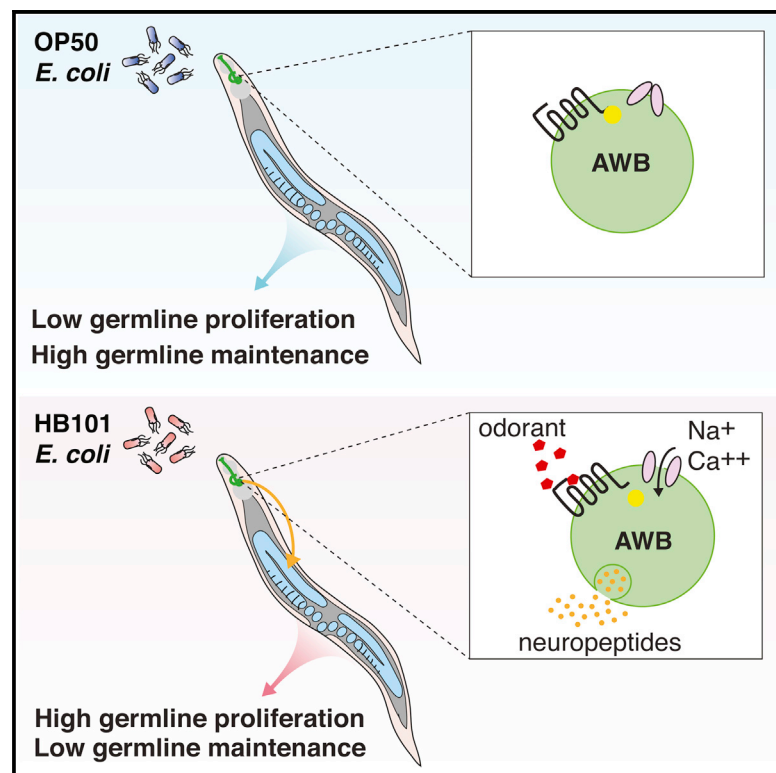


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Olfaction Modulates Reproductive Plasticity through Neuroendocrine Signaling in *Caenorhabditis elegans*

Graphical Abstract



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In Brief

Sowa et al. show that AWB olfactory neurons perceive odorant signals to adjust reproductive timing and senescence via neuropeptide signaling, revealing a neuroendocrine link between olfactory sensation and reproductive plasticity and the power of smell to regulate reproductive aging.

Highlights

- Animals adjust reproductive strategy in response to environmental variations
- AWB olfactory neurons regulate reproductive plasticity
- Neuropeptide signaling mediates the olfactory regulation of reproductive aging



Olfaction Modulates Reproductive Plasticity through Neuroendocrine Signaling in *Caenorhabditis elegans*

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SUMMARY

Reproductive plasticity describes the ability of organisms to adjust parameters such as volume, rate, or timing of progeny production in order to maximize successful reproduction under different environmental conditions. Reproductive plasticity in response to environmental variation has been observed in a wide range of animals [1–4]; however, the mechanisms involved in translating environmental cues into reproductive outcomes remain unknown. Here, we show that olfaction modulates reproductive timing and senescence through neuroendocrine signaling in *Caenorhabditis elegans*. On their preferred diet, worms demonstrate an increased rate of reproduction and an early onset of reproductive aging. Perception of the preferred diet's odor by AWB olfactory neurons elicits these adjustments by increasing germline proliferation, and optogenetic stimulation of AWB neurons is sufficient to accelerate reproductive timing in the absence of dietary inputs. Furthermore, AWB neurons act through neuropeptide signaling to regulate reproductive rate and senescence. These findings reveal a neuroendocrine nexus linking olfactory sensation and reproduction in response to environmental variation and indicate the significance of olfaction in the regulation of reproductive decline during aging.

RESULTS AND DISCUSSION

As soil-dwelling nematodes, *C. elegans* experience their diet of bacteria as a source of both sensory and nutritional inputs from the environment. To test whether *C. elegans* responds to environmental variations with different reproductive strategies, we examined the reproductive lifespan of wild-type *C. elegans* feeding on different bacterial diets, including *Comamonas* sp., *Bacillus megaterium*, and a number of *Escherichia coli* strains (OP50, HB101, HT115, and MG1655) and found distinct differences among them (Figure S1A).

In particular, the reproductive lifespan of wild-type *C. elegans* feeding on HB101 *E. coli* was ~65% shorter than that of worms feeding on OP50 *E. coli* (Figure 1A; Table S1), although these two *E. coli* strains are closely related. *C. elegans* feeding on OP50 and HB101 had comparable brood size (Figure 1B), development (Figure S1B), and lifespan [5, 6]. Both food intake rate (Figure S1C) and physical activity (Figure S1D) were slightly changed in worms raised on HB101. HB101 exposure at different developmental stages decreased reproductive lifespan to a similar extent (Figure 1C; Table S2), even at day 1 adult stage when the germline has fully developed and differentiated [7]. On the other hand, exposure to OP50 at different developmental stages induced reproductive lifespan extension, but a prolonged period of exposure was necessary to negate the effect of prior exposure to HB101 (Figure S1E; Table S2). Together, these results suggest that the difference in reproductive lifespan is not simply a result of altered germline development or maturation.

Wild-type *C. elegans* feeding on HB101 had an accelerated rate of reproduction for the first 2 days of adulthood, followed by early reproductive cessation (Figure 1D). Consistently, these animals showed 37% more EdU-positive S phase nuclei in the germline compared to their counterparts on OP50 after 1-hr pulse labeling (Figure 1E), indicating that HB101 exposure increases the rate of germline proliferation. Furthermore, wild-type *C. elegans* raised on OP50 and switched to HB101 at L4 stage or day 1 of adulthood also showed increased EdU-positive nuclei compared to OP50-only controls (Figure S1F), suggesting that the rate of germline proliferation is rapidly modulated upon HB101 exposure. *C. elegans* hermaphrodites cease reproduction upon self-sperm exhaustion and can resume reproduction when supplied with young sperm; however, fertility during late mating declines with hermaphrodite age and the concomitant failure of oocyte maintenance [8–10]. We mated post-self-reproductive wild-type hermaphrodites at different ages with young males and found that HB101 exposure significantly decreased success of late conception with fresh sperm (Figure 1F), suggesting an earlier onset of reproductive senescence. Thus, in responding to different bacterial environmental inputs, *C. elegans* adopt different reproductive strategies by adjusting both the rate of germline proliferation and the onset of reproductive aging. Interestingly, computational simulation suggested that the HB101-associated reproductive strategy is advantageous in an unsaturated environment where population size is much smaller than environmental capacity, whereas the OP50 one is more

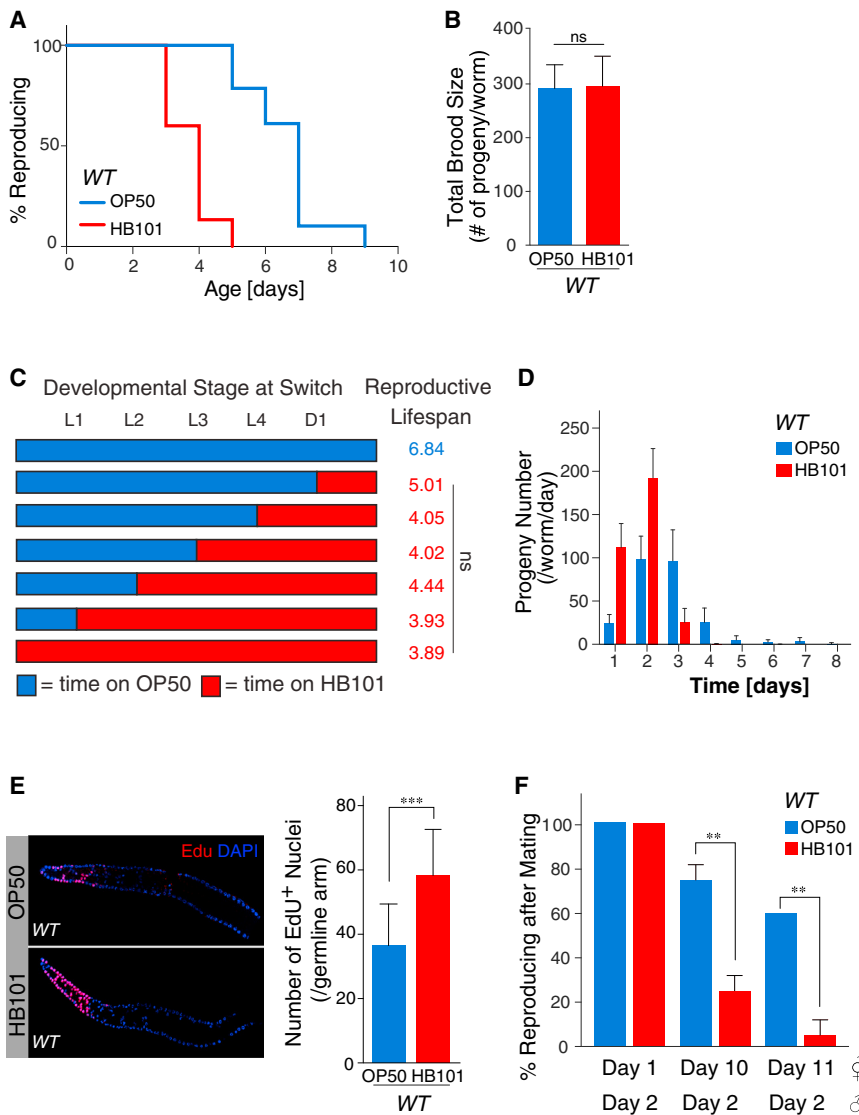


Figure 1. Reproductive Strategy Is Adjusted under Dietary Variations

(A) Wild-type (WT) worms feeding on *E. coli* HB101 show a significantly shorter reproductive lifespan compared to those on *E. coli* OP50 ($p < 0.001$; log rank test).

(B) Total progeny numbers of WT worms feeding on OP50 and HB101 are not significantly different ($p > 0.05$; Student's *t* test; $n = 20$). Data are represented as mean \pm SD.

(C) WT worms feeding on OP50 were switched to HB101 at the L1, L2, L3, L4, or day 1 adult stage, and their reproductive lifespans are not significantly different from that of WT worms feeding entirely on HB101 ($p > 0.05$; log rank test).

(D) WT worms feeding on HB101 have an accelerated rate of progeny production in early adulthood and an early onset of reproductive cessation. Data are represented as mean \pm SD.

(E) Germline proliferation assessment by EdU incorporation shows that WT worms feeding on HB101 have a significantly higher number of EdU-positive nuclei per germline arm compared to those feeding on OP50 ($***p < 0.001$; Student's *t* test). Data are represented as mean \pm SD.

(F) WT hermaphrodites were crossed with 2-day-old young males at indicated ages. Compared to OP50-feeding worms, the success rate of late conception is decreased in HB101-feeding worms by 3-fold at day 10 age and 12-fold at day 11 age ($**p < 0.01$; Fisher's exact test; $n = 20$). Data are represented as mean \pm SD.

See also Figure S1.

odor of HB101 alone significantly reduced the reproductive lifespan of wild-type worms feeding on OP50 (Figure 2C; Table S1). These findings indicate that volatile odorants derived from HB101 bacteria act as the signal to affect reproductive timing.

advantageous when the environment becomes saturated (Figure S1G).

Next, to investigate the origin and nature of the bacterial inputs, we measured the reproductive lifespans of *C. elegans* feeding on mixtures of HB101 and OP50. To our surprise, worms raised on bacterial mixtures containing diluted HB101 (1:1, 1:10, and 1:100) showed reproductive lifespans indistinguishable from those of worms feeding on HB101 alone (Figure 2A; Table S2). Feeding HB101 and OP50 strains expressing mCherry and GFP, respectively, confirmed that animals consumed both bacterial types in the mixture. The very low detection threshold for the reproductive response to HB101 and the relatively short time needed to initiate this response (Figure 1C) are consistent with the ability of *C. elegans* to detect volatile chemicals at low concentrations [11].

To test whether the regulatory cues associated with HB101 were volatile signals, we incubated worms in chambers designed to allow them to feed on one bacterial strain while smelling a second strain (Figure 2B). We found that exposure to the

C. elegans olfaction is mediated by G-protein-coupled receptors (GPCRs) on chemosensory neurons, which utilize a few different $G\alpha$ subunits [11, 12]. We found that mutants lacking *odr-3*, a $G\alpha$ subunit expressed in AWA, AWB, AWC, and ADF olfactory neurons [13], displayed an OP50-like reproductive lifespan on both OP50 and HB101 (Figure 3A; Table S1). We further analyzed mutants specifically defective in each of the neuron classes affected by the *odr-3(n2046)* mutation and found that the *lim-4(ky403)* mutant, which lacks a transcription factor necessary for AWB neuronal fate specification and thus functional AWB neurons [14], showed an OP50-like reproductive lifespan on both OP50 and HB101 (Figure 3B; Table S1). The *lim-4* mutant also failed to shorten reproductive lifespan when exposed to HB101 odors (Figure S2; Table S1). Mutations affecting AWA, AWC, or ADF neurons, however, did not alleviate reproductive lifespan differences on HB101 versus OP50 (Figure S3; Table S1). Restoring *odr-3* expression exclusively in AWB neurons was sufficient to rescue the reproductive lifespan difference in the *odr-3* mutant (Figure 3C; Table S1). Together,

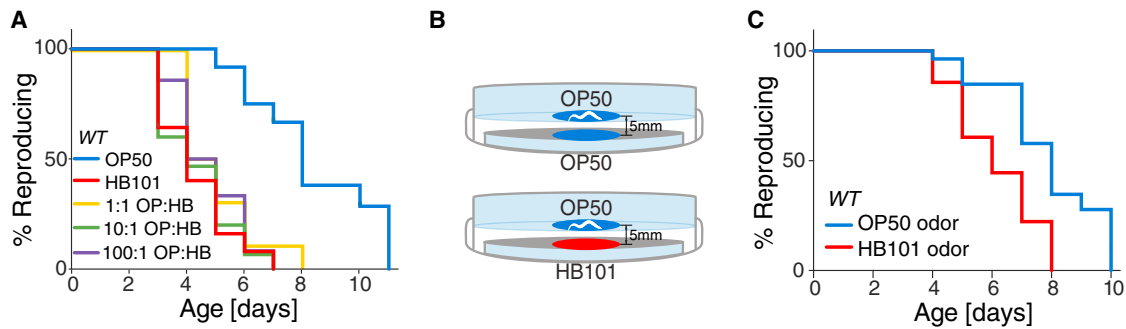


Figure 2. Volatile Sensory Cues Elicit Reproductive Timing Adjustment

(A) Reproductive lifespan of WT worms feeding on OP50 and HB101 mixed in varying ratios (1:1, 10:1, and 100:1 OP50:HB101) are indistinguishable from those on HB101 alone ($p > 0.05$; log rank test).

(B) Design of 3D-printed odor exposure chambers. Adult worms are fed on OP50 while exposed to the odorants of either HB101 or OP50. The distance between two agar surfaces is 5 mm.

(C) Exposure to HB101 odorants significantly reduces the reproductive lifespan of WT worms feeding on OP50 ($p < 0.01$; log rank test).

See also Figure S2.

these results suggest that AWB neurons are specifically required in perceiving HB101-derived sensory inputs to adjust reproductive timing. Furthermore, *odr-3* and *lim-4* mutants grown on HB101 showed low rates of germline proliferation similar to that of wild-type worms on OP50 (Figures 1E and 3D), indicating that AWB neurons modulate reproductive timing by affecting germline proliferation.

We next photoactivated AWB neurons, using a transgenic line (*AWB::ChR2 Tg*) expressing the blue-light-activated cation channel channelrhodopsin-2 (ChR2) [15] under the control of the AWB-specific *str-1* promoter [16]. Unstimulated *AWB::ChR2 Tg* animals showed a 43% longer reproductive lifespan on OP50 than on HB101 (Figure 3E; Table S1); however, this extension was completely abolished in *AWB::ChR2 Tg* animals upon blue light activation (Figure 3E; Table S1). Blue light exposure did not affect reproductive lifespan difference of wild-type animals on HB101 versus OP50 (Figure 3E; Table S1). Thus, AWB neuronal activation is sufficient to recapitulate the effect of HB101-derived sensory signals on reproductive timing.

C. elegans neurotransmission is mediated by both neurotransmitters and neuropeptides, which are released synaptically from small clear vesicles (SCVs) and non-synaptically from dense core vesicles (DCVs), respectively [17, 18]. We blocked SCV release specifically in AWB neurons by expressing tetanus toxin light chain [19] using the *str-1* promoter and found that inhibition of neurotransmitter signaling did not alleviate the reproductive lifespan difference on HB101 versus OP50 (Figure 4A; Table S1). Next, we inhibited neuropeptide release from AWB neurons by expressing dsRNA against *C. elegans* CAPS homolog UNC-31 (*AWB::unc-31^{KD}*), which is necessary for DCV, but not SCV, release [20]. *AWB::unc-31^{KD}* transgenic strains did not show differences in reproductive lifespan when grown on HB101 versus OP50 (Figure 4B; Table S1). Furthermore, *AWB::unc-31^{KD}* transgenic worms grown on OP50 and HB101 both showed increased success of late-life fertilization (Figure 4C). Thus, neuropeptide signaling of AWB neurons mediates the olfactory impacts on both reproductive rate and reproductive senescence.

Previous reports indicated that chemosensory neurons mediate *C. elegans* choice to different bacteria [21–23]. Given

the strong preference of wild-type worms for HB101 (Figure 4D) [24], we tested whether AWB neuropeptide signaling mediates this response. We found that the preference for HB101 was completely abolished in the *lim-4(ky403)* mutant and in the mutants *egl-3(gk238)*, *egl-21(n476)*, and *sbt-1(ok901)* that are defective in neuropeptide synthesis and processing [25–27] (Figure 4D). These results suggest that AWB olfactory neurons perceive environmental cues and consequently modulate both the organisms' attraction behavior and reproductive strategies via neuropeptide transmission.

Together, our results reveal that olfactory neuroendocrine signaling regulates reproductive strategies in response to environmental cues. Based on computational simulation, HB101- and OP50-related reproductive strategies correlate with survival advantages under unsaturated and saturated environments, respectively. Interestingly, experimental results show that animals prefer the HB101 environment, suggesting that the HB101-associated volatile signal may act as an indicator of a favorable environment with high capacity. The olfactory regulatory mechanism would give organisms flexibility for rapidly responding to environmental fluctuations and adjusting reproductive strategies to ensure the perpetuation of the species.

Our studies also suggest a reverse correlation between early- and late-life fertility under different environments. Although *C. elegans* continues to produce oocytes throughout adult life, the quality of oocytes decline with age [8], resulting in late-life fertility defects. Further study is needed to determine whether the late-life fertility defect associated with HB101 exposure is a direct consequence of the increased rate of germline proliferation in earlier life or whether the two effects could potentially be uncoupled. Notably, a similar trade-off has also been demonstrated in experimental evolution studies in both *C. elegans* [28] and *Drosophila* [29]. Therefore, early- and late-life fertility may be inextricably linked and their trade-off may be under the control of the olfactory nervous system. Olfactory regulation of early-life fertility via hormonal signaling is well known in mammals [30]; our findings therefore raise the possibility that olfactory neuroendocrine signaling could be also contributing to the regulation of reproductive aging in higher organisms.

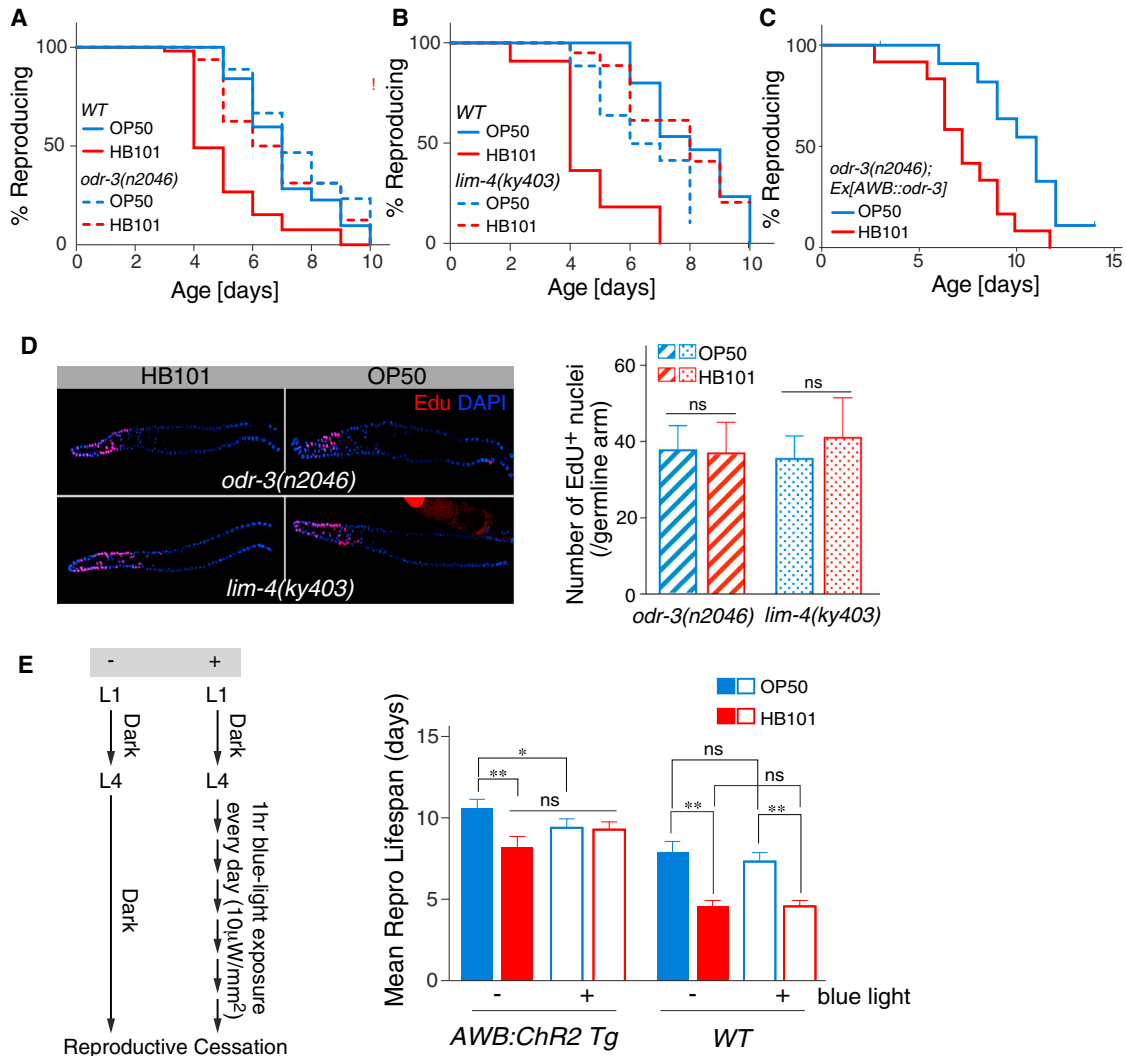


Figure 3. AWB Olfactory Neurons Regulate Reproductive Timing

(A) *odr-3(n2046)* olfaction-defective mutants feeding on OP50 and HB101 both show reproductive lifespans similar to that of WT worms feeding on OP50 ($p > 0.05$; log rank test; composite of two trials).

(B) *lim-4(ky403)* mutants that lack functional AWB neurons do not show a difference in reproductive lifespan on OP50 versus HB101, with both similar to WT reproductive lifespan on OP50 ($p > 0.05$; log rank test; composite of two trials).

(C) AWB-specific rescue of *odr-3* expression in the *odr-3(n2046)* mutants restores difference in reproductive lifespan ($p < 0.001$; log rank test).

(D) Neither the *odr-3(n2046)* nor *lim-4(ky403)* show differences in numbers of germline Edu^+ nuclei between OP50 and HB101 feeding conditions ($p > 0.05$; Student's *t* test). Data are represented as mean \pm SD.

(E) *AWB::ChR2* transgenic and WT worms with and without blue light stimulation (as shown in diagram) were compared for reproductive lifespan. Unstimulated *AWB::ChR2* *Tg* worms show reproductive lifespan differences on OP50 versus HB101 (** $p < 0.01$; log rank test). Blue light stimulation significantly reduces the reproductive lifespan of *AWB::ChR2* *Tg* worms feeding on OP50 (* $p < 0.05$; log rank test) but does not affect the reproductive lifespan of *AWB::ChR2* *Tg* worms feeding on HB101 or the reproductive lifespan of WT worms on either OP50 or HB101 ($p > 0.05$; log rank test). Data are represented as mean \pm SEM. See also Figure S3.

Interestingly, genetic interaction analysis showed that this olfactory regulation acts independently of the FOXO/insulin/IGF-1, TGF- β Sma/Mab, or caloric restriction pathways (Figures S3D–S3G), which are thus far the only characterized regulators of *C. elegans* reproductive aging [8, 9]. Thus, our findings suggest an ancient link between the olfactory and reproductive systems and reveal a distinct regulatory mechanism of reproductive aging via neuroendocrine signaling. Further studies will be needed to elucidate the genes and pathways implicated in this regulation,

which will provide an entry point for investigating the underlying mechanisms by which the olfactory nervous system modulates the processes of reproductive senescence in higher organisms.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2015.07.023>.

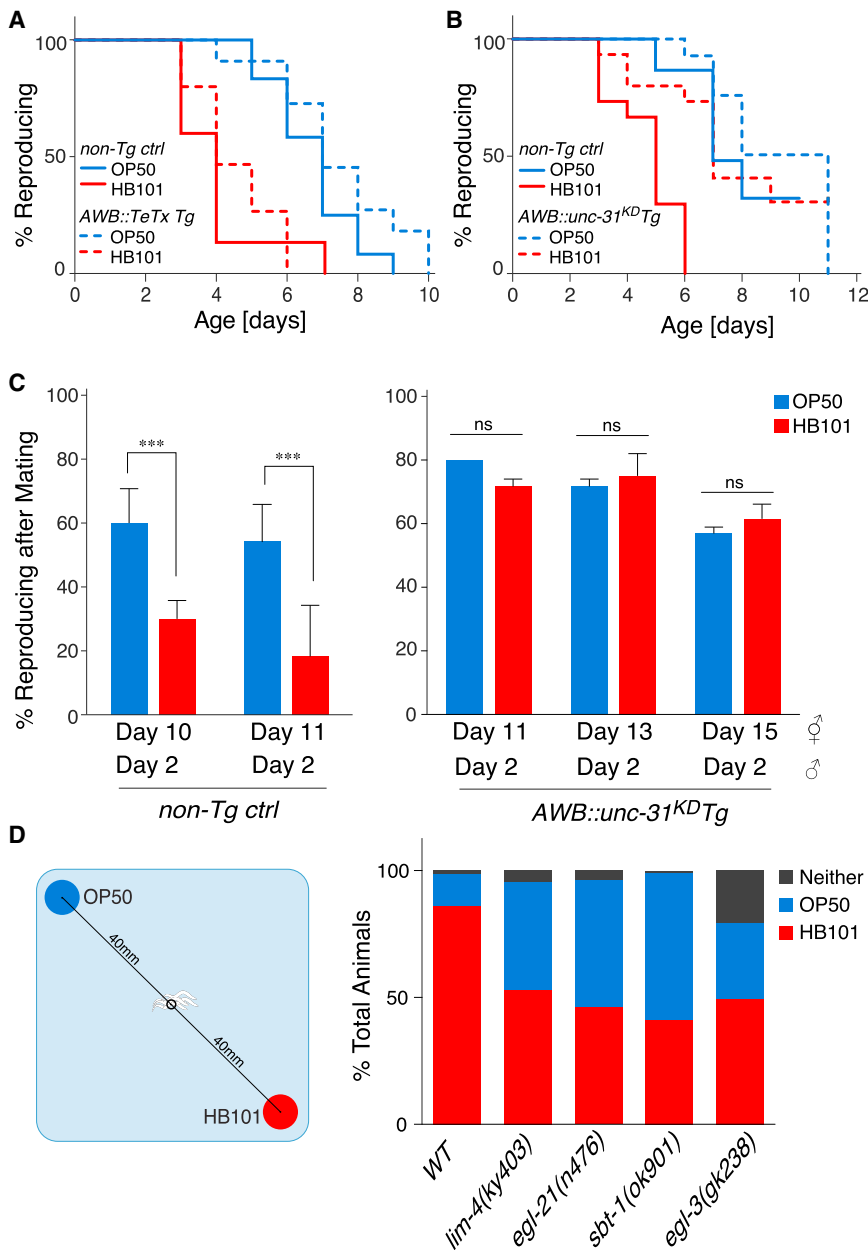


Figure 4. Neuropeptide Signaling Mediates Reproductive and Behavioral Responses

(A) *AWB::TeTx* transgenic strains and non-transgenic sibling controls show the same level of differences in reproductive lifespan on OP50 versus HB101 ($p < 0.01$; log rank test).

(B) *AWB::unc-31^{KD}* transgenic strains feeding on HB101 and OP50 do not show a significant difference in reproductive lifespan ($p > 0.05$; log rank test), whereas nontransgenic sibling controls show a significant difference ($p < 0.001$; log rank test).

(C) Nontransgenic control or *AWB::unc-31^{KD}* transgenic worms crossed with 2-day-old young WT males at indicated ages. Ten-day-old controls show 60% success rate of conception when raised on OP50, compared to 30% on HB101 ($***p < 0.001$; Fisher's exact test; $n = 65$). Data are represented as mean \pm SD. Eleven-day-old controls show 54% success rate of conception when raised on OP50, compared to 18% on HB101 ($***p < 0.001$; Fisher's exact test; $n = 50$). *AWB::unc-31^{KD}* transgenic worms show 76%, 73%, and 64% success rate of conception when raised on either HB101 or OP50 at day 11, day 13, and day 15, respectively ($p > 0.05$; Fisher's exact test; $n = 25$). Data are represented as mean \pm SD. (D) In the food choice assay, WT worms show 74% ($\pm 6\%$) preference for HB101. This preference is absent in the *lim-4(ky403)* mutant and in the neuropeptide-processing mutants *egl-3(gk238)*, *egl-21(n476)*, and *sbt-1(ok901)*. Representative experiment is an average of three technical replicates.

AUTHOR CONTRIBUTIONS

J.N.S., A.S.M., and M.C.W. conceived the study. J.N.S. and A.S.M. performed experiments and analysis. F.X. designed and tested computational simulations. J.N.S., A.S.M., F.X., and M.C.W. wrote the manuscript.

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