) III; him-5(e1490) V | This work | |
| him-5(e1490) V; Ex[srh-281p::mCasp1 + myo-3p::GFP] | This work | |
| pdfr-1(ok3425) III; him-5(e1490) V; Ex[srh-281p::mCasp1 + myo-3p::GFP] | This work (from UR1106 and UR930) | UR1376 |
| him-8(e1489) IV | CGC | CB1489 |
| pdfr-1(ok3425) III; him-8(e1489) IV | This work (from CB1489 and VC2609) | UR1223 |
| him-8(e1489) IV; qrls2[sra-9::mCasp1] V | This work (from CB1489 and PS6025 (CGC)) | UR1216 |
| pdfr-1(ok3425) III; him-8(e1489) IV; qrls2[sra-9::mCasp1] V | This work (from UR1223 and UR1216) | UR1231 |
| daf-7(e1372) pdfr-1(ok3425) III; him-5(e1490) V | This work (from UR930 and UR913) | UR1378 |
| him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work and D. Ferkey | UR987 |
| him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work and D. Ferkey | UR1379 |
| him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work and Janssen et al. | UR1380 |
| pdfr-1(ok3425) III; him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work (from UR930 and UR987) | UR1025 |
| pdfr-1(ok3425) III; him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work (from UR987 and UR1110) | UR1381 |
| pdfr-1(ok3425) III; him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work (from UR1025 and UR1375) | UR1381 |
| pdfr-1(ok3425) III; him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work (from UR954 and UR955) | UR1388 |
| pdfr-1(ok3425) III; him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work (from UR930 and UR978) | UR1389 |
| pdfr-1(ok3425) III; him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work and Janssen et al. | UR1390 |
| pdfr-1(ok3425) III; him-5(e1490) V; udeEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)] | This work and Janssen et al. | UR1391 |

### Oligonucleotides

See Table S1 for oligonucleotide information

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Prab-3::nCre | This work | N/A |
| Pmyo-3::nCre | This work | N/A |

(Continued on next page)
**RESOURCE AVAILABILITY**

**Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Douglas Portman (douglas.portman@rochester.edu).

**Materials availability**

Plasmids and nematode strains generated in the course of this work are freely available to interested academic researchers through the Lead Contact.

**Data and code availability**

- Source data obtained in the current study have not been deposited in a public repository but are available from the Lead Contact on request.
- This study did not generate code.
- Any additional information required to reanalyze the data reported in this work paper is available from the Lead Contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

All C. elegans strains were cultured using E. coli OP50 and NGM agar as described82,83. All strains were grown at 20°C except for those containing daf-7 mutations; these were cultured at 15°C from egg to L4 stage and then assayed at 20°C. him-5(e1490) is considered the wild-type for these studies. All strains used here contained this mutation unless otherwise noted.

**METHOD DETAILS**

**Behavioral Assays**

**Quadrant assay with controlled bacterial thickness**

Quadrant assays were performed as described previously34 with the following modifications. To prepare lawns of controlled thicknesses, plates were seeded with bacterial cultures of defined concentration. To prepare these, 2 mL of E. coli OP50, freshly cultured in LB media, was collected and centrifuged (12,000 g for 30 s). After determining its mass, the bacterial pellet was vigorously resuspended in sterile water (143 μL water for each 1 mg of E. coli) to create the T3 stock. T3 stock was diluted 0.1x to create T2 stock, which was then diluted 0.1x to create T1 stock. Each assay plate was seeded with 50 μL of stock suspension and incubated at 20°C for exactly 16 hr before t = 0 min. For three random T3 stocks, bacterial density was calculated by serial dilution to be 3.5x10⁸, 8.8x10⁸, and 2.8x10⁹ cfu/mL. The variation among these samples is likely amplified by repeated serial dilution, as the lawns created with these stocks appeared identical under a stereomicroscope after 16 hours of incubation. We visually inspected lawn quality for each batch of plates before use and found this procedure to reliably produce lawns appearing like those in Figure 1B.

For each assay, 30 mins before t = 0 min, ten animals were picked to the center of each plate, with the experimenter blind to genotype. At t = 0, four 1 μL drops of ascr#3 or vehicle (an equivalent volume of ethanol diluted in sterile water) were dropped into each
of the four quadrants. At t = 30, 60, 90, and 120 mins, the number of animals in each quadrant was scored. For each assay, the overall Quadrant Chemotaxis Index (QCI) was calculated as the mean of the four QCI values.

**Foraging assay**

On the day preceding the assay, L4 hermaphrodites were picked to OP50-seeded plates (30 animals per plate) and assay plates were prepared. Guided by a custom-made transparent template, the boundaries of a center square (“source patch”) and four peripheral rectangles (“outer patches”) were drawn on the bottom of each unseeded 10-cm NGM agar plate. The shape and size of the source patch is equivalent to a single quadrant in the quadrant assay. The shape and size of each outer patch is equivalent to two adjacent quadrants. The distance from the boundary of the source patch to the nearest boundary of an outer patch is 1.8 cm. T1 or T3 bacterial stocks were made as described above. For the source patch, 12.5 μL of T1 or T3 stock suspension was dropped and carefully spread. For each outer patch, 25 μL of T3 suspension was spread. All assay plates were then incubated at 20°C for exactly 16 hr before t = 0.

At t = 0, 10 μL ascr#3 (1 μM solution) or vehicle control (ethanol diluted in sterile water) was dropped onto the source patch under the stereomicroscope. Air bubbles were created and quickly moved around on the square food lawn to cover it completely with the solution. Once the lawn was dry (3 to 5 min), 25 WT hermaphrodites (strain DR466) and 25 ASI-ablated hermaphrodites (strain UR1110) were picked into the source patch. Each plate was incubated at 20°C for 3 hr. At t = 180 min, animals in the outer patches were counted and genotyped using GFP fluorescence.

**Molecular biology and generation of transgenic strains**

To express nCre in different cells, we created a series of nCre expression plasmids. Promoters were designed from sequence information from Wormbase and were amplified from purified *C. elegans* genomic DNA using Phusion DNA polymerase (New England Biolabs). nCre expression constructs containing these promoters and the *unc-54* 3′ UTR were created using Gateway cloning (Thermo Fisher Scientific). All constructs were confirmed by Sanger sequencing. See Table S1 for primer sequences.

To create conditional *pdfr-1* rescue strains, the nCre expression plasmid was first injected into the strain UR930 with the co-injection marker P*pha-6::mCherry*(mini), a gift from K. Nehrke. The resulting transgene was then crossed into the *kyEx4648* background (CX14488, generously provided by S. Flavell and C. Bargmann) and maintained by picking hermaphrodites with both intestinal and pharyngeal mCherry signals.

All new extrachromosomal array transgenes were created by microinjection of DNA of interest at 20 ng/μL together with co-injection marker at 50 ng/μL. At least two lines were assayed for each new array. Genetic ablation strains were confirmed by DiI staining (for ablation of ADL, ASK, and ASI) or using a fluorescent marker (for ablation of ADF). Strains were genotyped by PCR and/or Sanger sequencing (see Table S1 for details).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Unless otherwise indicated, statistical significance was assessed using a two-tailed Mann-Whitney t test with unequal variances (to compare two genotypes) or Tukey’s multiple comparison test (to compare more than two genotypes) post one-way or two-way ANOVA, corresponding to the number of factors. Asterisks indicate p values associated with these tests: *p* ≤ 0.05; **p** ≤ 0.005; ***p*** ≤ 0.001. For clarity, the brackets in each graph indicate all comparisons made; those with a statistically non-significant result (p > 0.05) are shown with dashed gray lines. For each group tested in the quadrant assay, we also carried out a one-sample t test to ask whether there was a significant aversive or attractive response to ascr#3 (i.e., to ask whether the QCI was statistically different from zero). The resulting p values are indicated with circles above each violin plot: * p ≤ 0.05; ** p ≤ 0.005; *** p ≤ 0.001.