



SYMPOSIUM

Neural Circuitry That Mediates Behavior Governing the Tradeoffs Between Survival and Reproduction in *Caenorhabditis elegans*

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From the symposium “Molecular and Neuroendocrine Approaches to the Study of Evolutionary Tradeoffs: Food, Sex, Stress, and Longevity” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 4–8, 2017 at New Orleans, Louisiana.

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Synopsis In all outcrossing sexual species there is a mechanism that brings two parents together. For animals, this reproductive requirement may at times conflict with other needs, such as foraging for food. This tension has been studied using the tiny (1 mm) nematode worm, *Caenorhabditis elegans*. In a trade off between certainty of survival and possibility of reproduction, the *C. elegans* male will abandon a food patch lacking mates and explore its environment to find one where mates are present. A quantitative behavioral assay has been used to study the behavioral mechanism of mate searching and nutritional, sexual, and neurohormonal pathways that influence the underlying drive state. Taking advantage of the known connectivity of the *C. elegans* nervous system, neural pathways have been identified that influence the male's behavior in the presence of food with and without mates.

Since its selection by Sydney Brenner in the late 1960s as a genetically accessible experimental model for studies of nervous system function and development, the tiny nematode worm *Caenorhabditis elegans* has proven to be valuable for studies of problems throughout biology (Emmons 2015). One feature of the species that promotes its usefulness for genetics is the fact that the female (XX) is a functional hermaphrodite with about 300 gametes developing as sperm. This means that animals studied in many laboratories can be genetically identical and homozygous at every locus and newly-induced recessive mutations can be recovered in two generations by selfing.

But after depleting its 300 self-sperm, the hermaphrodite can produce many more progeny by mating with a male (XO). This androdioecious mating system has arisen independently several times in the phylogenetic history of free-living nematodes and may be advantageous when resources are discontinuous and populations sparse—meaning an individual may often find itself alone when food is encountered. But outcrossing provides an evolutionary advantage

and so males persist (Morran et al. 2010). Unlike hermaphrodites, males must mate to pass on their genes. Hence, while the hermaphrodite prioritizes finding food, for a male this is insufficient—it needs to find a food source containing mates. These divergent priorities underlie differences in male and hermaphrodite behavior.

We might think a tiny, 1 mm, worm with a nervous system of fewer than 400 neurons would be capable of only simple behaviors. But in fact, the male displays a behavior that has to be considered a higher-order appetitive, goal-oriented behavior governed by a drive. An adult *C. elegans* male will leave a food source and search its environment until it finds one where mating partners are also present. We can say this behavior has the goal of finding mates with food because leaving a food patch without mating partners and exploring the environment will promote finding a food patch with mating partners, and a food patch with mates present stops the behavior. Hermaphrodites alone on the agar surface without food will not retain males—both food and mates are necessary.

In the way described by classical ethologists, this appetitive behavior is followed by the consummatory behavior, copulation, when the goal is reached. The behavior is governed by a drive because it occurs in response to absence of a stimulus, rather than presence of one. It is a sex drive because it is only exhibited by mature males, not larval males or hermaphrodites of any age, and is influenced by signals from the gonad and conserved neurohormone pathways known to be involved in reproduction in higher animals. The drive state that induces the behavior competes with a drive for nutrition—starved adult males do not leave food. Mate searching therefore requires decision making between the needs for nutrition and reproduction. The experimental strengths of the *C. elegans* system allow us to delve into this phenomenon at great depth. For an additional more detailed review, see [Barrios \(2014\)](#).

Caenorhabditis elegans males find their mates

Graduate student Jonathan Lipton (unpublished data) first performed a simple experiment that revealed the male's mate-searching strategy (Fig. 1). The experiment illustrated something that worm geneticists had long known: adult males, unlike adult hermaphrodites or juveniles of either sex, will not stay by themselves on a food patch. They disperse across an agar plate and even try to crawl up the sides of the plastic dish, eventually winding up dried out and dead. An assay was developed to quantitatively measure this tendency ([Lipton et al. 2004](#)) (Fig. 2). This "leaving assay" revealed that leaving is a stochastic process—some males leave within minutes of being placed on a lawn while others remain after 24 h, but all have the same, equal probability of leaving per unit time. The value of this probability depends on experimental conditions, genotype, or other variables and has been used to delve into the regulation and mechanism of mate-searching.

The behavioral basis of mate searching

What worm behavior is involved in staying on a food source or leaving it? Worm navigation in its environment involves forward locomotion driven by waves of sinusoidal body bends that travel from the head to the tail, backward locomotion by waves in the opposite direction, and deep body bends, often executed between backward and forward bouts, that change the direction of locomotion. Worms stay on food because they are sensitive to the food edge: if they detect it with sensory neurons in the nose, a reversal is induced. If they do get off the food, they

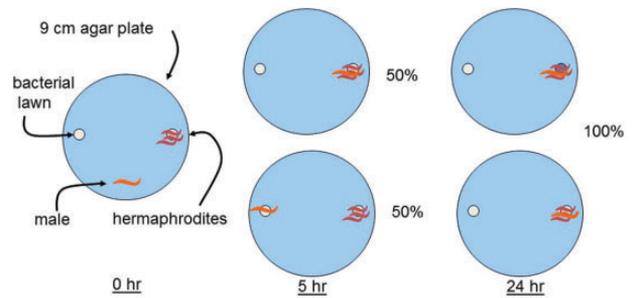


Fig. 1 A simple experiment (unpublished) showing that *C. elegans* males actively find their hermaphrodite mating partners. An agar plate is set up with two dots of bacterial food and a small group of hermaphrodites are placed on one of them, where they will remain and lay eggs produced with their self-sperm. A male is placed an equal distance away from both food patches. All worms are attracted to food odors and after 5 h the males will find the food patches. But half will wind up on the one without mates. This shows there is no long-range sex pheromone gradient across the plate that attracts males. Twenty-four hours later, however, all the males are with the hermaphrodites. The males that initially found the hermaphrodites remained with them (those plates show no worm tracks on the empty food patch), while those that went to the wrong patch left it. Their tracks show they wander about the agar surface until they hit the productive food patch, where they then remain. Sex-attractant pheromones that have been discovered since this experiment was first performed act at shorter range and are not detected by this protocol ([White et al. 2007](#); [Leighton and Sternberg 2016](#)).

execute a local area search, backing and turning frequently, in an attempt to return ([Gray et al. 2005](#)).

Close observation of the behavior of a male alone on a food patch reveals that its behavior differs in both of these properties from hermaphrodites or from males with mates ([Barrios et al. 2008](#)). Males alone on food, if they encounter the food edge, make wide excursions off the food before returning. If they fail to re-encounter the food, they terminate the local area search more quickly and begin long, uninterrupted bouts of forward locomotion that take them away.

Both of these factors are affected when hermaphrodites are present. On food with hermaphrodites, males are copulating only a fraction of the time; mostly they move about, foraging alone. But if they encounter the food edge they back up quickly, and if they get off they search longer before heading away straight. Physical contact with the hermaphrodite cuticle triggers these lasting behavioral changes. Although nematodes do produce attractive sex pheromones, mutant hermaphrodites that fail to do so still retain males, as do dead hermaphrodite carcasses. But if the carcasses are covered with a thin layer of agar, the males leave. Moreover, the surface cues are sex specific, since males retain other males much less efficiently than hermaphrodites do ([Barrios et al. 2008](#)).

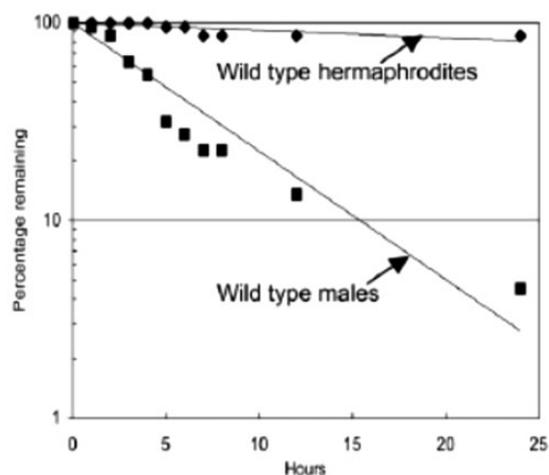


Fig. 2 The quantitative assay used to study *C. elegans* mate-searching behavior (Lipton et al. 2004). The “leaving” assay measures the rate at which males leave a food source. In a typical assay, a number of males (usually 20) are placed individually on a dot of bacteria in the center of an agar plate and their tracks on the agar surface (slight indentations) are examined at intervals. If a male has wandered a certain distance from the food source, it is scored as a “leaver.” When the fraction remaining is plotted on a semi-log plot, it yields a straight line, a “decay curve” that shows leaving occurs stochastically with a certain probability that is the same for all males and constant during the period of the assay. There is very little lag time; males appear to know very quickly (within 20 min) that there are no hermaphrodites present. The slope of the line yields a probability of leaving per hour, P_L . If hermaphrodites are present on the food, $P_L = 0$. P_L is measured under different conditions, for example after starvation of the males, after removal of the gonad or particular neurons with a laser microbeam, or in mutants, to study the mechanism and regulation of the behavior.

Reproductive and physiological status regulate expression of mate searching behavior

As might be expected, starved males are more reluctant to leave food. In the leaving assay with starved males, back-extrapolation of the leaving curve does not generate a line that crosses 100% remaining at time 0 (the time when the males are placed on the bacterial lawn), which indicates the leaving probably was a constant nearly from the start of the assay, but rather shows a lag; the leaving probability at first is near zero and leaving only begins after several hours on the food (Lipton et al. 2004). The satiety signal may involve insulin, since males mutant for the single insulin receptor, DAF-2, have significantly lower leaving probability (Lipton et al. 2004).

Similarly, absence of leaving behavior by larval males suggests that signals associated with sexual maturity stimulate leaving. Indeed, if the precursor cells to the germline or gonad are ablated in a young larval male, gonadless or germ-cell-depleted adult males remain on food (Lipton et al. 2004). The

gonad signal appears to involve a nuclear hormone receptor with homology to the vitamin D receptor and the thyroid hormone receptor, and its steroid ligand(s) (Kleemann et al. 2008). Mutations in the receptor gene *daf-12* are highly pleiotropic, affecting a number of aspects of *C. elegans* development, including lifespan, induction of the long-lived dispersal dauer larval developmental pathway, gonad development, resistance to heat stress, and so on. A theme is involvement of this pathway in the organismal choice between survival and reproduction. Thus, it is perhaps not surprising that mutations in *daf-12* reduce the rate of male mate-searching and promote remaining on food.

Two conserved neurohormone pathways promote sex drive

With the leaving assay, it was possible to carry out forward genetic screens to attempt to identify additional genes that promote leaving behavior. Males homozygous for the new mutation *bx142* remain indefinitely on food when alone, yet otherwise appear normal, and are able to copulate. The mutation was found to fall in a gene with homology to the receptor for the PDF (pigment dispersing factor) neurohormone (Barrios et al. 2012). Mutations in a gene for the cognate peptide ligand had the same defect. The PDF neurohormone, a member of the secretin family of neuropeptides, has effects on male sex drive in *Drosophila*, circadian rhythms in insects, and mood disorders in humans.

In a second, candidate gene approach, the nematode homolog of oxytocin/vasopressin pathways was examined, two pathways with important roles in regulating reproductive behaviors in mammals (Garrison et al. 2012). Mutations in the ligand and receptor genes of this pathway, termed the nematocin pathway, affected both appetitive and consummatory aspects of male behavior. Mate searching was reduced and copulatory efforts were defective. Multiple cells, both sensory neurons and interneurons, express the peptide and receptor genes of these two pathways and are present throughout the *C. elegans* nervous system. This is consistent with the dispersed nature of the circuits that affect sexual behavior, as described next.

The neural substrate for mate searching

Although the *C. elegans* nervous system appears to be simple by the standard that it contains fewer than 400 neurons (302 in the adult hermaphrodite, 385 in the adult male), in fact these neurons are richly cross-connected, creating a neural network of

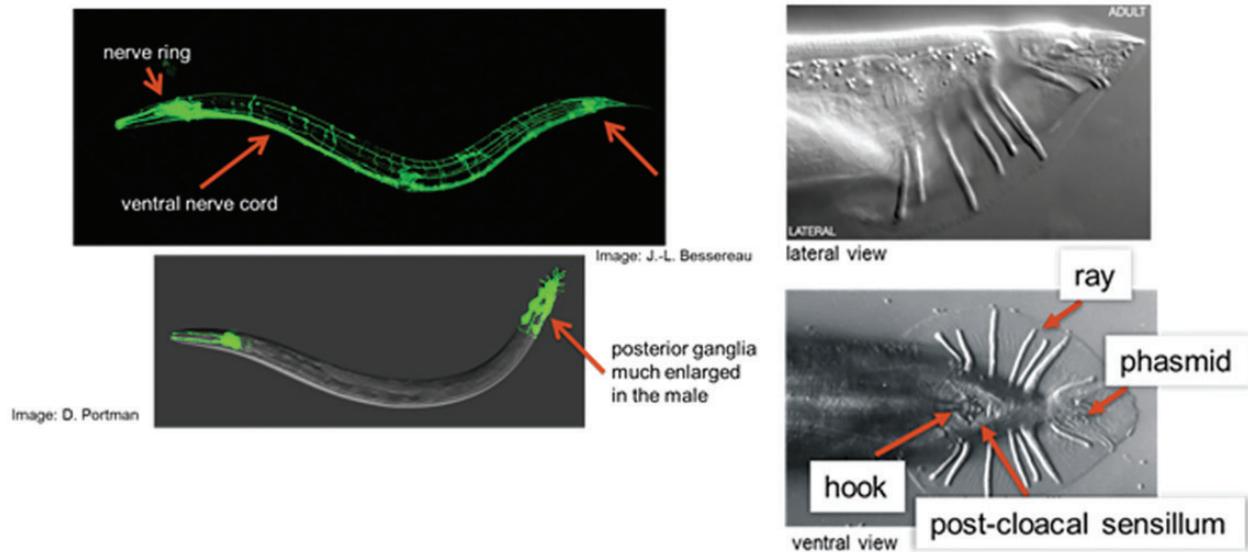


Fig. 3 The *C. elegans* nervous system. Left panels: fluorescent reporter genes reveal the tracts of the entire nervous system in the hermaphrodite (upper) and a sub-set of male-specific neurons in the male (lower). Right panels: Nomarski micrographs of the sexual specialization of the adult male tail, showing structures important for copulation. In the lower panel, four sets of sensilla are indicated. Each ray sensilla contains the sensory endings of two sensory neurons. These ray sensory neurons play a role in stimulating male mate-searching.

daunting complexity with over 5000 connected cell pairs (White et al. 1986; Jarrell et al. 2012; Emmons 2015) (Fig. 3). It is a unique property of the *C. elegans* nervous system that it is possible to completely define its neural and end organ connectivity with current electron microscope (EM) methodology. Worms have a stereotyped number of cells and all the neurons can be identified by light microscopy. In reconstructions from EM serial sections, neurites are traced until they come to a cell body, which identifies them. Pathways from diverse sensory inputs converge and diverge throughout the network. In general, it is not possible to discern independent neural pathways controlling motor neurons and muscles that lead to different behavioral outputs. Much of *C. elegans* locomotory behavior may emerge as alternate modes of activity of the network (Stephens et al. 2008, 2011).

Nevertheless, candidate neurons for a behavior of interest can be suggested by their structure and connectivity, and their activities tested *in vivo* by cell ablation studies. As an entry point into the problem of the neural substrate for mate searching, we asked which sensory neurons allow the males to detect hermaphrodites, causing them to stop mate searching. We reasoned that the targets of these neurons would be the circuits that promote mate searching. The first logical candidates to test were the male-specific ray sensory neurons, which allow the male to detect contact with the hermaphrodite in the first step of copulation (Liu and Sternberg 1995) (Fig. 3).

Indeed, ablation of these neurons, either by laser microsurgery or by genetic means, caused males to leave food even when hermaphrodites were present (Barrios et al. 2008). Surprisingly, when alone on food they also left food more slowly than intact, wildtype males. Thus, these sensory neurons stimulate leaving, and sensation of hermaphrodites abrogates this stimulation.

One major target of the ray sensory neurons is a set of three male-specific interneurons that send processes from the tail through the ventral nerve cord to make synapses with circuits in the anterior nerve ring. Reconstruction of the male nervous system has revealed that on reaching the head, these interneurons, the EF interneurons, target one major class of premotor interneurons, a class that promotes forward locomotion (unpublished data). These are one of two classes of opposing premotor interneurons that respectively play important roles in driving forward versus backward locomotion. Thus, the signal from the ray sensory neurons through the EF interneurons likely modulates the balance between forward and backward locomotion and the turns that occur during transitions.

There are several additional neurons that participate in the male circuits (are ray neuron targets) that also send processes through the ventral nerve cord to make connections in the head (Jarrell et al. 2012). In addition to targeting the motor system, some of these also target sensory neurons in the nose that may be involved in detecting the food edge

(unpublished data). Some of these neurons and their targets express the receptors or ligands in the PDF and nematocin pathways, consistent with the roles these pathways play in regulating leaving.

The rate at which males leave a food patch results from the balance between two competing drives: the drive to follow an attractive food gradient to a source of nutrition and a drive to explore the environment, leaving food if necessary, until a food patch with mates is arrived at. Mate searching is regulated not only by inputs to the motor system from male-specific neurons in the tail, but also by male-specific regulation of circuits that detect food. In one pair of head sensory neurons, transcription of the gene for a receptor for food odorants is downregulated in the male (Ryan et al., 2014). In another, at male maturation, transcription of the gene for the TGF β peptide is turned up in a head sensory neuron, which stimulates exploration (Hilbert and Kim 2017). While there is some cross connectivity at higher levels of the neural network between circuits from food-sensing head sensory neurons on the one hand and from male-specific sex circuits on the other, both pathways are most strongly targeted to the motor system (unpublished data). Thus, ultimately the motor system itself is an important point of convergence of competing nutritional and reproductive sensory streams.

As stated above, the *C. elegans* nervous system is so highly cross connected as a neural network that rarely are clear, isolated circuits evident underlying particular behaviors. This is particularly the case for controlling the balance between straight runs and reversal/turns, whether for navigation in chemotaxis, thermotaxis, or mate searching. Thus, it is not surprising to find that tracing the neural pathways defined by neurons or molecules with a demonstrable role in mate searching reveals connections that spread diffusely and widely through the nervous system. Male mate-searching is highly integrated with other behaviors and involves the activities of many neurons. The behavioral outcome of the system as a whole is under many sensory, physiological, hormonal, and sexual inputs. Its response to these inputs is adaptive and represents the decision-making function of the nervous system when the organism is confronted with competing drives that require a trade-off.

Acknowledgments

Work in the author's laboratory is supported by the US National Institutes of Health (R01 GM066897) and the G. Harold and Leila Y. Mathers Charitable Foundation.

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