Published in final edited form as:

Curr Opin Syst Biol. 2019 February ; 13: 23–30. doi:10.1016/j.coisb.2018.08.012.

A primer on pheromone signaling in *Caenorhabditis elegans* for systems biologists

Patrick T. McGrath¹ and Ilya Ruvinsky²

¹Department of Biological Sciences, Department of Physics; Georgia Institute of Technology, Atlanta, GA 30332. patrick.mcgrath@biology.gatech.edu

²Department of Molecular Biosciences; Northwestern University, Evanston, IL 60208. ilya.ruvinsky@northwestern.edu

Abstract

Individuals communicate information about their age, sex, social status, and recent life history with other members of their species through the release of pheromones, chemical signals that elicit behavioral or physiological changes in the recipients. Pheromones provide a fascinating example of information exchange: animals have evolved intraspecific languages in the presence of eavesdroppers and cheaters. In this review, we discuss the recent work using the nematode C. elegans to decipher its chemical language through the analysis of ascaroside pheromones. Genetic dissection has started to identify the enzymes that produce pheromones and the neural circuits that process these signals. Ecological experiments have characterized the biotic environment of C. elegans and its relatives, including ecological relationships with a variety of species that sense or release similar blends of ascarosides. Systems biology approaches should be fruitful in understanding the organization and function of communication systems in C. elegans.

Introduction

The exchange of secreted chemical signals among organisms is a common feature of life. Examples have been documented in bacteria [1], plants [2], fungi [3], and animals [4]. These molecules are particularly salient when exchanged by members of the same species, although the broadcast nature of the signals offers opportunities for eavesdropping and exploitation. Excreted chemosignals that evoke responses in members of the same species are referred to as pheromones [5]; these often consist of blends of molecules that determine a unique chemical dialect for each species and trigger changes in behavior, physiology, and development in the recipients.

C. elegans is an attractive model organism for the study of pheromone signaling. This millimeter-long nematode has a compact nervous system with barely more than a dozen pairs of chemosensory neurons and a roughly comparable number of interneurons [6]. Little

more than a decade ago, a major class of secreted pheromones, called ascarosides, were identified in *C. elegans* [7–9]. Since then, a number of metabolomic, phenotypic, and genetic studies have explored how these ascarosides are released and sensed in *C. elegans* and related nematodes.

Here, we briefly review the known phenomenology of *C. elegans* pheromones and focus on two topics that are central to a comprehensive understanding of communication in any species. The strengths of *C. elegans* as a model system will likely make these areas the major contributions of “the worm” to the discovery of general principles of inter-organismal chemical signaling. First, we discuss the efforts to place pheromone signaling in the context of the complex, natural habitats in which it takes place. Second, we consider the means by which the nervous system perceives and processes these signals, while integrating them with other relevant information. The field of systems biology has developed a rich plethora of experimental and conceptual approaches to address similar questions. We expect that their application to the fascinating world of chemical communication in *C. elegans* could considerably propel the field.

Pheromones in *C. elegans* and the “messages” they convey

Whereas several classes of molecules are known to act as chemosignals in *C. elegans*, by far the best studied are the so-called ascaroside pheromones. In this review, we primarily focus on this class of pheromones because the available data allow us to discuss general principles that we expect to be applicable to other classes of chemosignals. Scores of ascaroside pheromones, named after an ascarylose sugar found in the core of each molecule, have been identified in *C. elegans* (Figure 1). The diversity of ascarosides derives from the length of a short- to medium-chain fatty acid attached to this sugar and the presence of other moieties [10,11]. While several hundred ascarylose-containing molecules have been identified [12,13], only a small subset has been shown to evoke responses in the recipients (i.e., act as pheromones); functional roles of the remaining majority remain to be ascertained. Two different ascaroside naming schemes are used in the literature. In the first, following approaches in the biosynthetic literature, information regarding the chemical structure is contained in the name, e.g. asc- C9 (or even C9 in older literature) contains a 9-carbon fatty acid with an α – β double bond. Additional chemical modifications that can occur on the ascarylose sugar are indicated at the beginning of the name (e.g., indole-3-carbonyl, IC) and those that can occur on the fatty acid are indicated at the end of the name (e.g., methylketone, MK). The second nomenclature, following approaches in *C. elegans* literature, subsumes this complexity under a number reflecting the order of discovery, converting asc- C9 into ascr#3. Distinct abbreviations are used for ascarylose-containing molecules that contain additional moieties, such as when the sugar is decorated with an indol (icas#), octopamine succinate (osas#), hydroxybenzoyl (hbas#), or methyl-butenoyl (mbas#) [11,14]. Even more complex compounds have been detected in other nematodes, including the use of the isomer paratose in place of the ascarylose sugar, and the use of a sugar derivative of the nucleoside adenosine as a side chain [15].

The functions of *C. Production and perdurance of ascaroside pheromones in natural habitats**C. elegans* pheromones were first studied in the context of dauer formation, an

alternative developmental morph that facilitates survival of adverse conditions and dispersal [16]. “Dauer pheromones” convey population density, information that is assessed together with food availability by recently hatched larvae. It is now understood that the dauer pheromone is a blend of at least five synergistically-acting ascarosides [7–9,17–19]. In addition to biasing developmental plasticity, a subset of ascarosides can affect social behaviors, including mate finding/attraction [9,19,20], aggregation [9,13,14,20], repulsion [21], exploratory movement [22], and pseudo parent-offspring interaction [23].

There is also a growing recognition that ascarosides can alter organismal physiology, particularly the germline function [24–26], olfactory plasticity [27], and aspects of metabolism [28]. The totality of the physiological effects of pheromones manifests in the alteration of aging and lifespan, the mechanisms of which are only beginning to be understood [25,29–31]. In general, different combinations and concentrations of ascarosides regulate a number of traits.

Although, in most cases, the specific “meanings” of pheromone signals remain to be determined, the amount and identity of molecules released by animals depend on several biological factors, suggesting that the status of the sender is communicated. For example, pheromones could convey information about the age [32], stress [33], sex [20], diet [32], and population density [16] of the emitter. How is this achieved? One possibility is that certain aspect of pheromone production might require chemical precursors that are produced by other species in the environment. Release of a particular ascaroside could indicate the presence of this species (e.g. to communicate information about diet). Several recent studies suggest that changes in the composition of ascaroside blends may be achieved by altering the relative amounts of the biosynthetic enzymes involved in pheromone production [13,34–36]. For example, expression levels of key enzymes differ between males and hermaphrodites, consistent with the different abundance of sex-specific ascarosides [20]. There is an intriguing possibility that enzymes may directly read out metabolic state of the animals and change ascaroside production accordingly [35]. Recently, an enzyme was identified that could shorten the fatty acids side chain length in existing indolated ascarosides which signal for aggregation behavior [37]. The shortened ascarosides now induced receiving worms to disperse, suggesting that regulation of this enzyme could be used to modify the signals released by animals as conditions changed. Despite this recent progress, much work remains to be done to discover the mechanisms that coordinate the production of pheromone messages with specific environmental conditions.

Growing evidence suggests the existence of non-ascaroside pheromones in *C. elegans* because certain types of chemical communication are retained in the mutants defective in ascaroside production [38,39]. For example, hermaphrodites still faithfully report the presence of sperm in the gonad [38,40], both males [25] and hermaphrodites [41] produce substance(s) that accelerate larval development, indicators of alarm [42] and larval population density [43,44] are still being made. The molecular nature of these and other non-ascaroside signals remains to be discovered. Recent developments in this direction include a detailed characterization of the exo-metabolome of mutants that are unable to produce mature ascaroside pheromones [12].

Crosstalk and eavesdropping in natural environments

The purpose of pheromones is to convey social information, primarily regarding the location and status of potential mates and competitors, in complex biotic environments. The natural habitat of *C. elegans* is rotting fruits, flowers, and plant stems [45]. After a small handful of animals colonize a new locale, they consume bacteria growing on the decomposing matter. Given the generation time of ~3 days and brood sizes of hundreds of offspring, the population rapidly expands until the food sources are exhausted [46]. Caught in a nutritionally-deprived environment, many larvae become dauer and disperse, thus re-initiating the boom-and-bust cycle of population growth. In the wild, *C. elegans* hermaphrodites primarily reproduce by self-fertilization, infrequently outcrossing with rare males [47–49]. Consequently, local populations are highly inbred with the majority of individuals being homozygous for most of the genome. Only a small number of haplotypes stably persist within a geographic area over several years, comprising dozens of generations [46]. Different lineages display variation in release of and response to pheromones [50–53]. It remains important to understand whether this reflects independently evolving honest signaling (one that faithfully reflects the condition of the sender, e.g. [25]) that is private among kin or a weapon used to deceive competitors that occupy the same niche [54].

Pheromone messages are primarily intended for intraspecific use, but they could also be detected by the multiple nematode species that are found in the natural habitats occupied by *C. elegans* [55]. Several of the abundant ascaroside molecules are found in distantly-related nematodes [56–61], and at least in some instances, there is functional conservation of pheromone blends [24,25,57]. On the other hand, directed comparative screens are continuing to reveal species-specific compounds [15,56,62–64]. It is likely that both unique molecules and different ratios of conserved components define the species-specificity of pheromone blends. The extent to which pheromones of one species are intelligible to another and whether these chemical signals represent honest or deceptive communications largely remains an open question.

Examples from a variety of nematodes suggest that pheromones are used in interactions beyond the boundaries of this phylum. Because specific ascarosides indicate the nearby presence of nematodes, in response to these molecules, nematophagous fungi elaborate trapping devices to capture and consume their prey [65], whereas plants boost their immune defense in preparation for an imminent attack [59]. Ascarosides play important roles in the association between insects and nematodes – nematode pheromones could promote pupation in the beetle vector, while ascarosides released by adult beetles can attract nearby nematodes [60]. It appears that we have only begun to scratch the surface of a complex network of multi-directional communications mediated by ascarosides in nature.

Ascaroside processing by the nervous system

The nervous system detects pheromones, integrates different pheromone signals with other relevant information, and dispatches secondary messages [66] that ultimately modulate physiology and behavior (Figure 2). The logic of pheromone responses begins at the chemoreceptor. The eight ascaroside receptors identified so far belong to a large (1,341

genes) family of G-protein coupled receptors (GPCRs) related to the rhodopsin class GPCRs [67,68]. These eight receptors are expressed in a subset of bilaterally-paired chemosensory neurons that are open to the environment – named ASI, ASK, ASJ, and ADL [22,69–72]. Additional sensory neurons, such as the male-specific CEM and the ADF, have been implicated in ascaroside responses as well, but pheromone receptors expressed in these neurons are yet to be identified [9,39,73]. Two themes have started to emerge from these studies: 1) some receptors are tuned to a single ascaroside, while others to multiple ascarosides and 2) several receptors could detect the same ascaroside and regulate a given biological trait. If this “several ascarosides – several receptors” hypothesis is true, *C. elegans* may dedicate hundreds of GPCRs to ascaroside detection, perhaps in a condition- or trait-specific manner.

Expression patterns of most GPCRs remain to be determined, but a recent large-scale analysis of chemoreceptor expression [68] suggests that ASI, ASK, ASJ, and ADL will each express ~200–400 receptors, raising a question of how a single neuron could parse multiple inputs. One possible solution may be the increased complexity of the G-protein signaling within individual neurons. The *C. elegans* genome harbors 21 Gα proteins including single-copy, broadly-expressed orthologs of the four mammalian Gα families (Gs, Gi/o, Gq, and G12) and nematode-specific Gα genes that are expressed in restricted patterns in sensory neurons [74]. Pairing different Gα proteins with particular GPCRs in a given neuron, either in a dedicated complex or in a many-to-many relationship, as well as receptor heterodimerization, could establish specificity and independence of signaling by particular ascarosides. Whereas dedicated circuits may sense individual ascarosides [75], animals respond to the total pheromone blend. This process requires integration of inputs from several sensory neurons, possibly at the level of interneurons.

The complex relationship between ascarosides, receptors and the traits they control is well illustrated by distinct pheromone responses in strains that evolved under different conditions. For example, IC-ascC5/icas#9 differentially alters foraging behavior in N2 and MY14 strains [22,69]. The difference is due to variation in expression of two receptors, *srx-43* in the ASI and *srx-44* in the ASJ/ADL neurons, each state representing alternative successful strategies depending on the local distribution of food [22]. Experimental evolution [71] and population genetics [69] suggest that variation in chemoreceptor deployment may represent a general strategy for evolutionary plasticity in pheromone response.

As might be expected of signals that communicate social information, the same ascaroside molecules can elicit different responses in hermaphrodites and males. To some extent these differences are due to the function of sex-specific neurons, such as the CEMs in males [9,73]. Importantly, the genetic sex of the nervous system also modulates the activity of the neurons common to both sexes, resulting in consequential behavioral differences [76–79]. Even members of the same sex interpret social signals in the context of other relevant information, such as their current status (e.g. nutritional) and past experience [77,80]. This type of plasticity requires integration of inputs from neurons other than those that sense ascarosides and long-term modification of neuronal circuits that process and propagate ascaroside signals. For example, recent levels of environmental O₂ can change the valence of pheromones by altering the circuits centered on the RMG neuron [77]. This hub interneuron

[21] forms gap junctions with multiple sensory neurons, including those that sense O₂ and pheromones [21,81], and ensures context-appropriate behaviors. As we learn more about the circuits that mediate responses to different ascarosides, additional mechanisms may emerge that coordinate social signals with other types of relevant environmental information.

Conclusions and questions that systems biology approaches could help to answer

Despite the many recent advances highlighted above, our understanding of pheromone signaling in *C. elegans* is limited, particularly in the context of natural habitats and salient ecological interactions. We conclude this review by identifying two broad categories of problems that researchers in the field currently face and suggest ways in which systems biologists could contribute to this effort:

I. Production and perdurance of ascaroside pheromones in natural habitats –

How do pheromones communicate information about an individual's experience? How do individuals encounter pheromones in natural environments?

a. Goal 1: Reveal spatial distribution of pheromones

It is unclear to what degree animals release pheromones constitutively or situationally. Also unknown is whether individual molecules in pheromone blends are released independently or in coordinated pulses. Resolving these questions is necessary for understanding how *C. elegans* experience pheromone signals. Ascarosides are expected to diffuse readily in the environments in which nematodes live. This raises a question of how animals discriminate between signals produced by different senders – gradients emanating from a source or as discrete 'packets' of multiple ascarosides. Mass Spectrometry Imaging is a technique that can potentially be used to characterize the spatial distribution of compounds such as ascaroside pheromones.

b. Goal 2: Determine how pheromone distributions change over time

It is largely unclear what happens to ascaroside pheromones after they are released into the environment and whether or not they are subject to modification or degradation. Different chemical moieties and fatty acid chains suggest that the rates of degradation and diffusion of different ascarosides could be different. Experimental measurements of these rates could help to determine how animals distinguish between different pheromone sources and whether recipients could estimate when the sender released the signal based upon differential degradation or modification of different ascaroside components.

c. Goal 3: Characterize pheromones in natural samples

Critically, pheromones identified so far have been detected in laboratory settings. It is possible that different ascarosides are released in natural habitats, in response to other nematode species, pathogens, or diet. Additionally, the ascarosides from non-*elegans* sources that are present in *C. elegans* natural

habitats are still poorly characterized. Metabolomics can be used to survey the pheromones found in environmental samples that contain *C. elegans*.

II. Perception of pheromones by the neural circuits in the recipients –

How are the messages communicated by the sender deciphered by the receiver? How do animals distinguish between the honest messages released by their own species vs messages released by other species or by cheaters?

a. Goal 1: Experimentally link *C. elegans* chemoreceptors with ascaroside ligands

C. elegans likely dedicate dozens if not hundreds of chemoreceptor genes to sensing ascarosides, but the correspondence between ligands and receptors is still largely unknown. In *Drosophila* and humans, large-scale heterologous assays have been used to link a large number of receptors with specific odorants. Low-throughput heterologous approaches have been used in *C. elegans* to identify pheromone receptors but these experiments could be expanded to a genome-wide level. Promising candidates from heterologous essays could be readily tested *in vivo* using CRISPR.

b. Goal 2: *In silico* predict ascaroside/chemoreceptor pairs

In parallel, ligand docking and molecular dynamics can be used to predict the ascaroside pheromones bound to a given chemoreceptor, relying solely on protein sequence information. If successful, this approach could be extended to the growing number of related nematodes with genome sequences. How does ascaroside sensation change in relation to release of pheromones in different species? To what extent do species sense ascarosides that are produced by other species?

c. Goal 3: Characterize sensory neurons used for processing ascaroside signals

Identity of candidate ascaroside-binding GPCRs could be used to determine which neurons express them, providing a detailed map of the sensory portion of the nervous system dedicated to processing pheromone signals. Coupled with a similarly detailed (single-neuron resolution) map of Gα protein expression and experiments to identify physical contacts between GPCRs and Gα proteins, would help to test the hypotheses about molecular mechanisms that give rise to specificity and signal separation that characterize ascaroside detection by the nervous system.

Acknowledgements

We thank Rebecca Butcher and Frank Schroeder for comments on the manuscript and help with figures. This work was supported by NIH grant R01GM114170 to PTM and grants from NSF (IOS-1708518) and NIH (R01GM126125) to IR.

References

1. Bassler BL, Losick R: Bacterially speaking. *Cell* 2006, 125:237–246. [PubMed: 16630813]
2. Sekimoto H: Plant sex pheromones. *Vitam Horm* 2005, 72:457–478. [PubMed: 16492479]

3. Bardwell L: A walk-through of the yeast mating pheromone response pathway. *Peptides* 2005, 26:339–350. [PubMed: 15690603]
 4. Brennan PA, Zufall F: Pheromonal communication in vertebrates. *Nature* 2006, 444:308–315. [PubMed: 17108955]
 5. Karlson P, Luscher M: Pheromones': a new term for a class of biologically active substances. *Nature* 1959, 183:55–56. [PubMed: 13622694]
 6. White JG, Southgate E, Thomson JN, Brenner S: The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 1986, 314:1–340. [PubMed: 22462104]
 7. Butcher RA, Fujita M, Schroeder FC, Clardy J: Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. *Nat Chem Biol* 2007, 3:420–422. [PubMed: 17558398]
 8. Jeong PY, Jung M, Yim YH, Kim H, Park M, Hong E, Lee W, Kim YH, Kim K, Paik YK: Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. *Nature* 2005, 433:541–545. [PubMed: 15690045]
 9. Srinivasan J, Kaplan F, Ajredini R, Zachariah C, Alborn HT, Teal PE, Malik RU, Edison AS, Sternberg PW, Schroeder FC: A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. *Nature* 2008, 454:1115–1118. [PubMed: 18650807]
 10. Butcher RA: Decoding chemical communication in nematodes. *Nat Prod Rep* 2017, 34:472–477. [PubMed: 28386618]
 11. Ludewig AH, Schroeder FC: Ascaroside signaling in *C. elegans*. *WormBook* 2013:1–22.
 - 12**. Artyukhin AB, Zhang YK, Akagi AE, Panda O, Sternberg PW, Schroeder FC: Metabolomic “Dark Matter” Dependent on Peroxisomal beta-Oxidation in *Caenorhabditis elegans*. *J Am Chem Soc* 2018, 140:2841–2852.
- Comparative mass spectrometry revealed that production of hundreds of diverse and previously unknown compounds depends on the enzyme required for ascaroside synthesis. Altered metabolomes showed age- and tissue-specific profiles, suggesting multi-layer regulatory complexity
- . [PubMed: 29401383]
13. von Reuss SH, Bose N, Srinivasan J, Yim JJ, Judkins JC, Sternberg PW, Schroeder FC: Comparative metabolomics reveals biogenesis of ascarosides, a modular library of small-molecule signals in *C. elegans*. *J Am Chem Soc* 2012, 134:1817–1824. [PubMed: 22239548]
 14. Srinivasan J, von Reuss SH, Bose N, Zaslaver A, Mahanti P, Ho MC, O'Doherty OG, Edison AS, Sternberg PW, Schroeder FC: A modular library of small molecule signals regulates social behaviors in *Caenorhabditis elegans*. *PLoS Biol* 2012, 10:e1001237. [PubMed: 22253572]
 15. Bose NO A; von Reuss SH; Yim JJ; Ragsdale EJ; Sommer RJ; Schroeder FC: Complex Small-Molecule Architectures Regulate Phenotypic Plasticity in a Nematode. *Angew Chem Int Ed Engl*. 2012, 51:12438–12443. [PubMed: 23161728]
 16. Golden JW, Riddle DL: A pheromone influences larval development in the nematode *Caenorhabditis elegans*. *Science* 1982, 218:578–580. [PubMed: 6896933]
 17. Butcher RA, Ragains JR, Clardy J: An indole-containing dauer pheromone component with unusual dauer inhibitory activity at higher concentrations. *Org Lett* 2009, 11:3100–3103. [PubMed: 19545143]
 18. Butcher RA, Ragains JR, Kim E, Clardy J: A potent dauer pheromone component in *Caenorhabditis elegans* that acts synergistically with other components. *Proc Natl Acad Sci U S A* 2008, 105:14288–14292. [PubMed: 18791072]
 19. Pungalaya CS J; Fox BW; Malik RU; Ludewig AH; Sternberg PW; Schroeder FC. A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2009, 106:7708–7713. [PubMed: 19346493]
 20. Izrayelit Y, Srinivasan J, Campbell SL, Jo Y, von Reuss SH, Genoff MC, Sternberg PW, Schroeder FC: Targeted metabolomics reveals a male pheromone and sex-specific ascaroside biosynthesis in *Caenorhabditis elegans*. *ACS Chem Biol* 2012, 7:1321–1325. [PubMed: 22662967]

21. Macosko EZ, Pokala N, Feinberg EH, Chalasani SH, Butcher RA, Clardy J, Bargmann CI: A hub-and-spoke circuit drives pheromone attraction and social behaviour in *C. elegans*. *Nature* 2009, 458:1171–1175. [PubMed: 19349961]
- 22**. Greene JS, Brown M, Dobosiewicz M, Ishida IG, Macosko EZ, Zhang X, Butcher RA, Cline DJ, McGrath PT, Bargmann CI: Balancing selection shapes density-dependent foraging behaviour. *Nature* 2016, 539:254–258.
- Ascarosides suppress environmental exploration in favor of foraging. Several wild-isolated *C. elegans* strains do not alter foraging behavior in response to a particular ascaroside due to a mutation in the ascaroside receptor. The two haplotypes spanning the causal locus are maintained by balancing selection, each showing competitive advantage under a particular pattern of spatial distribution of food
[PubMed: 27799655]
23. Scott E, Hudson A, Feist E, Calahorra F, Dillon J, de Freitas R, Wand M, Schoofs L, O'Connor V, Holden-Dye L: An oxytocin-dependent social interaction between larvae and adult *C. elegans*. *Sci Rep* 2017, 7:10122. [PubMed: 28860630]
24. Aprison EZ, Ruvinsky I: Sex Pheromones of *C. elegans* Males Prime the Female Reproductive System and Ameliorate the Effects of Heat Stress. *PLoS Genet* 2015, 11:e1005729. [PubMed: 26645097]
25. Aprison EZ, Ruvinsky I: Sexually Antagonistic Male Signals Manipulate Germline and Soma of *C. elegans* Hermaphrodites. *Curr Biol* 2016, 26:2827–2833. [PubMed: 27618262]
26. McKnight K, Hoang HD, Prasain JK, Brown N, Vibbert J, Hollister KA, Moore R, Ragains JR, Reese J, Miller MA: Neurosensory perception of environmental cues modulates sperm motility critical for fertilization. *Science* 2014, 344:754–757. [PubMed: 24833393]
27. Yamada K, Hirotsu T, Matsuki M, Butcher RA, Tomioka M, Ishihara T, Clardy J, Kunitomo H, Iino Y: Olfactory plasticity is regulated by pheromonal signaling in *Caenorhabditis elegans*. *Science* 2010, 329:1647–1650. [PubMed: 20929849]
28. Hussey R, Stieglitz J, Mesgarzadeh J, Locke TT, Zhang YK, Schroeder FC, Srinivasan S: Pheromone-sensing neurons regulate peripheral lipid metabolism in *Caenorhabditis elegans*. *PLoS Genet* 2017, 13:e1006806. [PubMed: 28545126]
29. Ludewig AH, Izrayelit Y, Park D, Malik RU, Zimmermann A, Mahanti P, Fox BW, Bethke A, Doering F, Riddle DL, et al.: Pheromone sensing regulates *Caenorhabditis elegans* lifespan and stress resistance via the deacetylase SIR-2.1. *Proc Natl Acad Sci U S A* 2013, 110:5522–5527. [PubMed: 23509272]
30. Maures TJ, Booth LN, Benayoun BA, Izrayelit Y, Schroeder FC, Brunet A: Males shorten the life span of *C. elegans* hermaphrodites via secreted compounds. *Science* 2014, 343:541–544. [PubMed: 24292626]
31. Shi C, Runnels AM, Murphy CT: Mating and male pheromone kill *Caenorhabditis* males through distinct mechanisms. *Elife* 2017, 6.
32. Kaplan F, Srinivasan J, Mahanti P, Ajredini R, Durak O, Nimalendran R, Sternberg PW, Teal PE, Schroeder FC, Edison AS, et al.: Ascaroside expression in *Caenorhabditis elegans* is strongly dependent on diet and developmental stage. *PLoS One* 2011, 6:e17804. [PubMed: 21423575]
33. Joo HJ, Park S, Kim KY, Kim MY, Kim H, Park D, Paik YK: HSF-1 is involved in regulation of ascaroside pheromone biosynthesis by heat stress in *Caenorhabditis elegans*. *Biochem J* 2016, 473:789–796. [PubMed: 26759377]
34. Panda O, Akagi AE, Artyukhin AB, Judkins JC, Le HH, Mahanti P, Cohen SM, Sternberg PW, Schroeder FC: Biosynthesis of Modular Ascarosides in *C. elegans*. *Angew Chem Int Ed Engl* 2017, 56:4729–4733. [PubMed: 28371259]
35. Zhang X, Feng L, Chinta S, Singh P, Wang Y, Nunnery JK, Butcher RA: Acyl-CoA oxidase complexes control the chemical message produced by *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2015, 112:3955–3960. [PubMed: 25775534]

36. Zhang X, Wang Y, Perez DH, Jones Lipinski RA, Butcher RA: Acyl-CoA Oxidases Fine-Tune the Production of Ascaroside Pheromones with Specific Side Chain Lengths. *ACS Chem Biol* 2018, 13:1048–1056. [PubMed: 29537254]
- 37**. Zhou YW Y; Zhang X; Bhar S; Jones Lipinski RA; Han J; Feng L; Butcher RA: Biosynthetic tailoring of existing ascaroside pheromones alters their biological function in *C. elegans*. *Elife* 2018, A mechanism that links secreted chemical messages to changing environmental conditions. Upon starvation, *C. elegans* activates a pathway that modifies fatty acids of indolated ascarosides to convert them from aggregation to dispersal pheromones

DOI: 10.7554/eLife.33286.
38. Morsci NS, Haas LA, Barr MM: Sperm status regulates sexual attraction in *Caenorhabditis elegans*. *Genetics* 2011, 189:1341–1346. [PubMed: 21968192]
39. White JQ, Nicholas TJ, Gritton J, Truong L, Davidson ER, Jorgensen EM: The sensory circuitry for sexual attraction in *C. elegans* males. *Curr Biol* 2007, 17:1847–1857. [PubMed: 17964166]
40. Leighton DH, Choe A, Wu SY, Sternberg PW: Communication between oocytes and somatic cells regulates volatile pheromone production in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2014, 111:17905–17910. [PubMed: 25453110]
41. Ludewig AH, Gimond C, Judkins JC, Thornton S, Pulido DC, Micikas RJ, Doring F, Antebi A, Braendle C, Schroeder FC: Larval crowding accelerates *C. elegans* development and reduces lifespan. *PLoS Genet* 2017, 13:e1006717. [PubMed: 28394895]
42. Zhou Y, Loeza-Cabrera M, Liu Z, Aleman-Meza B, Nguyen JK, Jung SK, Choi Y, Shou Q, Butcher RA, Zhong W: Potential Nematode Alarm Pheromone Induces Acute Avoidance in *Caenorhabditis elegans*. *Genetics* 2017, 206:1469–1478. [PubMed: 28495959]
43. Artyukhin AB, Schroeder FC, Avery L: Density dependence in *Caenorhabditis* larval starvation. *Sci Rep* 2013, 3:2777. [PubMed: 24071624]
44. Chan IL, Rando OJ, Conine CC: Effects of Larval Density on Gene Regulation in *Caenorhabditis elegans* During Routine L1 Synchronization. *G3 (Bethesda)* 2018, 8:1787–1793.
45. Schulenburg H, Felix MA: The Natural Biotic Environment of *Caenorhabditis elegans*. *Genetics* 2017, 206:55–86. [PubMed: 28476862]
- 46**. Richaud A, Zhang G, Lee D, Lee J, Felix MA: The Local Coexistence Pattern of Selfing Genotypes in *Caenorhabditis elegans* Natural Metapopulations. *Genetics* 2018, 208:807–821. A natural population of *C. elegans* was sampled and genotyped for multiple years. Despite the opportunity for recombination, a few major haplotypes dominated, suggesting that strains of *C. elegans* evolve independently in the wild

[PubMed: 29242287]
47. Barriere A, Felix MA: High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. *Curr Biol* 2005, 15:1176–1184. [PubMed: 16005289]
48. Cutter AD: Nucleotide polymorphism and linkage disequilibrium in wild populations of the partial selfer *Caenorhabditis elegans*. *Genetics* 2006, 172:171–184. [PubMed: 16272415]
49. Haber M, Schungel M, Putz A, Muller S, Hasert B, Schulenburg H: Evolutionary history of *Caenorhabditis elegans* inferred from microsatellites: evidence for spatial and temporal genetic differentiation and the occurrence of outbreeding. *Mol Biol Evol* 2005, 22:160–173. [PubMed: 15371529]
50. Bose N, Meyer JM, Yim JJ, Mayer MG, Markov GV, Ogawa A, Schroeder FC, Sommer RJ: Natural variation in dauer pheromone production and sensing supports intraspecific competition in nematodes. *Curr Biol* 2014, 24:1536–1541. [PubMed: 24980503]
51. Diaz SA, Brunet V, Lloyd-Jones GC, Spinner W, Wharam B, Viney M: Diverse and potentially manipulative signalling with ascarosides in the model nematode *C. elegans*. *BMC Evol Biol* 2014, 14:46. [PubMed: 24618411]

52. Mayer MG, Sommer RJ: Natural variation in *Pristionchus pacificus* dauer formation reveals cross-preference rather than self-preference of nematode dauer pheromones. *Proc Biol Sci* 2011, 278:2784–2790. [PubMed: 21307052]
 53. Falcke JMB,N; Artyukhin AB; Rödelberger C; Markov GV; Yim JJ, Grimm D; Claassen MH; Panda O; Baccile JA; Zhang YK; Le HH; Jolic D; Schroeder FC; Sommer RJ: Linking Genomic and Metabolomic Natural Variation Uncovers Nematode Pheromone Biosynthesis. *Cell Chem Biol*. 2018, In press.
 54. Viney M, Harvey S: Reimagining pheromone signalling in the model nematode *Caenorhabditis elegans*. *PLoS Genet* 2017, 13:e1007046. [PubMed: 29095825]
 55. Felix MA, Duveau F: Population dynamics and habitat sharing of natural populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC Biol* 2012, 10:59. [PubMed: 22731941]
 56. Choe A, Chuman T, von Reuss SH, Dossey AT, Yim JJ, Ajredini R, Kolawa AA, Kaplan F, Alborn HT, Teal PE, et al.: Sex-specific mating pheromones in the nematode *Panagrellus redivivus*. *Proc Natl Acad Sci U S A* 2012, 109:20949–20954. [PubMed: 23213209]
 57. Choe A, von Reuss SH, Kogan D, Gasser RB, Platzer EG, Schroeder FC, Sternberg PW: Ascaroside signaling is widely conserved among nematodes. *Curr Biol* 2012, 22:772–780. [PubMed: 22503501]
 58. Kaplan F, Alborn HT, von Reuss SH, Ajredini R, Ali JG, Akyazi F, Stelinski LL, Edison AS, Schroeder FC, Teal PE: Interspecific nematode signals regulate dispersal behavior. *PLoS One* 2012, 7:e38735. [PubMed: 22701701]
 59. Manosalva P, Manohar M, von Reuss SH, Chen S, Koch A, Kaplan F, Choe A, Micikas RJ, Wang X, Kogel KH, et al.: Conserved nematode signalling molecules elicit plant defenses and pathogen resistance. *Nat Commun* 2015, 6:7795. [PubMed: 26203561]
 - 60*. Zhao L, Zhang X, Wei Y, Zhou J, Zhang W, Qin P, Chinta S, Kong X, Liu Y, Yu H, et al.: Ascarosides coordinate the dispersal of a plant-parasitic nematode with the metamorphosis of its vector beetle. *Nat Commun* 2016, 7:12341
- The pinewood nematode, *Bursaphelenchus xylophilus*, engages in a two-way ascaroside-mediated communication with its beetle vector. Nematode pheromones promote reproductive development of the beetle, whereas adult beetles secrete ascarosides that attract nematode larvae, facilitating dispersal
- . [PubMed: 27477780]
61. Chaudhuri J, Bose N, Tandonnet S, Adams S, Zucco G, Kache V, Parihar M, von Reuss SH, Schroeder FC, Pires-daSilva A: Mating dynamics in a nematode with three sexes and its evolutionary implications. *Sci Rep* 2015, 5:17676. [PubMed: 26631423]
 62. Dong C, Dolke F, von Reuss SH: Selective MS screening reveals a sex pheromone in *Caenorhabditis briggsae* and species-specificity in indole ascaroside signalling. *Org Biomol Chem* 2016, 14:7217–7225. [PubMed: 27381649]
 63. Dong C, Reilly DK, Bergame C, Dolke F, Srinivasan J, von Reuss SH: Comparative Ascaroside Profiling of *Caenorhabditis* Exometabolomes Reveals Species-Specific (omega) and (omega - 2)-Hydroxylation Downstream of Peroxisomal beta-Oxidation. *J Org Chem* 2018.
 64. Noguez JH, Conner ES, Zhou Y, Ciche TA, Ragains JR, Butcher RA: A novel ascaroside controls the parasitic life cycle of the entomopathogenic nematode *Heterorhabditis bacteriophora*. *ACS Chem Biol* 2012, 7:961–966. [PubMed: 22444073]
 65. Hsueh YP, Mahanti P, Schroeder FC, Sternberg PW: Nematode-trapping fungi eavesdrop on nematode pheromones. *Curr Biol* 2013, 23:83–86. [PubMed: 23246407]
 66. Park D, Hahm JH, Park S, Ha G, Chang GE, Jeong H, Kim H, Kim S, Cheong E, Paik YK: A conserved neuronal DAF-16/FoxO plays an important role in conveying pheromone signals to elicit repulsion behavior in *Caenorhabditis elegans*. *Sci Rep* 2017, 7:7260. [PubMed: 28775361]
 67. Thomas JH, Robertson HM: The *Caenorhabditis* chemoreceptor gene families. *BMC Biol* 2008, 6:42. [PubMed: 18837995]

68. Vidal B, Aghayeva U, Sun H, Wang C, Glenwinkel L, Bayer EA, Hobert O: An atlas of *Caenorhabditis elegans* chemoreceptor expression. *PLoS Biol* 2018, 16:e2004218. [PubMed: 29293491]
- 69**. Greene JS, Dobosiewicz M, Butcher RA, McGrath PT, Bargmann CI: Regulatory changes in two chemoreceptor genes contribute to a *Caenorhabditis elegans* QTL for foraging behavior. *Elife* 2016, 5
- cis*-regulatory variation in a locus with two adjacent pheromone receptors determines the differences in ascaroside-induced exploratory behavior between *C. elegans* strains. These genetic differences result in changes in expression level and remapping of the chemoreceptors to different sensory neurons, causing different foraging behaviors. Balancing selection maintains this variation and evidence suggests that many pheromone receptors may evolve in a similar regime
70. Kim K, Sato K, Shibuya M, Zeiger DM, Butcher RA, Ragains JR, Clardy J, Touhara K, Sengupta P: Two chemoreceptors mediate developmental effects of dauer pheromone in *C. elegans*. *Science* 2009, 326:994–998. [PubMed: 19797623]
71. McGrath PT, Xu Y, Ailion M, Garrison JL, Butcher RA, Bargmann CI: Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor genes. *Nature* 2011, 477:321–325. [PubMed: 21849976]
72. Park D, O'Doherty I, Somvanshi RK, Bethke A, Schroeder FC, Kumar U, Riddle DL: Interaction of structure-specific and promiscuous G-protein-coupled receptors mediates small-molecule signaling in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2012, 109:9917–9922. [PubMed: 22665789]
73. Narayan A, Venkatachalam V, Durak O, Reilly DK, Bose N, Schroeder FC, Samuel AD, Srinivasan J, Sternberg PW: Contrasting responses within a single neuron class enable sex-specific attraction in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2016, 113:E1392–1401. [PubMed: 26903633]
74. Jansen G, Thijssen KL, Werner P, van der Horst M, Hazendonk E, Plasterk RH: The complete family of genes encoding G proteins of *Caenorhabditis elegans*. *Nat Genet* 1999, 21:414–419. [PubMed: 10192394]
- 75**. Aprison EZ, Ruvinsky I: Counteracting Ascarosides Act through Distinct Neurons to Determine the Sexual Identity of *C. elegans* Pheromones. *Curr Biol* 2017, 27:2589–2599 e2583.
- Sexual identity (male vs. female/hermaphrodite) of pheromone blends in *C. elegans* is determined by the ratio of concentrations of two counteracting ascarosides. Distinct neurons mediate effects of these two molecules in the recipients, suggesting that the ratio is ascertained by activity of opposing neuronal circuits
- . [PubMed: 28844646]
- 76**. Fagan KA, Luo J, Lagoy RC, Schroeder FC, Albrecht DR, Portman DS: A Single-Neuron Chemosensory Switch Determines the Valence of a Sexually Dimorphic Sensory Behavior. *Curr Biol* 2018, 28:902–914 e905.
- Sex of the recipient animal shapes valence of pheromone signals. Genetic sex of a single sensory neuron could determine whether pheromones elicit attraction or repulsion
- . [PubMed: 29526590]
- 77**. Fenk LA, de Bono M: Memory of recent oxygen experience switches pheromone valence in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2017, 114:4195–4200.
- C. elegans* integrate pheromone signals with other sensory inputs. Worms find a mix of ascarosides either attractive or repulsive, depending on prior exposure to O₂. This experience reprograms a hub interneuron that alters the responses of chemosensory neurons that detect pheromones
- . [PubMed: 28373553]

78. Jang H, Kim K, Neal SJ, Macosko E, Kim D, Butcher RA, Zeiger DM, Bargmann CI, Sengupta P: Neuromodulatory state and sex specify alternative behaviors through antagonistic synaptic pathways in *C. elegans*. *Neuron* 2012, 75:585–592. [PubMed: 22920251]
79. White JQ, Jorgensen EM: Sensation in a single neuron pair represses male behavior in hermaphrodites. *Neuron* 2012, 75:593–600. [PubMed: 22920252]
- 80**. Hong M, Ryu L, Ow MC, Kim J, Je AR, Chinta S, Huh YH, Lee KJ, Butcher RA, Choi H, et al.: Early Pheromone Experience Modifies a Synaptic Activity to Influence Adult Pheromone Responses of *C. elegans*. *Curr Biol* 2017, 27:3168–3177 e3163.
- Exposure during early larval development increases avoidance of an ascaroside in adults, who normally find this molecule repulsive. The imprinting is mediated by an altered connection between the chemosensory neurons and their post-synaptic motor neuron partners
- . [PubMed: 28988862]
81. Jang H, Levy S, Flavell SW, Mende F, Latham R, Zimmer M, Bargmann CI: Dissection of neuronal gap junction circuits that regulate social behavior in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 2017, 114:E1263–E1272. [PubMed: 28143932]

Highlights

- Ascaroside pheromones of *C. elegans* are an attractive model to understand the principles of information exchange via chemical signals
- Nematodes release blends of ascarosides that are species-specific and reflect condition of the sender
- Ascaroside signaling is involved in communication with predators, hosts, and vectors associated with nematodes
- Neural circuits that sense ascarosides and modulate behavior, development, and physiology are being define

Figure 1.

Overview of the chemical structure of the ascaroside pheromones, summarizing the diversity of hundreds of unique molecules identified in nematode secretions. Each ascaroside contains an ascarylose sugar (black) attached to a fatty acid of variable length (blue). Additional moieties (R^1 , R^2 , red, and green) could decorate the ascarylose and the fatty acid.

Figure 2. Schematic representation of a relationship between ascarosides, their cognate receptors, sensory neurons, and the phenotypic effects they elicit. Colored squares underneath ascarosides and receptors correspond to the phenotypes regulated by them. Solid lines between ascarosides and receptors indicate inferences based on heterologous expression or pull-down assays; dotted lines indicate that genetic information was used to assign ascaroside/chemoreceptor pairing. The arrows connecting receptors and sensory neurons represent expression patterns. Estimated numbers of GPCRs expressed by each neuron (extrapolated from [68]) are shown.