Dispatches

Pheromones: Evolving Language of Chemical Communication in Nematodes

New research reveals how the intricate repertoire of ascarosides — small molecules acting as multifaceted pheromones in the nematode worm *Caenorhabditis elegans* — has evolved in divergent nematode taxa occupying contrasted ecological niches.

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Environmental sensing and inter-individual communication via pheromone systems are fundamental to most organisms, guiding their behaviour, development and physiology. Recent studies using the nematode *Caenorhabditis elegans* have unravelled a surprisingly wide spectrum of ascaroside metabolites that serve as pheromones in the regulation of developmental decisions and distinct behaviours. A new study published in this issue of *Current Biology* [1] now shows that ascaroside synthesis and signalling are conserved in phylogenetically distant nematode groups.

Ascarosides are nematode-specific signalling molecules, composed of the dideoxysugar ascarolyse, linked to fatty-acid-like side chains. Modification of side chain length and derivatization with various moieties generate structural and functional diversity of ascarosides [2] (Figure 1A). The process of ascaroside biosynthesis consequently relies on multiple metabolic pathways, involving peroxisomal beta-oxidation and carbohydrate and amino acid catabolism [2–4].

The study of ascaroside signalling has its roots in the discovery of the pheromone-mediated induction of the alternative developmental stage, the dauer, in *C. elegans* [5]. This morphologically specialised and developmentally arrested larval stage allows survival of adverse conditions and is induced in response to high population density, starvation and high temperature. Organic extracts from worms grown in such conditions were shown to be sufficient to induce dauer development, indicating the activity of a dauer pheromone [5]. The molecular composition of the dauer pheromone remained a mystery until 2005, when the first ascaroside (ascr#1), labelled daumone, was isolated [6]. Subsequent research showed that multiple ascarosides (ascr#1–3, 5, 8 and icas#9) act synergistically to form a dauer-inducing cocktail, with ascr#2 and ascr#3 being many times more potent than the originally identified daumone [7–9]. Production of these ascarosides varies across developmental stage and increases under dauer-inducing conditions, such as starvation; the *C. elegans* dauer itself, however, does not seem to secrete any significant amounts of ascarosides [9].

Several recent studies [10–13] have further implicated ascaroside signalling in diverse behaviours, including sex-specific attraction, repulsion, aggregation or olfactory plasticity. Remarkably, the same ascarosides regulating dauer formation are involved in behaviour. While eliciting dauer formation requires nano- to micromolar ascaroside concentrations, behavioural responses may be mediated by concentrations in the femto- to picomolar range. Srinivisan *et al.* [10] showed that a mixture of multiple ascarosides, in particular ascr#3 and ascr#8, act as strong male attractors at very low concentrations. At higher concentrations, the same mixture loses its appeal to males and instead repels hermaphrodites.

Adding to the complexity of their biology, ascarosides containing tryptophan-derived indole moeities — indole ascarosides — play a newly discovered role in affecting aggregation behaviour: *C. elegans* hermaphrodites are strongly attracted to femtomolar to picomolar concentrations of icas#3 and icas#9, causing aggregation or ‘clumping’ of individuals, an extensively studied behavioural phenomenon [13]. Ascarosides are thus structurally and functionally versatile signalling molecules. Signal activity and specificity for a given function depends on chemical structure and concentration of individual compounds, as well as on the combination of structurally different compounds, which moreover behave additively or synergistically. Specificity in ascaroside signalling may therefore be generated through combinatorial action of both qualitative and quantitative properties.

A key current quest is to identify how the nematode’s sensory system perceives and integrates such complicated ascaroside blends to trigger specific developmental and behavioural changes. The simple and well-characterized *C. elegans* nervous system has already allowed the identification of specific pheromone-sensing neurons involved in ascaroside-mediated mating and aggregation behaviours [10–13]. Dauer pheromone has been known to target chemoreceptors of the amphid neurons, but it was only recently that specific chemoreceptor genes of the G-protein-coupled-receptor family have been shown to be required for dauer induction in response to specific subsets of ascarosides [14,15]. The complexity of ascaroside signalling is likely to be matched by a diverse and large ascaroside receptor repertoire, given that individual neurons express multiple chemoreceptors and that the chemosensory gene repertoire in *C. elegans* is exceptionally broad [16] and remains to be fully explored.

Although long-chain ascarosides had been previously identified in ascarid nematodes [17], nothing was known about short-chain ascarosides potentially involved in pheromone signalling. As they report in this issue, Choe *et al.* [1] characterized ascaroside profiles in 19 evolutionarily divergent nematode species, sampled from different branches of the nematode phylogeny, including free-living and parasitic nematodes of mammals, insects and plants

(Figure 1B). Using a high performance liquid chromatography/mass spectrometry-based screen (HPLC/MS), various ascarosides could be recovered from most species, indicating that ascaroside production is conserved within this highly diversified phylum. Spectra and quantities of ascaroside production exhibited, however, substantial differences between species.

Although the evidence is not yet conclusive, these differences could be due to species- or clade-specific trends in ascaroside synthesis and/or be linked to ecological specialization of certain nematode life styles. For example, all assayed entomopathogenic *Steinerma* species consistently revealed high production of ascr#9, as did other insect-associated nematodes. Furthermore, ascaroside structural diversity may be taxon-specific: the indole-containing ascaroside, icas#9, was only found in rhabditid nematodes, such as *Caenorhabditis*. In four of the 19 examined species, including the name-giving parasitic ascarid nematodes, no signalling ascarosides could be recovered in significant amounts. This could indicate that certain major taxonomic groups, such as the mermithid nematodes, lack synthesis of ascaroside signalling molecules or that these groups produce a structurally divergent set of ascarosides that awaits discovery.

In addition to demonstrating the evolutionary conservation of ascaroside biosynthesis, Choe *et al.* [1] present evidence that uncovered ascarosides indeed act as signalling molecules in divergent nematode species. In several behavioural assays, synthetic ascarosides elicited species- and sex-specific behavioural responses, such as attraction and repulsion, and individuals of certain species were attracted by pheromones typical for an unrelated species, yet repelled by those from another. Taken together, these chemotaxis assays indicate that different nematode species respond to specific but sometimes overlapping sets of ascarosides. To what degree ascaroside signalling among unrelated nematodes could be biologically relevant remains unclear but this could be tested with species that co-occur in the same natural habitat and that compete for the same resources.

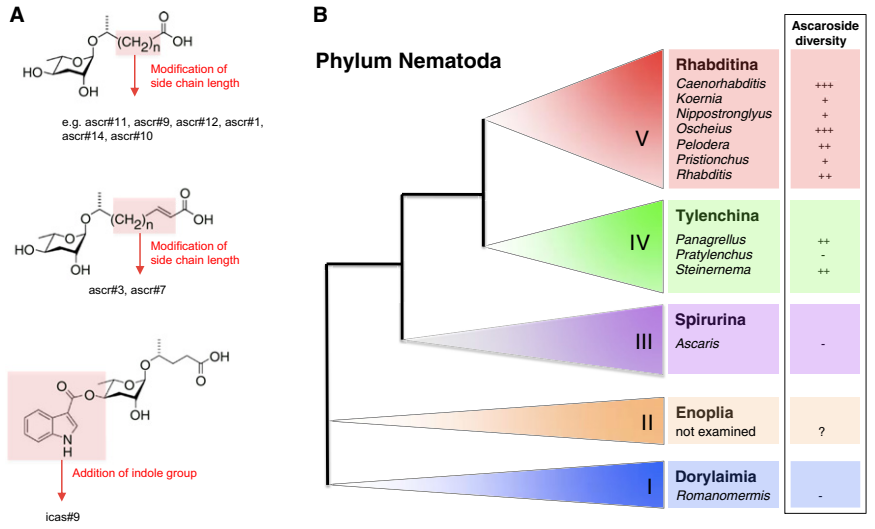

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Figure 1. Ascaroside signalling in nematodes.

(A) Examples illustrating the structural diversity of ascaroside signalling molecules. Minor modification of molecular building blocks, such as side chains, may modulate functional roles of ascarosides [1]. (B) A highly schematic view of the major nematode clades, indicating the 12 different genera examined for ascaroside synthesis by Choe *et al.* [1]. Adapted with permission from Blaxter [19].

To evaluate the extent of intraspecific variation in ascaroside synthesis, Choe *et al.* [1] also compared ascaroside profiles among genetically distinct wild isolates of *C. elegans*. While the range of expressed ascarosides was similar, isolates differed in the secreted quantity of specific ascarosides. It will be of major interest to analyse how such genotype-specific ascaroside profiles relate to natural variation in ascaroside receptor diversity and ultimate effects on development and behaviour. McGrath *et al.* [15] conducted such an integrative analysis and demonstrated how a set of G-coupled receptors encoded by *serpentine receptor class g* (*srg*) genes act as ascaroside receptors in dauer formation, and how they have been lost during laboratory evolution due to environmental conditions favouring the loss of dauer formation. This evolutionary change in ascaroside sensitivity has occurred through deletion of the same pheromone receptors independently in two *C. elegans* strains, and through deletion of a paralogous set of receptors in an isolate of *C. briggsae*.

This impressive example of parallel evolution illustrates how ascaroside signalling may evolve very rapidly at the receptor level. This view is also supported by genomic analyses indicating drastic evolutionary divergence of chemoreceptor gene families in the genus *Caenorhabditis* [16], and high levels of sequence variation among *C. elegans* wild isolates [18]. Understanding the evolution of ascaroside signal and receptor diversity at the intraspecific level is not only technically feasible through genetic analysis but also the biologically most relevant object of study, given that competition for resources and mates primarily acts among conspecifics.

Small-molecule signalling via ascarosides in the phylum Nematoda — reminiscent of bacterial quorum sensing systems — enables detection of complex environmental situations to modulate behaviour and development. The recent discoveries on nematode ascarosides provide unprecedented experimental access to investigate pheromone communication and its evolution. This study system will be of great value to neurobiology and chemical ecology, and further promises to help developing novel, highly specific molecules to control parasitic nematodes of biomedical and agricultural relevance. Ascarosides may be small but they are certain to make a big impact on research for many years to come.

References

1. Choe, A., von Reuss, S.H., Kogan, D., Gasser, R.B., Platzer, E.G., Schroeder, F.C., and Sternberg, P.W. (2012). Ascaroside signaling is widely conserved among nematodes. Curr. Biol. *22*, 772–780.
2. von Reuss, S.H., Bose, N., Srinivasan, J., Yim, J.J., Judkins, J.C., Sternberg, P.W., and Schroeder, F.C. (2012). Comparative metabolomics reveals biogenesis of ascarosides, a modular library of small-molecule signals in *C. elegans*. J. Am. Chem. Soc. *134*, 1817–1824.
3. Butcher, R.A., Ragains, J.R., Li, W., Ruvkun, G., Clardy, J., and Mak, H.Y. (2009). Biosynthesis of the *Caenorhabditis elegans* dauer pheromone. Proc. Natl. Acad. Sci. USA *106*, 1875–1879.
4. Pungaliya, C., Srinivasan, J., Fox, B.W., Malik, R.U., Ludewig, A.H., Sternberg, P.W., and Schroeder, F.C. (2009). A shortcut to identifying small molecule signals that regulate behavior and development in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *106*, 7708–7713.
5. Golden, J.W., and Riddle, D.L. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science *218*, 578–580.
6. Jeong, P.Y., Jung, M., Yim, Y.H., Kim, H., Park, M., Hong, E., Lee, W., Kim, Y.H., Kim, K., and Paik, Y.K. (2005). Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. Nature *433*, 541–545.
7. Butcher, R.A., Fujita, M., Schroeder, F.C., and Clardy, J. (2007). Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. Nat. Chem. Biol. *3*, 420–422.
8. Butcher, R.A., Ragains, J.R., Kim, E., and Clardy, J. (2008). A potent dauer pheromone component in *Caenorhabditis elegans* that acts synergistically with other components. Proc. Natl. Acad. Sci. USA *105*, 14288–14292.
9. Kaplan, F., Srinivasan, J., Mahanti, P., Ajredini, R., Durak, O., Nimalendran, R., Sternberg, P.W., Teal, P.E., Schroeder, F.C., Edison, A.S., *et al*. (2011). Ascaroside expression in *Caenorhabditis elegans* is strongly dependent on diet and developmental stage. PLoS One *6*, e17804.
10. Srinivasan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H.T., Teal, P.E., Malik, R.U., Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2008). A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. Nature *454*, 1115–1118.
11. Yamada, K., Hirotsu, T., Matsuki, M., Butcher, R.A., Tomioka, M., Ishihara, T., Clardy, J., Kunitomo, H., and Iino, Y. (2010). Olfactory plasticity is regulated by pheromonal signaling in *Caenorhabditis elegans*. Science *329*, 1647–1650.
12. Macosko, E.Z., Pokala, N., Feinberg, E.H., Chalasani, S.H., Butcher, R.A., Clardy, J., and Bargmann, C.I. (2009). A hub-and-spoke circuit drives pheromone attraction and social behaviour in *C. elegans*. Nature *458*, 1171–1175.
13. Srinivasan, J., von Reuss, S.H., Bose, N., Zaslaver, A., Mahanti, P., Ho, M.C., O'Doherty, O.G., Edison, A.S., Sternberg, P.W., and Schroeder, F.C. (2012). A modular library of small molecule signals regulates social behaviors in *Caenorhabditis elegans*. PLoS Biol. *10*, e1001237.
14. Kim, K., Sato, K., Shibuya, M., Zeiger, D.M., Butcher, R.A., Ragains, J.R., Clardy, J., Touhara, K., and Sengupta, P. (2009). Two chemoreceptors mediate developmental effects of dauer pheromone in *C. elegans*. Science *326*, 994–998.
15. McGrath, P.T., Xu, Y., Ailion, M., Garrison, J.L., Butcher, R.A., and Bargmann, C.I. (2011). Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor genes. Nature *477*, 321–325.
16. Thomas, J.H., and Robertson, H.M. (2008). The *Caenorhabditis* chemoreceptor gene families. BMC Biol. *6*, 42.
17. Bartley, J.P., Bennett, E.A., and Darben, P.A. (1996). Structure of the ascarosides from *Ascaris suum*. J. Nat. Prod. *59*, 921–926.
18. Stewart, M.K., Clark, N.L., Merrihew, G., Galloway, E.M., and Thomas, J.H. (2004). High genetic diversity in the chemoreceptor superfamily of *C. elegans*. Genetics *169*, 1985–1996.
19. Blaxter, M.L. (2011). Nematodes: The worm and its relatives. PLoS Biol. *9*, e1001050.

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Endosymbiotic Evolution: RNA Intermediates in Endosymbiotic Gene Transfer

More than a billion years of endosymbiotic evolution has resulted in extensive gene relocation between the genetic compartments of eukaryotic cells. A new study uses chloroplast genome transformation to shed light on the mechanisms involved.

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In the 40 years since Lynn Margulis [1,2] revived the endosymbiotic theory of eukaryotic evolution after decades of neglect, experimental results have been universally supportive of the concepts. Convincing evidence has been provided both by inference from bioinformatic analyses of nuclear genomes and by experimental recapitulation of some of the key processes [3]. Since the engulfment of the prokaryote ancestors of mitochondria and, later, plastids (chloroplasts) by a precursor of the nucleated cell, many genes have migrated to the nucleus of the host at the expense of the endosymbiont genomes. As a result, extant cytoplasmic organellar genomes encode very few proteins, making it necessary for chloroplasts and mitochondria to import thousands of nucleus-encoded gene products required for their biogenesis and function. A new study in this issue of *Current Biology* [4] uncovers mechanistic detail regarding how gene transfer to the nucleus occurs.

The majority of functional endosymbiotic gene transfer (EGT) probably occurred relatively soon after the formation of the first conglomerate cells, and the process appears to have gone as far as it will for mitochondrial genes in animal cells, though some protists with altered biochemistry lack a mitochondrial genome altogether. Interestingly, EGT is still ticking over for both mitochondrial and chloroplast genes in plant systems. Furthermore, some steps in the EGT process still occur in essentially all eukaryotes, where they continue to have a major influence on nuclear evolution [3]. Simple and shuffled tracts of genetic information from cytoplasmic organelles, resulting from DNA transfer *per se*, are universally seen in the nuclear genome of essentially all organisms examined [5–7]. Thus, the processes responsible for EGT and associated DNA transfer are fundamental to eukaryote evolution, the production of genetic diversity, and the emergence of multicellular organisms.

A small sample of the thousands of nuclear genes that have entered plant nuclei by EGT [8] have been examined in detail, and some transfer events appear to have involved an RNA intermediate [9–11]. This suggestion is based on the observation that some nuclear genes contributing to some plant mitochondrial proteomes are more similar to spliced and edited mitochondrial mRNAs than to the equivalent gene copies that remain in the mitochondrial genome in some related plant species. There is clear