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

Jintao Luo and Douglas S. Portman*

Department of Biomedical Genetics
Del Monte Institute for Neuroscience
University of Rochester School of Medicine and Dentistry
Rochester, NY 14642

*Lead Contact and Corresponding Author: 601 Elmwood Ave., Box 633, Rochester, NY 14642; douglas.portman@rochester.edu

Keywords: *C. elegans*, behavior, decision making, neurogenetics, neuromodulation, pheromones, sexual dimorphism, foraging, sensory behavior

1 SUMMARY

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

25

26 INTRODUCTION

27 Feeding and foraging provide ideal opportunities to understand the mechanisms
28 by which neural circuits give rise to flexible innate behaviors. Despite wide variation in
29 the mechanics of foraging, the logic that guides decisions about whether to exploit or
30 abandon a food resource is remarkably conserved. In animals as diverse as bees, birds,
31 and fish, foraging decisions weigh the current benefit of feeding against the costs of
32 exploration and the potential benefits of new food sources [1, 2]. Optimal foraging
33 requires that animals assess not only local food abundance and quality but also other
34 factors, such as population density, nutritional needs, and the risk of predation, to make
35 the most advantageous decisions. Experimental studies of foraging behavior have
36 provided important insights into the neural mechanisms underlying these calculations as
37 well as the genetic mechanisms that have optimized them [3].

38 The nematode *C. elegans*, a bacterivore, feeds on microbes that colonize
39 decaying vegetation [4]. Laboratory studies have shown that *C. elegans* modulates its
40 feeding and exploratory behaviors in ways that are consistent with optimal foraging
41 theory: foraging and dispersal in adults varies with food abundance [5, 6], nutritional
42 value [7, 8], pathogenicity and toxicity [9, 10], and experience [11, 12]. Studies of wild
43 isolates have demonstrated substantial natural variation in foraging behavior, owing in
44 some cases to altered responses to sensory signals [6, 13-17]. Furthermore, foraging and
45 dispersal are sexually dimorphic and sensitive to nutritional state, as solitary males will
46 abandon a high-quality food source in search of mates, but suppress this behavior after
47 food deprivation [18-22].

48 Foraging behaviors in many species are sensitive to presence of conspecifics.
49 These can sometimes be competitors that reduce the marginal value of a crowded food
50 source, but in other contexts, collective foraging can be more advantageous than
51 solitary foraging [23]. There is evidence for both of these possibilities in *C. elegans*:
52 worms will leave a food source more often if it is crowded [17], but some pheromones
53 inhibit dispersal and exploratory behaviors [6, 16]. Further, many natural isolates feed in
54 groups [13] and theoretical models suggest that collective foraging may be favored in
55 patchy food environments [24]. These findings indicate that the role of population
56 density in *C. elegans* foraging and dispersal may not be fixed. Indeed, in the wild,
57 balancing selection can simultaneously maintain alleles that promote both strategies
58 [16, 17].

59 Because of the “boom-and-bust” life cycle of *C. elegans*, in which food
60 availability and population density can vary rapidly and dramatically [25], worms likely
61 integrate information about these two variables when assessing the current and future
62 quality of a food source. In *C. elegans*, population density is mainly signaled by
63 ascarosides, derivatives of the dideoxy sugar ascarylose that serve as a modular
64 chemical language in nematodes [26-28]. A prominent member of this group is *ascr#3*
65 (also called “C9” and “asc-ΔC9”), which, together with other ascarosides, signals

66 population density early in larval development to influence the decision to enter the
67 stress-induced dauer stage [29, 30]. *ascr#3* is also a sex pheromone, as it is produced
68 predominantly by adult hermaphrodites and elicits strong male-specific attraction [31-
69 34]. In contrast, adult hermaphrodite behavioral responses to *ascr#3* are typically
70 aversive, though this depends on the context of *ascr#3* presentation as well as animals'
71 internal state and previous experience [32, 34-38].

72 In our previous studies of sexual dimorphism in *ascr#3* responses [33], we
73 encountered variability in hermaphrodite *ascr#3* avoidance responses, leading us to
74 wonder whether flexible responses to population density signals might have adaptive
75 value. Here, by systematically varying the thickness of the food lawn, we show that
76 variability in *ascr#3* avoidance can result from food abundance. We find that the
77 conserved neuropeptide receptor PDFR-1 is necessary for this plasticity; moreover,
78 signaling by ligand(s) produced by *pdf-1* appears to integrate information about food
79 abundance into the *ascr#3* avoidance circuit. Unexpectedly, *ascr#3* avoidance in this
80 context does not require the ADL neurons, which are known to have an important role
81 in acute, off-food *ascr#3* avoidance [34, 35], but instead depend on the ASI sensory
82 neurons. The PDFR-1 signal likely acts downstream of ASI by modulating a distributed
83 set of interneurons. Interestingly, we find that this modulation is sex-specific: although
84 males possess a latent, ASI- and *pdf-1*-dependent *ascr#3* avoidance mechanism, it is
85 not modulated by food thickness. Finally, we find that this mechanism does indeed have
86 a potential role in worm fitness: *ascr#3* can promote ASI-dependent dispersal from a
87 food source, and this effect is markedly enhanced when food is sparse. Together, these
88 studies identify a neurogenetic mechanism that sex-specifically integrates two distinct
89 sensory stimuli, providing a means by which population density cues can adaptively
90 modulate feeding and foraging decisions in the adult *C. elegans* hermaphrodite.

91

92

93 RESULTS

94 *Hermaphrodite pheromone avoidance is modulated by food abundance*

95 To explore the relationship between food and pheromone signals in *C. elegans*
96 adults, we used a quadrant-format behavioral assay [33] (Figure 1A). This assay, carried
97 out in the presence of bacterial food, allows robust quantitation of medium-term
98 ("chronic," 1-2 hr) attractive and aversive responses to non-volatile chemical stimuli. To
99 control the abundance of food on the assay plate, we prepared standardized lawns of *E.*
100 *coli* OP50 of three different densities (Figure 1B; see Methods for details). "T3" is a
101 smooth, uniform lawn providing ample but not excessive food, similar to standard
102 culture conditions. "T2" is a thin, slightly patchy lawn. "T1" is a very thin lawn with many
103 food-free patches, mimicking a food source approaching depletion.

104 We first examined behavioral responses to ascr#3 under thin food conditions
105 (Figure 1C and S1). In hermaphrodites, we observed modest but consistent avoidance of
106 1–5 μM ascr#3, but no response to lower concentrations (0.1 and 0.01 μM) was seen. In
107 males, 1–5 μM ascr#3 elicited robust attraction, but there was little or no response to
108 lower concentrations (0.01 μM and 0.1 μM). Thus, as expected, hermaphrodites and
109 males exhibit qualitatively distinct responses to ascr#3 when assayed with sparse food,
110 and the concentration-dependence of these responses was similar.

111 Next, we examined ascr#3-evoked behavior under different food-abundance
112 conditions. In adult hermaphrodites, consistent ascr#3 avoidance was apparent in the
113 context of thin lawns (T1 and T2), but, surprisingly, we found that animals were
114 indifferent to this stimulus in the presence of thicker T3 food (Figure 1D). In contrast,
115 adult males displayed a robust preference for the ascr#3-containing quadrants under all
116 three conditions, with no apparent effect of food thickness (Figure 1D). Thus, food
117 thickness modulates the intensity of ascr#3 aversion in hermaphrodites but has no
118 apparent effect on ascr#3 attraction in males.

119 The modulation of ascr#3 aversion by food abundance could reflect active
120 integration of food and pheromone signals by the hermaphrodite nervous system, with
121 the appetitive value of thick food outweighing the aversive effects of ascr#3.
122 Alternatively, thick food might simply block ascr#3 detection or response. We found that
123 animals exhibited avoidance of more concentrated ascr#3 (5 μM) on thick food (Figure
124 1E), consistent with the idea that the hermaphrodite nervous system actively integrates
125 the opposing signals of food abundance and pheromone to influence navigation.

126

127 ***PDF neuropeptide signaling modulates pheromone avoidance***

128 We next sought to identify the mechanisms underlying food-dependent plasticity
129 in ascr#3 avoidance. Because the conserved neuropeptide receptor PDFR-1 [39] has
130 previously been associated with both food-dependent and sex-specific behavioral states
131 [19, 40], we examined the behavior of *pdf-1* mutants. Interestingly, on thin food, *pdf-1*
132 null mutant hermaphrodites displayed markedly enhanced ascr#3 avoidance compared
133 to controls (Figure 2A, S2A). Single null mutations in *pdf-1* and *pdf-2*, which encode
134 ligands for PDFR-1 [39], caused little change in ascr#3 avoidance. However, we observed
135 enhanced avoidance in *pdf-1; pdf-2* double mutants (Figure 2A), suggesting functional
136 redundancy between these ligands. We detected no significant difference between the
137 ascr#3 avoidance behavior of *pdf-1* and *pdf-1; pdf-2* mutants, but we cannot rule out
138 the possibility that additional ligands contribute to the regulation of PDFR-1 activity.

139 Because larval exposure to ascr#3 can alter adult ascr#3 responses [37], our
140 results could point to a role for *pdf-1* in this experience-dependent plasticity. However,
141 we found that the ascr#3 avoidance measured by the quadrant assay was independent
142 of previous ascr#3 exposure, as the loss of *daf-22*, an enzyme necessary for synthesis of

143 *ascr#3* and other short-chain ascarosides [41], did not change the behavior of WT or
144 *pdf-1* hermaphrodites (Figure S2B).

145

146 ***PDFR-1* signaling intensity likely encodes food thickness information**

147 Interestingly, we found that the *ascr#3* responses of *pdf-1* mutants were
148 insensitive to food thickness: *pdf-1* hermaphrodites showed strong aversion to *ascr#3*
149 regardless of food abundance, with no apparent difference between thin and thick food
150 conditions (Figure 2B). Because responses to *ascr#3* are also modulated by the
151 neuropeptide receptor *npr-1* [34, 35], we examined the behavior of *npr-1* mutants in
152 different food contexts. On thin food, *npr-1* hermaphrodites displayed *ascr#3* avoidance
153 comparable to WT; furthermore, *ascr#3* avoidance remained sensitive to food
154 abundance, as it was significantly blunted in the presence of thick food (Figure 2B).
155 Thus, *pdf-1*, but not *npr-1*, is required for modulation of *ascr#3* aversion by food
156 abundance.

157 Consistent with this requirement for *pdf-1*, the food-dependence of *ascr#3*
158 avoidance was eliminated in *pdf-1*; *pdf-2* double mutants (Figure 2C). Surprisingly, food-
159 dependence was also absent in *pdf-1* single mutants (Figure 2D), but it remained intact
160 in *pdf-2* mutants (Figure 2E). Thus, although ligands encoded by both genes appear to
161 have roles in the modulation of *ascr#3* aversion, *pdf-1* has a specific role in linking food
162 thickness to the strength of the aversive response.

163 We noted that the difference in *ascr#3* avoidance between WT and *pdf-1*
164 hermaphrodites appeared to be greater on thick food than on thin food (Figure 2B). This
165 suggests that the intensity of PDFR-1 signaling in WT animals might encode information
166 about food thickness. To test this, we asked whether an increase in *pdf-1* signaling
167 would be sufficient to cause the behavior of animals assayed on thin food to be more
168 like that typically seen on thick food. To bring this about, we used previously validated
169 transgenes carrying extra copies of wild-type *pdf-1* or *pdf-2* [39]. Consistent with our
170 hypothesis, we found that *pdf-1* overexpression reduced *ascr#3* avoidance on thin food
171 (Figures 2F, G). Overexpression of *pdf-2*, in contrast, had no apparent effect (Figure 2G),
172 suggesting that *pdf-1*, but perhaps not *pdf-2*, conveys food thickness information. In
173 agreement with this possibility, overexpression of *pdf-1* had no apparent effect on
174 behavior in the presence of thick food (Figure 2G). As expected, *pdf-1* was required for
175 the effect of *pdf-1* overexpression, as *pdf-1*; *Ex[pdf-1(++)]* animals behaved comparably
176 to *pdf-1* mutants in both food conditions tested (Figure 2G). These findings, together
177 with the loss-of-function data described above, indicate that the intensity of PDFR-1
178 signaling, driven primarily by *pdf-1* activity, provides an instructive internal
179 representation of food thickness that modulates *ascr#3* avoidance.

180

181 ***The ASI sensory neurons are an essential component of the ascr#3 avoidance circuit***

182 To better understand how *pdf-1* modulates *ascr#3* avoidance, we sought to
 183 characterize the neuronal requirements for this behavior. Previous studies have found
 184 that acute aversive and attractive responses to *ascr#3* in the absence of food are
 185 mediated by the sensory neurons ADL and ASK, respectively [32, 34, 35, 42]. However,
 186 we found that genetic ablation of neither of these pairs had an apparent effect on the
 187 chronic *ascr#3* avoidance measured by the quadrant assay, either in wild-type or *pdf-1*
 188 hermaphrodites (Figures 3A, B). Thus, the longer-term, on-food responses we examine
 189 here are likely mediated by mechanisms distinct from those controlling acute *ascr#3*
 190 responses in the absence of food. This is reminiscent of our previous finding that male
 191 *ascr#3* attraction in the quadrant assay depends primarily on ADF [33], rather than on
 192 ASK or the male-specific CEM neurons, both of which are important for *ascr#3* attraction
 193 in other contexts [32, 35].

194 We next considered the ASI neurons, which can detect ascarosides in other
 195 contexts, including the decision to enter the stress-resistant dauer stage [43] and the
 196 response to the exploration-suppressing pheromone *icas#8* [16, 44]. We found *ascr#3*
 197 avoidance was abolished upon ASI ablation, both in wild-type and *pdf-1* backgrounds
 198 (Figures 3C), regardless of food thickness (Figures 3D, E). The requirement for ASI in
 199 both wild-type and *pdf-1* mutants indicates that the strong *ascr#3* avoidance of *pdf-1*
 200 mutants likely results from increased activity of the same mechanism that generates
 201 avoidance in wild-type hermaphrodites.

202 These results suggested that ASI might directly detect *ascr#3*. However, using
 203 validated GCaMP transgenes and a range of pheromone concentrations, others have
 204 seen no evidence of *ascr#3*-evoked calcium responses in ASI (E. DiLoreto and J.
 205 Srinivasan, pers. comm.; K. Kim and P. Sengupta, pers. comm.). This is consistent with
 206 previous findings that ascarosides can engage signaling mechanisms that act over longer
 207 timescales and are not accompanied by calcium transients [15, 30, 44]. The simplest
 208 interpretation of our results is that ASI directly detects *ascr#3*; however, it is also
 209 possible that ASI is required downstream of *ascr#3* detection or in parallel with it.

210 We also considered the possibility that ASI detects or implements the thin-food
 211 state, since ASI-ablated hermaphrodites mimic thick-food behavior even in the thin-food
 212 environment (Figure 3C). However, this appeared not to be the case, as ASI ablation in
 213 *pdf-1* mutants eliminated pheromone avoidance on thin food, rather than
 214 recapitulating the moderate *ascr#3* repulsion typical of *pdf-1* hermaphrodites on thick
 215 food (Figure 3C).

216 To understand the role of ASI in *ascr#3* aversion, we examined the TGF β -
 217 superfamily ligand DAF-7 [45, 46], one of several neuromodulators produced by ASI.
 218 Interestingly, we found that *daf-7* mutant hermaphrodites exhibited no response to
 219 *ascr#3* on either thin or thick food, reminiscent of ASI-ablated hermaphrodites (Figure
 220 3F). Further, *daf-7* suppressed the enhanced *ascr#3* avoidance of *pdf-1* hermaphrodites

on thin food (Figure 3G). Although the behavioral variability of *pdf-1; daf-7* double mutants was exceptionally high, these results indicate that the function of ASI in *ascr#3* avoidance might be mediated by *daf-7* and that PDFR-1 might modulate the neural circuit that the *daf-7* signal engages.

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

We next sought to identify the site of action of *pdf-1*. Previous work has shown that *pdf-1* is expressed in multiple head and tail neurons, as well as peripheral tissues such as body wall muscle [19, 39, 47]. Using a previously described intersectional strategy [40], we tested the ability of neuron-specific nCre constructs, together with a conditionally activatable floxed *Ppdf-1::Inv[PDFR-1::SL2::GFP]* transgene, to rescue the enhanced *ascr#3* avoidance phenotype of *pdf-1* hermaphrodites. This transgene produces functional *pdf-1* and *GFP* transcripts only in neurons in which both the *Ppdf-1* promoter and nCre are active (Figure 4A).

Consistent with the idea that *pdf-1* acts in the nervous system, pan-neural nCre expression was sufficient to completely rescue the *pdf-1* defect, but muscle expression of nCre was not (Figures 4B, C). Next, we tested a combination of three nCre transgenes ("*tmg::nCre*," driven by *Ptdc-1*, *Pmod-1*, and *Pglr-3*) that was previously shown to provide partial rescue of the persistent dwelling behavior of *pdf-1* mutants [40]. However, we observed no rescue of the *ascr#3* avoidance phenotype 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 *pdf-1* is unlikely to function in ASI, an interpretation strengthened by the absence of a GFP signal in animals carrying an ASI/AWA-specific *Pgpa-4::nCre* transgene together with the conditional *pdf-1* transgene (data not shown).

We next generated and tested several other cell-type-specific nCre constructs in combination with the conditional *pdf-1* transgene. These strains either exhibited no detectable GFP (*Pmec-10*, touch neurons) or failed to rescue the *ascr#3* avoidance phenotype (*Pmod-1 + Posm-6*, GFP seen in several sensory neurons and interneurons; *Ptdc-1 + Pglr-3 + Pnmr-1*, GFP in many interneurons; and *Pgcy-28.d*, GFP in AIA interneurons) (data not shown). However, with a different combination, *Pgcy-28.d::nCre + Pnmr-1::nCre*, we observed partial but consistent and significant reduction of the enhanced *ascr#3* avoidance of *pdf-1* mutants in both thin-food and thick-food conditions (Figures 4F, G). 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). From these results, we conclude that PDFR-1 does not have one discrete site of action. Rather, its distributed role suggests that PDFR-1 coordinately modulates multiple components of the *ascr#3* avoidance circuit in response to PDF neuropeptides.

260

261 ***The modulatory effects of food thickness are sex-specific***

262 Males and hermaphrodites have markedly different responses to *ascr#3* [32]. In
263 males, sex-specific detection of *ascr#3* by the sex-shared ADF neurons promotes
264 attraction in the quadrant assay [33]. Consistent with this, we found that *pdfr-1* and ASI
265 are dispensable for male *ascr#3* attraction (Figures 5A, B).

266 When the influence of ADF is removed by genetic feminization or ablation, males
267 display aversive responses to *ascr#3*, similar to those of wild-type hermaphrodites [33].
268 Therefore, we asked whether this underlying aversive drive is mediated by a sex-shared
269 mechanism. Consistent with this, we found that simultaneous ablation of ADF and ASI
270 ablation eliminated all response to *ascr#3* in males (Figure 5C). Further, loss of *pdfr-1*
271 enhanced the *ascr#3* avoidance of ADF-ablated males, and this enhanced avoidance also
272 required ASI (Figure 5D). Together, these findings indicate that the *ascr#3* avoidance
273 behavior revealed by ADF ablation is mediated by a mechanism shared by both sexes.

274 Despite this common mechanism, however, we found that the behavior of ADF-
275 ablated males was not completely equivalent to that of hermaphrodites. In particular,
276 even in the presence of thick food, ADF-ablated males continued to manifest clear
277 aversion to *ascr#3* (Figure 5E). Further, we observed no effect of food thickness on
278 *ascr#3* attraction in *pdfr-1* males or on *ascr#3* aversion in ADF-ablated *pdfr-1* males
279 (Figures 5F, G). Thus, under these conditions, the modulation of *ascr#3* avoidance by
280 food thickness is a hermaphrodite-specific feature of the nervous system.

281 One possible explanation for this sex-specificity is that genetic sex might
282 functionally configure shared circuitry, such that the integration of food thickness and
283 *ascr#3* signals is enabled only in hermaphrodites. Alternatively, sex-specific components
284 of the nervous system or a signal from non-neuronal tissues might bring about this sex
285 difference. To investigate this, we examined hermaphrodites in which the nervous
286 system was genetically masculinized by pan-neuronal expression of the male sexual
287 regulator *fem-3* [48]. This manipulation has previously been shown to functionally sex-
288 reverse many behaviors mediated by shared circuits, including chemosensation and
289 locomotion [22, 33, 49-51]. To prevent masculinized ADF neurons from driving *ascr#3*
290 attraction in these hermaphrodites [33], we examined ADF-ablated *Prab-3::fem-3*
291 hermaphrodites. Interestingly, pan-neuronal masculinization did not disrupt the
292 modulatory effects of food thickness: thick food eliminated *ascr#3* aversion in these
293 animals, just as it does in WT hermaphrodites (Figure 5H). This suggests a role for sex-
294 specific neurons and/or non-neuronal tissues in programming the nervous system's
295 integration of food and pheromone signals.

296

297 ***Foraging decisions that integrate food abundance and population density cues require*** 298 ***ASI***

299 Finally, we explored the potential ecological significance of food- and *pdf-1*-
300 dependent modulation of *ascr#3* avoidance. In particular, we wondered whether this
301 plasticity might allow hermaphrodites to incorporate population density into their
302 assessment of the value of an existing food resource. When food is abundant, it might
303 be adaptive for hermaphrodites to disregard population density, allowing them to
304 rapidly exploit a plentiful resource. When food is scarce, however, population density
305 might have higher salience, as the amount of competition for a very limited resource
306 could be a key predictor of the future potential of a food patch.

307 To explore this, we measured the propensity of wild-type and ASI-ablated
308 hermaphrodites to disperse from food sources of variable quality. We designed an assay
309 in which a thin (T1) or thick (T3) food source, supplemented with vehicle control or 1
310 μ M *ascr#3*, is placed at the center of a 10 cm agar plate (Figure 6A). Surrounding this,
311 but separated from the source patch by 1.8 cm, are four rectangular patches of thick
312 food without *ascr#3*, providing favorable alternative environments. At the beginning of
313 the assay, 50 young adult hermaphrodites (25 wild-type and 25 ASI-ablated,
314 distinguishable by a fluorescent marker) were deposited on the source patch. After 3 hr,
315 we scored the number of animals of each genotype that had migrated to the peripheral
316 thick food lawns.

317 When the source was a patch of thin food, we found that relatively few animals
318 migrated out of it, regardless of whether ASI had been ablated (Figure 6B). However,
319 supplementing the source patch with *ascr#3* brought about a marked increase in
320 dispersal rate, with nearly 50% of animals migrating to the outer patches on average
321 (Figure 6B). When ASI was ablated, however, addition of *ascr#3* did not increase
322 dispersal rates above baseline (Figure 6B). Thus, consistent with our findings in the
323 quadrant assay, *ascr#3* serves as a potent driver of dispersal when food is scarce.
324 Furthermore, the effects of *ascr#3* are completely dependent on the presence of ASI.

325 When the source patch was thick food, we again observed relatively few animals
326 dispersing (Figure 6B). Adding *ascr#3* to a thick-food source patch again caused an
327 increase in dispersal, but the magnitude of this effect was far smaller than on thin food
328 (Figure 6B). Furthermore, the effect of *ascr#3* on dispersal was eliminated by ASI
329 ablation. Thus, even on thick food, *ascr#3* can be perceived as an aversive cue through
330 an ASI-dependent mechanism. However, the ability of *ascr#3* to promote dispersal from
331 a food source is markedly blunted when food is abundant.

332 Together, these results show that flexibility in *ascr#3* aversion allows
333 hermaphrodites to modulate feeding and foraging decisions according to population
334 density and food abundance, two key determinants of the quality of a food patch. The
335 ASI-dependence of this plasticity strongly suggests that it arises through the same *pdf-1*-
336 dependent integration mechanism that modulates *ascr#3* aversion in the quadrant

337 assay. Further, because it provides a means for animals to optimize their dispersal from
338 a food source, this mechanism likely has adaptive value in the wild.

339

340

341 **DISCUSSION**

342 Foraging decisions are central to an animal's survival. In the presence of a food
343 source, animals must weigh the value of continuing to exploit this resource against the
344 risks and benefits of abandoning it. Guided by genetically specified rules, this calculation
345 must consider both the quality and amount of existing food as well as its rate of
346 depletion, a primary determinant of which is local population density. Previous studies
347 have demonstrated that all of these factors can modulate the propensity of *C. elegans*
348 hermaphrodites to disperse from an existing resource [5, 6, 17]. Here, we show that the
349 *C. elegans* nervous system actively integrates information about food abundance and
350 population density to modulate behavioral decisions. Further, we identify a mechanism
351 underlying this integration and find that it enables plasticity in feeding and foraging
352 behaviors. Thus, our results provide new insight into the neural mechanisms that
353 implement context-dependent behavioral choices in *C. elegans* and the genetically
354 specified rules by which these mechanisms operate.

355 In our proposed mechanism (Figure 6C), two parallel sensory inputs of food
356 thickness and ascr#3 concentration allow hermaphrodites to modulate the behavioral
357 response to pheromones according to food thickness. When food is sparse, ascr#3
358 avoidance is manifested, but when food is more abundant, ascr#3 avoidance is
359 suppressed. Thus, population density becomes particularly influential only when food
360 depletion is imminent. This may allow hermaphrodites to disperse from a crowded,
361 rapidly depleting food source before it is completely exhausted, avoiding potential
362 starvation (Figure 6D). When food is abundant, however, population density is less
363 relevant, likely allowing animals to continue to exploit an existing resource. Thus, based
364 on environmental conditions, the hermaphrodite nervous system assigns a weight to
365 population density in its calculations about the future potential of a given food source.

366 In the context of the on-food behavioral assay used here, we find that ascr#3 is
367 detected through at least two distinct sensory mechanisms. Importantly, the circuits
368 mediating ascr#3 responses appear to be context-specific, as the behaviors elicited by
369 ascr#3 in other settings (*e.g.*, the acute drop test) have distinct neuronal and genetic
370 requirements [32, 34, 35]. Here, one of the two ascr#3 inputs depends on the ASI
371 sensory neurons and drives an aversive response. While we found no indication that ASI
372 exhibits a calcium response to ascr#3 stimulation, previous work indicates that several
373 classes of sensory neurons in *C. elegans*, including ASI, can detect ascaroside
374 pheromones directly without displaying a calcium transient [15, 30, 44]. Interestingly,
375 these responses seem to act over a longer timescale than typical calcium-based

376 neuronal signals, suggesting that they mediate “primer” effects of pheromones [16]; this
377 is consistent with the 2-hr timescale of our quadrant assay. Thus, we favor the idea that
378 the aversive drive elicited by *ascr#3* is signaled directly by ASI, but our results are also
379 consistent with the possibility that ASI is required for the effects of an *ascr#3* signal from
380 other sensory neuron(s). We find that ASI’s function depends on the TGF β -superfamily
381 signal DAF-7, which, under typical conditions, is produced in hermaphrodites
382 predominantly by ASI [45]. Whether the DAF-7 signal acts instructively or permissively
383 with respect to *ascr#3* detection will be an important question for future research.

384 Our results indicate that a separate sensory stream, acting in parallel with these
385 *ascr#3* inputs, signals food abundance. While we do not yet know where this
386 information originates, our findings strongly suggest that it is represented internally by
387 the level of activity of the neuropeptide receptor PDFR-1. Multiple lines of evidence
388 support this idea. Animals lacking *pdfr-1* function, or the function of the two ligand-
389 encoding genes *pdf-1* and *pdf-2*, are completely insensitive to the modulatory effects of
390 food thickness. These animals display constitutively strong *ascr#3* aversion that depends
391 on ASI. Because this aversion is even stronger than that seen under thin-food (T1)
392 conditions, we infer that low levels of PDFR-1 signaling are active even when animals are
393 feeding on thin food, slightly blunting the aversive response. Under thick-food (T3)
394 conditions, *pdfr-1* and *pdf-1*; *pdf-2* hermaphrodites still strongly avoid *ascr#3*, even
395 though wild-type hermaphrodites are essentially indifferent to it. This indicates that
396 when wild-type animals are feeding on thick food, high levels of PDFR-1 signaling
397 completely inhibit *ascr#3* aversion. Our results are most consistent with the idea that
398 ligands produced by both *pdf-1* and *pdf-2* are involved in this signal, but that *pdf-1*-
399 derived ligand(s) likely have a more important role. Importantly, increasing PDFR-1
400 signaling by overexpressing of *pdf-1* in animals feeding on thin food is sufficient to cause
401 them to behave as though they were in the presence of thick food—that is, they are
402 now indifferent to *ascr#3*—but overexpressing of *pdf-1* in animals feeding on thick food
403 has no effect on *ascr#3* response.

404 While a great deal of work has been done to understand internal signals related
405 to satiety and food deprivation in *C. elegans*, far less is known about how the worm’s
406 nervous system detects and encodes the *amount* of available food. Interestingly, the
407 TGF β -superfamily ligand DAF-7 is important for coupling food abundance to aging and
408 the regulation of germline progenitor proliferation [52, 53]. Our studies here indicate
409 that *daf-7* might act upstream of *pdf-1*; understanding this will be an important area of
410 future research. Further, PDF-2 has recently been implicated in linking nutritional status
411 information from the intestine to locomotor state [54], raising the intriguing possibility
412 that PDF-1 and PDF-2 communicate external and internal information about nutrition,
413 respectively, to PDFR-1.

414 We find that PDFR-1 signaling does not have a single site of action with respect
415 to the repression of *ascr#3* aversion. Rather, this signal appears to act in a distributed
416 set of interneurons to modulate motor function. Interestingly, *pdfr-1* is also known to

417 modulate the balance between roaming and dwelling motor states, another behavior
418 well known to be affected by food thickness [8, 40]. However, we found that restoring
419 *pdf-1* function to a subset of neurons that mediate these effects was unable to rescue
420 the increased *ascr#3* aversion of *pdf-1* mutants. Future work will be required to
421 understand how *pdf-1* signals modulate the *ascr#3* aversion circuit downstream of ASI.

422 How might *pdf-1* signaling be regulated by food abundance? Because *pdf-1*
423 appears to be more important for this function than *pdf-2*, we propose that the
424 secretion of one or more *pdf-1*-encoded neuropeptides is regulated by a food thickness
425 signal. Previous studies [55] as well as recent single-cell RNAseq data [47] indicate that
426 sensory neurons are not a major site of *pdf-1* expression, indicating that sensory signals
427 may be linked to *pdf-1* indirectly. Previous work suggests at least four distinct
428 mechanisms that could couple the detection of food abundance to *pdf-1* activity. The
429 physical presence of bacterial food can be detected by the deirid sensory neuron ADE
430 [56]; dopamine released by this neuron might in turn regulate PDF-1 secretion. Bacterial
431 food is also a rich source of chemical cues [57]; the detection of these by amphid
432 chemosensory neurons could indirectly regulate *pdf-1*. Further, in bacteria-rich
433 environments, the local concentrations of O₂ and CO₂ are decreased and increased,
434 respectively, allowing worms to use the activity of gas-sensing neurons as proxy signals
435 for food abundance [58-62]. *pdf-1* signaling could be downstream of such a signal. A
436 final possibility has to do with the physical structure of the sparse-food environment. In
437 our thin-food T1 conditions, bacteria are present in patches interspersed with many
438 small food-free gaps. As animals navigate this environment, the frequency with which
439 they cross food boundaries is markedly higher than in an abundant-food environment.
440 Recent work has shown that worms can use this information to assess the physical
441 structure of food patches [12]; an intriguing possibility is that this might occur at least in
442 part via modulation of PDF-1 release and/or PDFR-1 signaling.

443 In males, the previously described sex-specific ability of the ADF sensory neurons
444 to detect *ascr#3* [33] overrides ASI-dependent *ascr#3* aversion. Thus, in wild-type males,
445 ablation of ASI has no effect on *ascr#3* attraction. However, disabling the male-specific
446 function of ADF by genetic feminization or ablation reveals a latent aversion to *ascr#3*
447 [33]. Here, we find that this circuit appears to be equivalent to the one that acts in
448 hermaphrodites, in that it requires ASI and is repressed by PDFR-1 signaling.
449 Surprisingly, however, the aversive behavior driven by this mechanism is insensitive to
450 food thickness: even in the presence of thick food, ADF-ablated males displayed ASI-
451 dependent avoidance of *ascr#3*. Thus, some aspect of the food thickness signal—either
452 its existence or its ability to modulate the aversive signal—is hermaphrodite-specific.
453 Interestingly, this hermaphrodite-specific feature was not eliminated when the
454 hermaphrodite nervous system was genetically masculinized. Several possible
455 mechanisms are consistent with this finding: the integration of food abundance and
456 pheromone signals might require hermaphrodite-specific cues from outside the nervous
457 system; it might require hermaphrodite-specific neurons; or it might be blocked by
458 male-specific neurons. Further, why males lack this sensory integration is unclear. One

possibility is that, rather than encoding food thickness, *pdf-1* signaling conveys some other dimension of information in males. The important role of *pdf-1* in promoting mate-searching behavior in males [19, 63] strongly supports this possibility.

Neuromodulation is an ancient and widespread feature of nervous systems that allows external and internal information to reconfigure the properties of neural circuits [64]. Even in simple nervous systems, myriad neuromodulatory signals exist: the *C. elegans* genome, for example, contains over 100 *flp*- and *nlp*-family neuropeptide genes, each of which can produce multiple ligands [65] (worm.peptide-gpcr.org). While significant progress has been made in determining the effects of these signals on physiology and behavior, understanding the functional significance of the modulation they bring about can be more challenging. Here, we make important progress in this area by providing evidence that PDF-1/PDFR-1 signaling serves as an internal representation of food abundance that regulates the strength of the behavioral response to stimulation by *ascr#3*. In *C. elegans*, *Drosophila*, and mammals, PDF-family neuropeptides (and/or the neurons that release them) have diverse functions, but in many cases, they modulate arousal and the sensitivity to external stimuli [66-69]. Our results suggest that modulation of attention to sensory stimuli could be an ancient function of this family of neuropeptides.

METHODS

Nematode Culture

All *C. elegans* strains were cultured using *E. coli* OP50 and NGM agar as described [70, 71]. 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. See Table S1 for a complete list of strains used in this work. *him-5(e1490)* is considered the wild-type for these studies. All strains contained this mutation except as noted in Table S1.

Plasmid Constructs and 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 S2 for primer sequences.

To create conditional *pdf-1* rescue strains, the nCre. expression plasmid was first injected into the strain UR930 with the co-injection marker P_{vha-6}::mCherry(mini),

495 a gift from K. Nehrke. The resulting transgene was then crossed into the *kyEx4648*
496 background (CX14488, generously provided by S. Flavell and C. Bargmann [40]) and
497 maintained by picking hermaphrodites with both intestinal and pharyngeal mCherry
498 signals.

499 All new extrachromosomal array transgenes were created by microinjection of
500 DNA of interest at 20 ng/μL together with co-injection marker at 50 ng/μL. At least two
501 lines were assayed for each new array. Genetic ablation strains were confirmed by Dil
502 staining (for ablation of ADL, ASK, and ASI) or using a fluorescent marker (for ablation of
503 ADF). Strains were genotyped by PCR and/or Sanger sequencing (see Table S2 for
504 details).

505 ***Quadrant assay with controlled bacterial thickness***

506 Quadrant assays were performed as described previously [33] with the following
507 modifications. To prepare lawns of controlled thicknesses, plates were seeded with
508 bacterial cultures of defined concentration. To prepare these, 2 mL of *E. coli* OP50,
509 freshly cultured in LB media, was collected and centrifuged (12,000 g for 30 sec). After
510 determining the mass of the bacterial pellet, it was vigorously resuspended in sterile
511 water (143 μL water for each 1 mg of *E. coli*) to create the T3 stock. T3 stock was diluted
512 0.1x to create T2 stock, which was then diluted 0.1x to create T1 stock. Each assay plate
513 was seeded with 50 μL of stock suspension and incubated at 20°C for exactly 16 hr
514 before t = 0 min. For three random T3 stocks, bacterial density was calculated by serial
515 dilution to be 3.5x10⁸, 8.8x10⁸, and 2.8x10⁹ cfu/mL. After 16 hours of incubation, the
516 lawns created with these stocks appeared identical under a stereomicroscope.

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

523 ***Foraging assay***

524 On the day preceding the assay, L4 hermaphrodites were picked to OP50-seeded
525 plates (30 animals per plate) and assay plates were prepared. Guided by a custom-made
526 transparent template, the boundaries of a center square (“source patch”) and four
527 peripheral rectangles (“outer patches”) were drawn on the bottom of each unseeded
528 10-cm NGM agar plate. The shape and size of the source patch is equivalent to a single
529 quadrant in the quadrant assay. The shape and size of each outer patch is equivalent to
530 two adjacent quadrants. The distance from the boundary of the source patch to the
531 nearest boundary of an outer patch is 1.8 cm. T1 or T3 bacterial stocks were made as
532 described above. For the source patch, 12.5 μL of T1 or T3 stock suspension was

533 dropped and carefully spread. For each outer patch, 25 μ L of T3 suspension was spread.
534 All assay plates were then incubated at 20 °C for exactly 16 hr before $t = 0$.

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

542 **Statistical analysis**

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

554

555

556 **ACKNOWLEDGEMENTS**

557 We are grateful to current and past members of the Portman lab, the University
558 of Rochester Invertebrate Biology Group, and the Western New York Worm Group for
559 discussion and critical feedback. We are particularly grateful to E. DiLoreto and J.
560 Srinivasan, as well as K. Kim and P. Sengupta, for communicating unpublished results,
561 and to F. Schroeder for providing synthetic ascr#3. We thank S. Flavell, C. Bargmann, T.
562 Hirotsu, and D. Ferkey for generously providing transgenic strains. Some strains used in
563 this work were provided by the *Caenorhabditis* Genetics Center, which is funded by NIH
564 Office of Research Infrastructure Programs (P40 OD010440). These studies were funded
565 by NIH R01 GM130136 to D.P.

566

567

568 **AUTHOR CONTRIBUTIONS**

569 Conceptualization: J.L. and D.P.; Investigation: J.L.; Writing — Original Draft: J.L.;
570 Writing — Review & Editing: D.P.; Supervision: D.P.; Funding Acquisition: D.P.

571

572

573 DECLARATION OF INTERESTS

574 The authors declare no competing interests.

575

576

577 FIGURE LEGENDS

578 **Figure 1. Hermaphrodite avoidance of *ascr#3* depends on food abundance.** (A) The
579 quadrant-format *ascr#3* chemotaxis assay [33]. (B) Photomicrographs of the edges of
580 representative bacterial food lawns. T3, a thick food lawn; T2, a food lawn of
581 intermediate thickness; and T1, a sparse, patchy food lawn. (C) Quadrant Chemotaxis
582 Index (QCI) values for adults of both sexes in the quadrant assay in response to a range
583 of *ascr#3* concentrations in the presence of thin food (T1). (D) QCIs for adult of both
584 sexes in response to 1.0 μ M *ascr#3* in the presence of three different food abundances
585 (T1, T2, and T3). (E) QCIs for adult hermaphrodites to 1.0 and 5.0 μ M *ascr#3* in the
586 presence of thick food (T3). For all quadrant assay data shown here and in the following
587 figures, data points are colored by sex (hermaphrodites in red and males in blue) and
588 represent the QCI from a single assay containing $n = 10$ worms. Violin plots illustrate the
589 distribution of the data and are shaded according to food thickness (T1 = lighter; T3 =
590 darker). The median and 25%/75% quartile intervals are indicated by thick and thin
591 black lines, respectively. Statistical comparisons between groups are indicated with
592 black brackets and asterisks ($*p \leq 0.05$; $**p \leq 0.005$; $***p \leq 0.001$) or dotted gray
593 brackets ($p > 0.05$). Open circles above each column of data indicate the results of one-
594 sample t -tests, carried out to determine whether the observed QCI differs significantly
595 from zero ($^{\circ}p \leq 0.05$; $^{\circ\circ}p \leq 0.005$; $^{\circ\circ\circ}p \leq 0.001$).

596 **Figure 2. PDFR-1 signaling couples food abundance to the repression of *ascr#3***
597 **avoidance.** (A) QCIs for WT, *pdf-1*, *pdf-1*, *pdf-2*, and *pdf-1; pdf-2* mutant
598 hermaphrodites with T1 food. (B) QCIs for WT, *pdf-1*, and *npr-1* hermaphrodites with
599 T1 and T3 food. (C-E) QCIs for *pdf-1*; *pdf-2* (C), *pdf-1* (D), and *pdf-2* (E) mutant
600 hermaphrodites with three different food conditions. (F) QCIs for WT hermaphrodites
601 and hermaphrodites overexpressing *pdf-1* (*pdf-1(++)*) and *pdf-2* (*pdf-2(++)*) with T1 food.
602 (G) QCIs for WT, *pdf-1(++)*, *pdf-1*, and *pdf-1; pdf-1(++)* hermaphrodites with T1 and T3
603 food. See legend to Figure 1 for details of statistical analyses.

604 **Figure 3. The ASI neurons mediate *pdf-1*-dependent *ascr#3* avoidance in**
605 **hermaphrodites. (A, B)** QCI for control and ADL-ablated (ADL $\otimes$) (A) or ASK-ablated
606 (ASK $\otimes$) (B) hermaphrodites in a wild-type or *pdf-1* background with T1 food. **(C)** QCI
607 for WT and ASI-ablated (ASI $\otimes$) hermaphrodites in a wild-type or *pdf-1* background with
608 T1 food. **(D, E)** QCI for ASI $\otimes$ (D) or *pdf-1*; ASI $\otimes$ (E) hermaphrodites with three food
609 conditions. **(F)** QCI for WT, *pdf-1*, and *daf-7* hermaphrodites with T1 and T3 food. **(G)**
610 QCI for WT, *pdf-1*, *pdf-1*; ASI $\otimes$ and *pdf-1*; *daf-7* hermaphrodites with T1 food. See
611 legend to Figure 1 for details of statistical analyses.

612 **Figure 4. *pdf-1* acts in a distributed set of interneurons to repress *ascr#3* avoidance in**
613 **hermaphrodites. (A)** The strategy for Cre-based conditional rescue of *pdf-1*, developed
614 previously [40]. **(B-G)** QCI for hermaphrodites carrying the conditional *pdf-1* transgene
615 *kyEx4648* with the indicated *pdf-1* genotypes at the endogenous *pdf-1* locus (+, wild-
616 type; -, mutant) without or with transgenes driving nCre expression pan-neuronally
617 ("*Neur::nCre*") (B), in muscle ("*Musc::nCre*") (C), in a previously described set of
618 interneurons in which *pdf-1* functions to regulate motor state [40] ("*tmg::nCre*") (D), in
619 sensory neurons ("*Sens::nCre*") (E), and in AIA and neurons expressing *nmr-1* ("*AIA+nmr-1::nCre*")
620 (F, G), with T1 (B-F) or T3 (G) food. See legend to Figure 1 for details of
621 statistical analyses.

622 **Figure 5. A latent ASI-dependent *ascr#3* avoidance mechanism in males is independent**
623 **of food abundance. (A, B)** QCI for WT and *pdf-1* (A) or ASI $\otimes$ (B) males with T1 food. **(C)**
624 QCI for ADF $\otimes$ and ADF $\otimes$; ASI $\otimes$ males with T1 food. **(D)** QCI for ADF $\otimes$, ADF $\otimes$; *pdf-1*,
625 ADF $\otimes$; ASI $\otimes$, and *pdf-1*; ADF $\otimes$; ASI $\otimes$ males with T1 food. **(E-G)** QCI for ADF $\otimes$ (E), *pdf-1*
626 (F), and ADF $\otimes$; *pdf-1* (G) males with T1, T2, and T3 food. **(H)** QCI for WT
627 hermaphrodites, ADF $\otimes$ hermaphrodites, ADF $\otimes$ hermaphrodites carrying the pan-
628 neuronal masculinization transgene *Prab-3::fem-3*, and ADF $\otimes$ males with T1 and T3
629 food. See legend to Figure 1 for details of statistical analyses.

630 **Figure 6. Modulation of *ascr#3* aversion by food thickness enables context-dependent**
631 **flexibility in *C. elegans* foraging decisions. (A)** The behavioral assay used to measure
632 the effects of *ascr#3* and food thickness on foraging decisions. See text for details. **(B)**
633 Foraging rates, shown as the frequency of animals dispersing to the outer food sources,
634 of WT and ASI $\otimes$ hermaphrodites when placed on a source patch of thick (T3) or thin (T1)
635 food, without (-) or with (+) *ascr#3*. Each data points represents a single genotype in a
636 single assay containing *n* = 25 worms per genotype. Filled and open circles indicate
637 control and ASI-ablated animals, respectively. Violin plots illustrate the distribution of
638 the data and are shaded according to food thickness at the source patch (T1 = lighter; T3
639 = darker). The median and 25%/75% quartile intervals are indicated by thick and thin
640 black lines, respectively. Statistical comparisons between groups are indicated with
641 black brackets and asterisks (**p* $\leq$ 0.05; ***p* $\leq$ 0.005; ****p* $\leq$ 0.001) or dotted gray
642 brackets (*p* > 0.05). **(C)** A neural circuit model showing the parallel inputs of food
643 abundance, *ascr#3* via ASI (a non-sex-specific input that population density), and *ascr#3*
644 via ADF (a male-specific input that mate availability). Hermaphrodite- and male-specific

645 aspects are shown in red and blue, respectively. **(D)** A proposed model illustrating the
 646 adaptive value of context-dependent flexibility in *C. elegans* foraging behavior based on
 647 the integration of food-thickness and population density information via *ascr#3*. See
 648 text for details.

649 **Figure S1. Supplementary data related to Figure 1. (A, B)** QCI for WT hermaphrodites
 650 (A) and males (B) in response to 1.0 μ M *ascr#3* with T1 food. QCI values from a given
 651 assay are connected across timepoints by a line. The violin plot at the right shows
 652 average QCI values (the mean of the four timepoints for each assay). These data are
 653 taken from Figure 1D.

654 **Figure S2. Supplementary data related to Figure 2. (A)** QCI for WT, *pdf-1(ok3425)*, and
 655 *pdf-1(bx142)* hermaphrodites with T1 food. **(B)** QCI for WT, *daf-22*, *pdf-1*, and *daf-22*;
 656 *pdf-1* hermaphrodites with T1 food. See legend to Figure 1 for details on statistical
 657 analyses.

658

659

660 REFERENCES

661

- 662 1. Charnov, E.L. (1976). Optimal foraging, the marginal value theorem. *Theor Popul Biol* 9, 129-
 663 136.
- 664 2. Pyke, G.H. (1984). Optimal Foraging Theory: A Critical Review. *Annual Review of Ecology and*
 665 *Systematics* 15, 523-575.
- 666 3. Calhoun, A.J., and Hayden, B.Y. (2015). The foraging brain. *Current Opinion in Behavioral*
 667 *Sciences* 5, 24-31.
- 668 4. Frezal, L., and Felix, M.A. (2015). *C. elegans* outside the Petri dish. *Elife* 4.
- 669 5. Milward, K., Busch, K.E., Murphy, R.J., de Bono, M., and Olofsson, B. (2011). Neuronal and
 670 molecular substrates for optimal foraging in *Caenorhabditis elegans*. *Proceedings of the*
 671 *National Academy of Sciences of the United States of America* 108, 20672-20677.
- 672 6. Harvey, S.C. (2009). Non-dauer larval dispersal in *Caenorhabditis elegans*. *J Exp Zool B Mol*
 673 *Dev Evol* 312B, 224-230.
- 674 7. Shtonda, B.B., and Avery, L. (2006). Dietary choice behavior in *Caenorhabditis elegans*. *The*
 675 *Journal of experimental biology* 209, 89-102.
- 676 8. Ben Arous, J., Laffont, S., and Chatenay, D. (2009). Molecular and sensory basis of a food
 677 related two-state behavior in *C. elegans*. *PloS one* 4, e7584.

- 678 9. Zhang, Y., Lu, H., and Bargmann, C. (2005). Pathogenic bacteria induce aversive olfactory
679 learning in *Caenorhabditis elegans*. *Nature* 438, 179-184.
- 680 10. Melo, J.A., and Ruvkun, G. (2012). Inactivation of conserved *C. elegans* genes engages
681 pathogen- and xenobiotic-associated defenses. *Cell* 149, 452-466.
- 682 11. Pradhan, S., Quilez, S., Homer, K., and Hendricks, M. (2019). Environmental Programming of
683 Adult Foraging Behavior in *C. elegans*. *Current biology : CB* 29, 2867-2879 e2864.
- 684 12. Calhoun, A.J., Tong, A., Pokala, N., Fitzpatrick, J.A., Sharpee, T.O., and Chalasani, S.H. (2015).
685 Neural Mechanisms for Evaluating Environmental Variability in *Caenorhabditis elegans*.
686 *Neuron* 86, 428-441.
- 687 13. de Bono, M., and Bargmann, C.I. (1998). Natural variation in a neuropeptide Y receptor
688 homolog modifies social behavior and food response in *C. elegans*. *Cell* 94, 679-689.
- 689 14. Bendesky, A., Tsunozaki, M., Rockman, M.V., Kruglyak, L., and Bargmann, C.I. (2011).
690 Catecholamine receptor polymorphisms affect decision-making in *C. elegans*. *Nature* 472,
691 313-318.
- 692 15. Greene, J.S., Dobosiewicz, M., Butcher, R.A., McGrath, P.T., and Bargmann, C.I. (2016).
693 Regulatory changes in two chemoreceptor genes contribute to a *Caenorhabditis elegans* QTL
694 for foraging behavior. *Elife* 5.
- 695 16. Greene, J.S., Brown, M., Dobosiewicz, M., Ishida, I.G., Macosko, E.Z., Zhang, X., Butcher,
696 R.A., Cline, D.J., McGrath, P.T., and Bargmann, C.I. (2016). Balancing selection shapes
697 density-dependent foraging behaviour. *Nature* 539, 254-258.
- 698 17. Gloria-Soria, A., and Azevedo, R.B. (2008). *npr-1* Regulates foraging and dispersal strategies
699 in *Caenorhabditis elegans*. *Current biology : CB* 18, 1694-1699.
- 700 18. Lipton, J., Kleemann, G., Ghosh, R., Lints, R., and Emmons, S.W. (2004). Mate searching in
701 *Caenorhabditis elegans*: a genetic model for sex drive in a simple invertebrate. *The Journal of*
702 *neuroscience : the official journal of the Society for Neuroscience* 24, 7427-7434.
- 703 19. Barrios, A., Ghosh, R., Fang, C., Emmons, S.W., and Barr, M.M. (2012). PDF-1 neuropeptide
704 signaling modulates a neural circuit for mate-searching behavior in *C. elegans*. *Nature*
705 *neuroscience* 15, 1675-1682.
- 706 20. Barrios, A., Nurrish, S., and Emmons, S.W. (2008). Sensory regulation of *C. elegans* male
707 mate-searching behavior. *Current biology : CB* 18, 1865-1871.
- 708 21. Wexler, L.R., Miller, R.M., and Portman, D.S. (2020). *C. elegans* Males Integrate Food Signals
709 and Biological Sex to Modulate State-Dependent Chemosensation and Behavioral
710 Prioritization. *Current biology : CB*.
- 711 22. Ryan, D.A., Miller, R.M., Lee, K., Neal, S.J., Fagan, K.A., Sengupta, P., and Portman, D.S.
712 (2014). Sex, age, and hunger regulate behavioral prioritization through dynamic modulation
713 of chemoreceptor expression. *Current biology : CB* 24, 2509-2517.

- 714 23. Giraldeau, L.-A., and Caraco, T. (2000). Social foraging theory, (Princeton, N.J.: Princeton
715 University Press).
- 716 24. Ding, S.S., Muhle, L.S., Brown, A.E.X., Schumacher, L.J., and Endres, R.G. (2020). Comparison
717 of solitary and collective foraging strategies of *Caenorhabditis elegans* in patchy food
718 distributions. *Philos Trans R Soc Lond B Biol Sci* 375, 20190382.
- 719 25. Felix, M.A., and Duveau, F. (2012). Population dynamics and habitat sharing of natural
720 populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC biology* 10, 59.
- 721 26. Ludewig, A.H., and Schroeder, F.C. (2013). Ascaroside signaling in *C. elegans*. *WormBook* :
722 the online review of *C. elegans* biology, 1-22.
- 723 27. Park, J.Y., Joo, H.J., Park, S., and Paik, Y.K. (2019). Ascaroside Pheromones: Chemical Biology
724 and Pleiotropic Neuronal Functions. *Int J Mol Sci* 20.
- 725 28. McGrath, P.T., and Ruvinsky, I. (2019). A primer on pheromone signaling in *Caenorhabditis*
726 *elegans* for systems biologists. *Curr Opin Syst Biol* 13, 23-30.
- 727 29. Butcher, R.A., Fujita, M., Schroeder, F.C., and Clardy, J. (2007). Small-molecule pheromones
728 that control dauer development in *Caenorhabditis elegans*. *Nature chemical biology* 3, 420-
729 422.
- 730 30. Kim, K., Sato, K., Shibuya, M., Zeiger, D.M., Butcher, R.A., Ragains, J.R., Clardy, J., Touhara,
731 K., and Sengupta, P. (2009). Two chemoreceptors mediate developmental effects of dauer
732 pheromone in *C. elegans*. *Science* 326, 994-998.
- 733 31. Narayan, A., Venkatachalam, V., Durak, O., Reilly, D.K., Bose, N., Schroeder, F.C., Samuel,
734 A.D., Srinivasan, J., and Sternberg, P.W. (2016). Contrasting responses within a single neuron
735 class enable sex-specific attraction in *Caenorhabditis elegans*. *Proceedings of the National*
736 *Academy of Sciences of the United States of America* 113, E1392-1401.
- 737 32. Srinivasan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H., Teal, P., Malik, R., Edison, A.,
738 Sternberg, P., and Schroeder, F. (2008). A blend of small molecules regulates both mating
739 and development in *Caenorhabditis elegans*. *Nature* 454, 1115-1118.
- 740 33. Fagan, K.A., Luo, J., Lagoy, R.C., Schroeder, F.C., Albrecht, D.R., and Portman, D.S. (2018). A
741 Single-Neuron Chemosensory Switch Determines the Valence of a Sexually Dimorphic
742 Sensory Behavior. *Current biology* : CB 28, 902-914 e905.
- 743 34. Jang, H., Kim, K., Neal, S.J., Macosko, E., Kim, D., Butcher, R.A., Zeiger, D.M., Bargmann, C.I.,
744 and Sengupta, P. (2012). Neuromodulatory state and sex specify alternative behaviors
745 through antagonistic synaptic pathways in *C. elegans*. *Neuron* 75, 585-592.
- 746 35. Macosko, E.Z., Pokala, N., Feinberg, E.H., Chalasani, S.H., Butcher, R.A., Clardy, J., and
747 Bargmann, C.I. (2009). A hub-and-spoke circuit drives pheromone attraction and social
748 behaviour in *C. elegans*. *Nature* 458, 1171-1175.

- 749 36. Fenk, L.A., and de Bono, M. (2017). Memory of recent oxygen experience switches
750 pheromone valence in *Caenorhabditis elegans*. *Proceedings of the National Academy of*
751 *Sciences of the United States of America* *114*, 4195-4200.
- 752 37. Hong, M., Ryu, L., Ow, M.C., Kim, J., Je, A.R., Chinta, S., Huh, Y.H., Lee, K.J., Butcher, R.A.,
753 Choi, H., et al. (2017). Early Pheromone Experience Modifies a Synaptic Activity to Influence
754 Adult Pheromone Responses of *C. elegans*. *Current biology : CB* *27*, 3168-3177 e3163.
- 755 38. Ryu, L., Cheon, Y., Huh, Y.H., Pyo, S., Chinta, S., Choi, H., Butcher, R.A., and Kim, K. (2018).
756 Feeding state regulates pheromone-mediated avoidance behavior via the insulin signaling
757 pathway in *Caenorhabditis elegans*. *The EMBO journal* *37*, e98402.
- 758 39. Janssen, T., Husson, S.J., Lindemans, M., Mertens, I., Rademakers, S., Ver Donck, K., Geysen,
759 J., Jansen, G., and Schoofs, L. (2008). Functional characterization of three G protein-coupled
760 receptors for pigment dispersing factors in *Caenorhabditis elegans*. *J Biol Chem* *283*, 15241-
761 15249.
- 762 40. Flavell, S.W., Pokala, N., Macosko, E.Z., Albrecht, D.R., Larsch, J., and Bargmann, C.I. (2013).
763 Serotonin and the neuropeptide PDF initiate and extend opposing behavioral states in *C.*
764 *elegans*. *Cell* *154*, 1023-1035.
- 765 41. Pungaliya, C., Srinivasan, J., Fox, B.W., Malik, R.U., Ludewig, A.H., Sternberg, P.W., and
766 Schroeder, F.C. (2009). A shortcut to identifying small molecule signals that regulate
767 behavior and development in *Caenorhabditis elegans*. *Proceedings of the National Academy*
768 *of Sciences of the United States of America* *106*, 7708-7713.
- 769 42. Srinivasan, J., von Reuss, S.H., Bose, N., Zaslaver, A., Mahanti, P., Ho, M.C., O'Doherty, O.G.,
770 Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2012). A modular library of small
771 molecule signals regulates social behaviors in *Caenorhabditis elegans*. *PLoS biology* *10*,
772 e1001237.
- 773 43. Bargmann, C.I., and Horvitz, H.R. (1991). Control of larval development by chemosensory
774 neurons in *Caenorhabditis elegans*. *Science* *251*, 1243-1246.
- 775 44. McGrath, P.T., Xu, Y., Ailion, M., Garrison, J.L., Butcher, R.A., and Bargmann, C.I. (2011).
776 Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor
777 genes. *Nature* *477*, 321-325.
- 778 45. Ren, P., Lim, C.S., Johnsen, R., Albert, P.S., Pilgrim, D., and Riddle, D.L. (1996). Control of *C.*
779 *elegans* larval development by neuronal expression of a TGF-beta homolog. *Science* *274*,
780 1389-1391.
- 781 46. Schackwitz, W.S., Inoue, T., and Thomas, J.H. (1996). Chemosensory neurons function in
782 parallel to mediate a pheromone response in *C. elegans*. *Neuron* *17*, 719-728.
- 783 47. Taylor, S.R., Santpere, G., Weinreb, A., Barrett, A., Reilly, M.B., Xu, C., Varol, E., Oikonomou,
784 P., Glenwinkel, L., McWhirter, R., et al. (2020). Molecular topography of an entire nervous
785 system. *bioRxiv*, 2020.2012.2015.422897.

786 48. Mehra, A., Gaudet, J., Heck, L., Kuwabara, P.E., and Spence, A.M. (1999). Negative regulation
787 of male development in *Caenorhabditis elegans* by a protein-protein interaction between
788 TRA-2A and FEM-3. *Genes Dev* 13, 1453-1463.

789 49. Mowrey, W.R., Bennett, J.R., and Portman, D.S. (2014). Distributed Effects of Biological Sex
790 Define Sex-Typical Motor Behavior in *Caenorhabditis elegans*. *The Journal of neuroscience :*
791 *the official journal of the Society for Neuroscience* 34, 1579-1591.

792 50. Lee, K., and Portman, D. (2007). Neural sex modifies the function of a *C. elegans* sensory
793 circuit. *Current biology : CB* 17, 1858-1863.

794 51. White, J., Nicholas, T., Gritton, J., Truong, L., Davidson, E., and Jorgensen, E. (2007). The
795 sensory circuitry for sexual attraction in *C. elegans* males. *Current biology : CB* 17, 1847-
796 1857.

797 52. Entchev, E.V., Patel, D.S., Zhan, M., Steele, A.J., Lu, H., and Ch'ng, Q. (2015). A gene-
798 expression-based neural code for food abundance that modulates lifespan. *Elife* 4, e06259.

799 53. Dalfo, D., Michaelson, D., and Hubbard, E.J. (2012). Sensory regulation of the *C. elegans*
800 germline through TGF-beta-dependent signaling in the niche. *Current biology : CB* 22, 712-
801 719.

802 54. O'Donnell, M.P., Chao, P.H., Kammenga, J.E., and Sengupta, P. (2018). Rictor/TORC2
803 mediates gut-to-brain signaling in the regulation of phenotypic plasticity in *C. elegans*. *PLoS*
804 *genetics* 14, e1007213.

805 55. Janssen, T., Husson, S.J., Meelkop, E., Temmerman, L., Lindemans, M., Verstraelen, K.,
806 Rademakers, S., Mertens, I., Nitabach, M., Jansen, G., et al. (2009). Discovery and
807 characterization of a conserved pigment dispersing factor-like neuropeptide pathway in
808 *Caenorhabditis elegans*. *J Neurochem* 111, 228-241.

809 56. Sawin, E.R., Ranganathan, R., and Horvitz, H.R. (2000). *C. elegans* locomotory rate is
810 modulated by the environment through a dopaminergic pathway and by experience through
811 a serotonergic pathway. *Neuron* 26, 619-631.

812 57. Worthy, S.E., Haynes, L., Chambers, M., Bethune, D., Kan, E., Chung, K., Ota, R., Taylor, C.J.,
813 and Glater, E.E. (2018). Identification of attractive odorants released by preferred bacterial
814 food found in the natural habitats of *C. elegans*. *PloS one* 13, e0201158.

815 58. Witham, E., Comunian, C., Ratanpal, H., Skora, S., Zimmer, M., and Srinivasan, S. (2016). *C.*
816 *elegans* Body Cavity Neurons Are Homeostatic Sensors that Integrate Fluctuations in Oxygen
817 Availability and Internal Nutrient Reserves. *Cell Rep* 14, 1641-1654.

818 59. Gray, J.M., Karow, D.S., Lu, H., Chang, A.J., Chang, J.S., Ellis, R.E., Marletta, M.A., and
819 Bargmann, C.I. (2004). Oxygen sensation and social feeding mediated by a *C. elegans*
820 guanylate cyclase homologue. *Nature* 430, 317-322.

- 821 60. Cheung, B.H., Cohen, M., Rogers, C., Albayram, O., and de Bono, M. (2005). Experience-
822 dependent modulation of *C. elegans* behavior by ambient oxygen. *Current biology : CB* 15,
823 905-917.
- 824 61. Hallem, E.A., Spencer, W.C., McWhirter, R.D., Zeller, G., Henz, S.R., Ratsch, G., Miller, D.M.,
825 3rd, Horvitz, H.R., Sternberg, P.W., and Ringstad, N. (2011). Receptor-type guanylate cyclase
826 is required for carbon dioxide sensation by *Caenorhabditis elegans*. *Proceedings of the*
827 *National Academy of Sciences of the United States of America* 108, 254-259.
- 828 62. Hallem, E.A., and Sternberg, P.W. (2008). Acute carbon dioxide avoidance in *Caenorhabditis*
829 *elegans*. *Proceedings of the National Academy of Sciences of the United States of America*
830 105, 8038-8043.
- 831 63. Hilbert, Z.A., and Kim, D.H. (2018). PDF-1 neuropeptide signaling regulates sexually
832 dimorphic gene expression in shared sensory neurons of *C. elegans*. *Elife* 7, e36547.
- 833 64. Marder, E., O'Leary, T., and Shruti, S. (2014). Neuromodulation of circuits with variable
834 parameters: single neurons and small circuits reveal principles of state-dependent and
835 robust neuromodulation. *Annu Rev Neurosci* 37, 329-346.
- 836 65. Hobert, O. (2013). The neuronal genome of *Caenorhabditis elegans*. *WormBook : the online*
837 *review of C. elegans biology*, 1-106.
- 838 66. Ardiel, E.L., Yu, A.J., Giles, A.C., and Rankin, C.H. (2017). Habituation as an adaptive shift in
839 response strategy mediated by neuropeptides. *NPJ Sci Learn* 2, 9.
- 840 67. Choi, S., Chatzigeorgiou, M., Taylor, K.P., Schafer, W.R., and Kaplan, J.M. (2013). Analysis of
841 NPR-1 Reveals a Circuit Mechanism for Behavioral Quiescence in *C. elegans*. *Neuron* 78, 869-
842 880.
- 843 68. Fu, Y., Tucciarone, J.M., Espinosa, J.S., Sheng, N., Darcy, D.P., Nicoll, R.A., Huang, Z.J., and
844 Stryker, M.P. (2014). A cortical circuit for gain control by behavioral state. *Cell* 156, 1139-
845 1152.
- 846 69. Dubowy, C., and Sehgal, A. (2017). Circadian Rhythms and Sleep in *Drosophila melanogaster*.
847 *Genetics* 205, 1373-1397.
- 848 70. Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71-94.
- 849 71. Stiernagle, T. (2006). Maintenance of *C. elegans*. *WormBook : the online review of C.*
850 *elegans biology*, 1-11.
- 851 72. Meelkop, E., Temmerman, L., Janssen, T., Suetens, N., Beets, I., Van Rompay, L.,
852 Shanmugam, N., Husson, S.J., and Schoofs, L. (2012). PDF receptor signaling in
853 *Caenorhabditis elegans* modulates locomotion and egg-laying. *Molecular and cellular*
854 *endocrinology* 361, 232-240.
- 855 73. Beverly, M., Anbil, S., and Sengupta, P. (2011). Degeneracy and neuromodulation among
856 thermosensory neurons contribute to robust thermosensory behaviors in *Caenorhabditis*

857 elegans. The Journal of neuroscience : the official journal of the Society for Neuroscience 31,
858 11718-11727.

859 74. Hamakawa, M., Uozumi, T., Ueda, N., Iino, Y., and Hirotsu, T. (2015). A role for Ras in
860 inhibiting circular foraging behavior as revealed by a new method for time and cell-specific
861 RNAi. BMC biology 13, 6.

862 75. Krzyzanowski, M.C., Woldemariam, S., Wood, J.F., Chaubey, A.H., Brueggemann, C., Bowitch,
863 A., Bethke, M., L'Etoile, N.D., and Ferkey, D.M. (2016). Aversive Behavior in the Nematode *C.*
864 *elegans* Is Modulated by cGMP and a Neuronal Gap Junction Network. PLoS genetics 12,
865 e1006153.
866
867

868 Table S1. Strains used in this work.

Strain	Genotype	Figure	Source
DR466	<i>him-5(e1490) V</i>		CGC
N2	<i>C. elegans</i> wild isolate		CGC
CX4148	<i>npr-1(ky13) X</i>		CGC [13]
UR699	<i>cat-2(e1112) II</i>		CGC
VC2609	<i>pdfr-1(ok3425) III</i>		CGC
UR930	<i>pdfr-1(ok3425) III; him-5(e1490) V</i>		This work (from DR466 and VC2609)
UR954	<i>pdf-1(tm1996) III; him-5(e1490) V</i>		This work (from DR466 and LSC27 [39])
UR955	<i>him-5(e1490) V; pdf-2(tm4780) X</i>		This work and [72]
UR1360	<i>pdf-1(tm1996) III; him-5(e1490) V; pdf-2(tm4780) X</i>		This work (from UR954 and UR955)
UR1361	<i>him-5(e1490) V; lstEx2[PDF-1(+); elt-2::GFP]</i>		This work (from DR466 and LSC84)
LSC84	<i>lstEx2[pdf-1p::pdf-1::3'UTR + elt-2p::GFP]</i>		CGC and [39]
LSC54	<i>lstEx3[pdf-2p::pdf-2::3'UTR + elt-2p::GFP]</i>		CGC and [39]
UR978	<i>daf-22(m130) II; him-5(e1490) V</i>		This work (from DR466 and DR476, both from CGC)
UR1362	<i>daf-22(m130) II; pdfr-1(ok3425) III; him-5(e1490) V</i>		This work (from UR930 and UR978)
UR1363	<i>pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx595[rab-3p::Cre + vha-6p::mCherry #1]</i>		This work and [40]
UR1364	<i>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]</i>		This study and [40]
UR1365	<i>pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx597[myo-3p::Cre + vha-6p::mCherry #1]</i>		This study and [40]
UR1366	<i>pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx598[myo-3p::Cre + vha-6p::mCherry #2]</i>		This study and [40]
UR1367	<i>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]</i>		This study and [40]
UR1368	<i>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]</i>		This study and [40]
EM938	<i>pdfr-1(bx142) III; him-5(e1490) V</i>		[19]
UR1369	<i>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]</i>		This study and [40]
UR1370	<i>pdfr-1(ok3425) III; him-5(e1490) V; kyEx4648[pdfr-1::inv[pdfr-1.d-sl2-GFP]]; fsEx602[mod-1p::Cre + tdc-1p::Cre + glr-3::Cre + vha-6p::mCherry #2]</i>		This study and [40]
UR1371	<i>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]</i>		This study and [40]
UR1372	<i>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]</i>		This study and [40]

UR1373	<i>pdf-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]</i>		This study and [40]
UR1374	<i>pdf-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]</i>		This study and [40]
UR1110	<i>him-5(e1490) V; oyls84[Pgpa-4::TU#813 + Pgcy-27::TU#814 + Pgcy-27::GFP + Punc-122::dsRed]</i>		This study (from DR466 and PY7505 [73])
UR1375	<i>pdf-1(ok3425) III; him-5(e1490) V; oyls84[Pgpa-4::TU#813 + Pgcy-27::TU#814 + Pgcy-27::GFP + Punc-122::dsRed]</i>		This work (from UR1110 and UR930)
UR913	<i>daf-7(e1372) III; him-5(e1490) V</i>		This study
UR1106	<i>him-5(e1490) V; Ex[srh-281p::mCasp1 + myo-3p::GFP]</i>		This study and [74]
UR1376	<i>pdf-1(ok3425) III; him-5(e1490) V; Ex[srh-281p::mCasp1 + myo-3p::GFP]</i>		This study (from UR1106 and UR930)
CB1489	<i>him-8(e1489) IV</i>		CGC
UR1223	<i>pdf-1(ok3425) III; him-8(e1489) IV</i>		This study (from CB1489 and VC2609)
UR1216	<i>him-8(e1489) IV; qrls2[sra-9::mCasp1] V</i>		This study (from CB1489 and PS6025 (CGC))
UR1231	<i>pdf-1(ok3425) III; him-8(e1489) IV; qrls2[sra-9::mCasp1] V</i>		This study (from UR1223 and UR1216)
UR1378	<i>daf-7(e1372) pdf-1(ok3425) III; him-5(e1490) V</i>		This study (from UR930 and UR913)
UR987	<i>him-5(e1490) V; udEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)]</i>		This study and [75]
UR1379	<i>him-5(e1490) fsIs15[rab-3p::FEM-3(+):outtron::mCherry + unc-122p::GFP] V; udEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)]</i>		This study and [22, 75]
UR1025	<i>pdf-1(ok3425) III; him-5(e1490) V; udEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)]</i>		This study (from UR930 and UR987)
UR1380	<i>him-5(e1490) V; oyls84[Pgpa-4::TU#813 + Pgcy-27::TU#814 + Pgcy-27::GFP + Punc-122::dsRed]; udEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)]</i>		This study (from UR987 and UR1110)
UR1381	<i>pdf-1(ok3425) III; him-5(e1490) V; oyls84[Pgpa-4::TU#813 + Pgcy-27::TU#814 + Pgcy-27::GFP + Punc-122::dsRed]; udEx428[elt-2p::GFP + srh-142p::GFP + srh-142p::CED-3(p15) + srh-142p::CED-3(p17)]</i>		This study (from UR1025 and UR987)
UR1388	<i>pdf-1(tm1996) III; him-5(e1490) V; pdf-2(tm4780) X</i>		This study (from UR954 and UR955)
UR1389	<i>daf-22(m130) II; pdf-1(ok3425) III; him-5(e1490) V</i>		This study (from UR930 and UR978)
UR1390	<i>him-5(e1490) V; lStEx2[pdf-1p::pdf-1::3'UTR + elt-2::GFP]</i>		This study and [39]
UR1391	<i>pdf-1(ok3425) III; him-5(e1490) V; lStEx2[pdf-1p::pdf-1::3'UTR + elt-2::GFP]</i>		This study and [39]

869

870

871 Table S2. Oligonucleotide primers used in this work.

Genotyping primers	Sequence of primer (5'->3')	Method
<i>pdf-1(ok3425)</i>	F1: GTGATCCTCGTCGAAGTTCG	Three-Primer PCR
	F2: AACGTTACCGTATCCAGCAATG	
	R1: TTCCGAAATCTGCTGCATTAC	
<i>pdf-1(tm1996)</i>	F1: GACCTTCGAGTGAAGTCGA	Three-Primer PCR
	F2: CTCAGGAATAGGAGTGCTCC	
	R1: TCAAGGTCTCGCCTCTAGGA	
<i>pdf-2(tm4780)</i>	F1: CCGCAGTAGCACATCTCTCA	Three-Primer PCR
	F2: CAGATGCGTTACGGCAACTC	
	R1: GTTTCGGTGTACAGGTGAC	
<i>daf-22(m130)</i>	F1: CATCCGTGTTGCTGAGAA	PCR, Sanger sequencing
	R1: TGGACTGTGCAGCTCCAG	
<i>daf-7(e1372)</i>	F1: CAATGGACCCAGAAATGAAGTC	PCR, <i>EcoRV</i> digestion
	R1: GTTGAATGCTGATACGTTCCGA	
Promoter amplification primers	Sequence of primer (5'->3') (only annealing region is shown)	
<i>rab-3p</i>	F: ATCTTCAGATGGGAGCAGTGGACTG R: TCTACAGTAGCCCTATTTTCAGATG	
<i>myo-3p</i>	F: CCCGACAAAACATGAGTATTTCTTT R: CCCACGACCACTAGATCCATCTAGA	
<i>nmr-1p</i>	F: TTCTGAAAGTATAAGATTGACTGA R: CGACAACTTTAGTTTGTACAGA	
<i>gcy-28d p</i>	F: TACAATTGTAGTGAGCTTCGAATTT R: AATGGAATGGTGAGATGAGTGCGAA	
<i>mod-1p</i>	F: GTCCTTTAACTTTCTGCTAAAAGTG R: GGTGCCAATGCGGTGAAAGAAAATT	
<i>tdc-1p</i>	F: CGGAAATTTAGGTAACCAAGAAGC R: TTCAGGACCGCCCAATGGTTTACG	
<i>glr-3p</i>	F: TCGGAAATGCGGAAGTCTTTCCGA R: AATCTTCAATATTTGCTATTAACAT	
<i>osm-6p</i>	F: TGAATGGAAGACGAGAGACAGTTGG R: CTATTACCTTCATTAGTATACATCT	

872

873

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure S1

Figure S2