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Caenorhabditis elegans Pheromones Regulate Multiple Complex Behaviors

Arthur S. Edison
Box 100245, Department of Biochemistry & Molecular Biology, McKnight Brain Institute, and National High Magnetic Field Laboratory, University of Florida, Gainesville FL 32610-0245

Summary of recent advances

A family of small molecules called ascarosides act as pheromones to control multiple behaviors in the nematode *Caenorhabditis elegans*. At picomolar concentrations, a synergistic mixture of at least three ascarosides produced by hermaphrodites causes male-specific attraction. At higher concentrations, the same ascarosides, perhaps in a different mixture, induce the developmentally arrested stage known as dauer. The production of ascarosides is strongly dependent on environmental conditions, although relatively little is known about the major variables and mechanisms of their regulation. Thus, male mating and dauer formation are linked through a common set of small molecules whose expression is sensitive to a given microenvironment, suggesting a model by which ascarosides regulate the overall life cycle of *C. elegans*.

Introduction

The nematode *Caenorhabditis elegans* is one of the best-studied animals on earth. It has both self-fertilizing hermaphrodites and males, about 1000 cells, a simple nervous system with about 300 neurons, and is a nearly perfect genetic model organism with a 3.5 day development time from fertilized egg to adult [1]. Numerous fundamental biological and medical discoveries have been made using this model laboratory organism, including the development of the complete cell lineage [2], the structure of the nervous system [3], the discovery of the molecular basis of axon guidance [4], apoptosis [5,6], the identification of a ras protein involved in vulva development [7], the discovery of microRNAs [8,9], and RNAi [10]. *C. elegans* has also been a valuable model organism in fundamental areas such as chemotaxis and olfaction [11], aging [12], and in the *in vivo* application of the green fluorescent protein [13]. Until recently,

Email: aedison@ufl.edu.

Sidebar: Nomenclature) The original daumone was named according to its function in promoting dauer formation. This is reasonable for a single compound with a single function. A second nomenclature in use is to name the compounds by the length of the carbon side-chains on the ascarylose sugar. Under this nomenclature, daumone is C7. This system avoids limitations when naming according to its function, but it has its own limitations when dealing with modifications such in ascr#4, the glycosylated form of ascr#2 or ascr#9, an indole derivative (Table 1). Another limitation would be if a new compound were identified with, for example, a six carbon carboxylic acid chain rather than a methyl ketone. For these reasons, I favor the system in which the ascarosides are named sequentially in order of their discovery, without reference to function or molecular architecture. In this system, daumone is ascr#1, C6 is ascr#2, C9 is ascr#3, and the glycosylated form of c6 is ascr#4. This system was developed to be easily searchable and to be distinct from nomenclature for phenotypes, genes or proteins [30]. Note that there are no spaces in the names in order to improve automated text searching. Frank Schroeder has established a *C. elegans* Small Metabolic Molecule Identifiers Database (http://www.smmid.org/) that has up-to-date information on pheromones and other metabolic signaling molecules in nematodes.

relatively little was known about the small molecules that *C. elegans* uses for chemical signaling between individuals. However, a significant body of knowledge has been discovered in just the past four years, and the relatively new field of *C. elegans* chemical biology is enhancing the vast amount of genetic, anatomical, and cellular information that has made it such an outstanding model organism.

The word "pheromone" was proposed in 1959 by Karlson and Lüscher [14]. The word is derived from the Greek φερειν (to transfer, carry, bear) and ορμων (to excite, set in motion). Karlson and Lüscher recognized the need to distinguish pheromones from hormones, and they define pheromones as "substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction, for example, a definite behaviour or a developmental process" [14]. Wyatt has recently published a wonderful essay on the 50-year perspective of pheromone research [15]. Much of what we know about pheromones comes from insects, from the initial discovery nearly 50 years ago of the silk moth bombykol, a straight-chain16 carbon primary alcohol with 2 double bonds [16], to recent work identifying the cockroach blattellaquinone, a modified quinone [17]. Both bombykol and blattellaquinone are sex pheromones, but they are completely different chemically. Remarkably, many species of moth and female Asian elephants both use the same compound, (Z)-7-dodecen-1-yl acetate (a 12 carbon chain with one double bond and an acetate group), as a sex pheromone [18]. The extensive work in insect pheromones has lead to both great practical advances (for example, pheromone traps for moths are available at any home improvement store) and a deep understanding of the molecular genetics underlying insect pheromone behavioral responses [19].

Vanillic acid is a sex pheromone in the parasitic soybean cyst nematode [20], but this review focuses almost exclusively on the ascarosides, a family of small molecule pheromones that control two basic *C. elegans* traits: developmental arrest at the dauer stage and male mating behavior (Fig 1A). I will start by briefly summarizing dauer arrest and male mating behavior, and the bulk of the review will focus on the identification and biological characterization of *C. elegans* ascarosides (Table 1), starting with the discovery of daumone in 2005 [21]. I will briefly relate the *C. elegans* ascarosides to those originally found in the parasitic nematode *Ascaris* (Fig 1B), the animal from which the group of compounds got its name. I will close with a summary of what is known about *C. elegans* pheromones and propose a model to put these compounds into an ecological context (Fig 2).

Dauer

Dauer has been recently reviewed [22] and will be only briefly summarized here. In the presence of ample food and low worm density, *C. elegans* progresses through four larval stages (L1 to L4) to become a reproductive adult in about 3.5 days. If the ratio of food to worm density becomes too low, L2 animals enter the dauer stage [23]. Animals in dauer cease feeding and development, become radially shrunken, move only when prodded, and are capable of a specialized behavior called nictation, a waving motion of the anterior body in the air that is thought to aid in dispersal through increased interactions with insects [24]. Dauer animals can survive for months, and when conditions become favorable for growth (high food, low pheromone), dauer larvae resume normal development as L4 larvae [24,25].

Golden and Riddle showed that supernatants of liquid cultures of *C. elegans* could induce dauer formation [23]. In this initial publication, Golden and Riddle indicated that the "pheromone is present in very small amounts, and that it may be a hydroxylated, short-chain fatty acid or a mixture of closely related compounds" [23]. As I will show below, they got it right.

C. elegans Mating Behavior

Simon and Sternberg published the first evidence for a chemical cue produced by *C. elegans* hermaphrodites that attracts males [26]. They demonstrated that males were attracted to a region of an agar plate that had been conditioned by an *unc-52* hermaphrodite, which is essentially motionless at the young adult stage but otherwise normal and able to reproduce. They also showed that the cue was diffusible by analyzing reversal frequencies of males as they approached the conditioned spot. A vulvaless mutant hermaphrodite could also attract males, showing that the cue was not secreted by or dependent on a vulva, and the sensory mutations *osm-5* and *osm-6* eliminated the male response. This effect was not reciprocal, as a conditioned spot from *unc-52* males would not elicit a response by N2 hermaphrodites. Finally, Simon and Sternberg showed that several *C. elegans* isolates produced the mating cue, demonstrating that it is robust and evolutionarily stable [26].

White and coworkers have also shown that *C. elegans* hermaphrodites produce a male-specific mating cue and have identified male-specific cells that are necessary for the response [27]. In contrast to Simon who used immobile hermaphrodites as the source of cue [26], White et al. used extracts from hermaphrodite liquid cultures and showed that *C. elegans* males were attracted to spots on a plate from the hermaphrodite cultures [27], consistent with the results of Simon and Sternberg [26]. Using a combination of genetics and laser ablation studies, White et al. also showed that the male response was mediated by a TRPV (transient receptor potential vanilloid) channel that is produced by the gene products of *osm-9*, *ocr-1*, and *ocr-2*. They also demonstrated that the male-specific cephalic companion (CEM) neurons, along with the AWA and AWC olfactory neurons, were necessary. Interestingly, they were able to overexpress *fem-3*, which allowed a fraction of CEM neurons to avoid apoptosis in hermaphrodites, and they showed that these “masculinized” hermaphrodites behaved like males in response to hermaphrodite-conditioned media [27].

Chasnov and colleagues have published two papers [28,29] that are in apparent disagreement with the Simon and Sternberg [26] and White et al. [27] studies described above. In these studies, Chasnov et al. compared the male-specific attraction and mating behavior between different *Caenorhabditis* species that are either hermaphrodite/male (*C. elegans* and *C. briggsae*) or female/male (*C. remanei* and *Caenorhabditis* sp. strain CB5161). In both studies, Chasnov et al. showed that *C. elegans* males had a greater attraction to females of other species compared to *C. elegans* hermaphrodites, and they concluded that *C. elegans* hermaphrodites have lost their ability to produce a mating pheromone [28,29]. There are several experimental differences between Chasnov et al. studies [28,29] and the Simon and Sternberg [26] or White et al. [27] studies. It is likely that females in species such as *C. remanei* and *Caenorhabditis* sp. strain CB5161 produce chemical signals for male attraction that are not present in *C. elegans*, which could potentially explain the observed stronger attraction of *C. elegans* males to females of these other species. As I will describe below, the *C. elegans* mating pheromone has now been identified [30], and I will describe a few properties of the pheromone that may also help to account forthese discrepancies.

C. elegans Dauer Pheromone Identification

Over twenty years passed between the demonstration that dauer is regulated by a pheromone [23] and the isolation and identification of the first dauer pheromone from *C. elegans*. The first chemical identification of a *C. elegans* pheromone was the discovery of “daumone” by Jeong and coworkers in 2005 [21]. These investigators conducted an activity-guided purification of the dauer pheromone by growing large-scale liquid cultures in a 300 L fermenter and extracting the aqueous culture with ethyl acetate. They conducted chromatographic separations, and the fractions were tested for their activity with a dauer formation bioassay. When they obtained a

significantly pure fraction, they used standard NMR and mass spectrometry techniques to identify ascr#1 (Table 1: a.k.a. daumone and C7: see side bar for a discussion on nomenclature) [21]. For a short time it was assumed by many *C. elegans* investigators that the dauer pheromone story was nearly complete.

Jeong and coworkers provided the first clue that there was more to the dauer story. The investigators reported that during the activity-guided fractionation, they were able to purify daumoneby >15,000-fold based on dauer formation activity. However, the same activity-guided fractionation purified daumone by 128,300-fold based on mass [21]. Thus, 88% of the activity was lost during the purification, and the investigators speculated that perhaps "purified or synthetic daumone might be more active when it is associated with other unidentified molecules (for example, membrane lipids)" [21].

Butcher et al. found that at least some of Jeong and coworkers "other unidentified molecules" were additional, more potent, dauer pheromones [31]. Using a similar approach taken by Jeong et al., Butcher et al. used a dauer recovery assay to purify ascr#2 (a.k.a. C6). In contrast to the dauer formation assay, which is used to test the ability of a compound to cause worms to enter dauer, the dauer recovery assay uses worms that are already in dauer and tests for compounds that keep the animals in dauer; consequently, these two assays may be sensitive to different compounds. As seen in Fig 1, ascr#1 and ascr#2 are similar and have a common feature of the ascarylose sugar. Butcher and colleagues then looked for additional ascarosides in a targeted assay using primarily NMR spectroscopy, and they were able to identify ascr#3, yet another ascaroside. They then tested the activities of all the compounds in a dauer formation assay and discovered that ascr#2 and ascr#3 were approximately 100 times more potent in dauer formation than ascr#1, which was also identified in their targeted identification [31]. Thus, Butcher et al. both verified Jeong and coworker's identification of daumone but also showed that it is, at best, a minor component in dauer formation.

Baiga et al. have made fluorescent derivatives of ascr#1 and ascr#3 and have used these compounds as probes to track the uptake and localization of dauer compounds in *C. elegans* [32]. They showed that the fluorescent probes had activity in dauer formation; that the probes first concentrate on the cuticle of the pharynx and, for the ascr#3 derivative, move to amphid neurons; that the fluorescence could be seen in the cuticle and amphid neurons of L4 animals after dauer recovery; and that the probes can penetrate *C. elegans* eggs [32]. Gallo and Riddle [33] recently compared the physiological properties of synthetic ascr#1 [21] to natural *C. elegans* pheromone extracts [34]. Consistent with Butcher et al., Gallo and Riddle found that ascr#1 is not a major component of the dauer-forming pheromone and that at concentrations reported to induce dauer formation [21], ascr#1 is toxic and will lower the life span of animals that are exposed to it [33].

Mating Pheromone Identification: Overlap with Dauer

While others were looking for dauer pheromone, Srinivasan, Kaplan and coworkers were conducting activity-guided fractionation of the mating pheromone in *C. elegans* [30]. Whereas Jeong et al. and Butcher et al. both used standard liquid bacterial/worm co-cultures to make the starting material, the Srinivasan and Kaplan team started with synchronized liquid co-cultures but then transferred worms at defined developmental stages to water to collect exclusively worm-derived compounds that exclude bacterial contaminants. This strategy accomplished three important goals. First, it greatly simplified the male-specific chemotaxis bioassay, because worms are attracted to many bacterial small molecules. Second, it simplified the chemical composition of the starting material for identification. Third, it allowed for the collection of material produced specifically at each developmental stage, as opposed to synchronized cultures that accumulate all compounds over time. The male activity only showed

up at L4, young adult, and adult stages, increasing confidence that it was a biologically relevant signal [30].

The activity-guided fractionation of the mating pheromone was complicated by the complete loss of activity in individual fractions following an ion-exchange chromatography step [30]. Srinivasan, Kaplan et al. found that no individual chromatographic fraction was sufficient to generate a male-specific response but that recombining fractions recovered activity. This indicated that the male-attracting pheromone consisted of more than one compound. The major component of one of these fractions was identified as a new ascaroside based on the known ascr#2 but modified with glucose. This compound was named ascr#4 (Table 1). The next step was to analyze the known dauer pheromones ascr#1, ascr#2, and ascr#3 for male-specific attraction, and they then discovered that at low concentrations ascr#2 and ascr#3 [31], but not ascr#1 [21], also functioned as mating pheromones [30]. They showed that ascr#2, ascr#3, and ascr#4 act synergistically and are active at picomolar concentrations in specifically attracting males. The mating response was bell-shaped, and at higher concentrations necessary for dauer formation, males were no longer attracted. At higher concentrations of pheromone, hermaphrodites were strongly repelled. Srinivasan, Kaplan et al. also found that the amphid single-ciliated sensory neuron type K (ASK) and the male-specific CEM neuron, are required for male attraction by ascr#3 [30].

As noted above, Chasnov and colleagues questioned the existence of a *C. elegans* hermaphrodite mating cue [28,29]. At least two characteristics of the pheromone might help to account for this discrepancy. First, the male mating response is bell-shaped, with a maximal response near 1 picomole of material. There is a relatively narrow range of activity in male response, and it would be easy to have either too much or too little material. Second, the male response is strongly synergistic, involving not only ascr#2, ascr#3, and ascr#4 described above but at least one additional ascaroside ascr#8 described below [35]. The ratios of the compounds are important, and it is also clear (see below) that both development and environmental conditions significantly influence the expression of ascarosides.

More Ascarosides

In an attempt to discover why Jeong et al. identified ascr#1 [21] and Butcher et al. identified ascr#2 and ascr#3 [31] as dauer pheromones, Butcher and coworkers examined the temperature dependence of ascaroside expression and did activity-guided fractionations using dauer formation assays of cultures grown at 20 C (like Jeong et al.) and 25°C (like Butcher et al.). Like their earlier study [31], they found that ascr#2 and ascr#3 were most abundant at 25°C. However, they also found that at 20°C, ascr#5 (a.k.a. C3) and ascr#1 were expressed, but ascr#1 was not active in their dauer recovery assays [36]. They went on to characterize the dauer formation activity and found modest synergy between ascr#5 and either ascr#2 or ascr#3, especially at 20°C. More recently, Butcher and colleagues have identified a novel indole-containing dauer pheromone that they named indolecarboxyl-ascaroside C5 and which I have named ascr#9 (Table 1) [37]. Ascr#9 is interesting, both structurally and functionally. It is a relatively minor component of the dauer pheromone mixture, but it has dauer-forming activity at low nanomolar concentrations and has a bell-shaped curve, with activity dropping significantly at higher concentrations [37]. This is similar to the mating activity profile observed by Srinivasan, Kaplan and coworkers with ascr#2 and ascr#3 [30] and is the only current example of this sort of dauer-formation profile [37].

Pungaliya and coworkers took a different approach to finding additional ascarosides in *C. elegans* [35]. They applied a new method developed by Schroeder et al. called differential analysisby 2D NMR spectroscopy (DANS), which compares material from two different biological sources [38], for example a mutant animal with wild type. In the *C. elegans* study,

Pungaliya et al. compared liquid cultures generated from wild type N2 and *daf-22* animals [35], which had previously been shown to lack the short-chain ascarosides [30,39]. By comparing NMR data of N2 and *daf-22* animals, they were able to identify ascr#5, ascr#6.1, ascr#6.2 (a diastereomer of 6.1), ascr#7, and ascr#8 (Fig 1). They synthesized the new ascarosides and tested them for activity using both dauer formation and male attraction assays and found that ascr#8 potently attracts males and is synergistic with ascr#2 and combinations of ascr#2 and ascr#3 (but not ascr#3 alone) [35]. Significantly, the addition of ascr#8 was able to fully account for male mating activity [35], which was only partially reconstructed with ascr#2, ascr#3, and ascr#4 [30]. Ascr#8 is a unique ascaroside in that it features an amide linkage and a *p*-aminobenzoic acid (PABA) subunit.

The *C. elegans* mating pheromone is a fascinating example of the synergistic action of small molecules, as a blend of three different ascarosides produces full activity at concentrations at which each individual component is completely inactive. At higher concentrations ascarosides also synergistically induce dauer formation, perhaps to a lesser extent than mating. Whereas synergistic action of sex pheromone components is well known from other animal species, especially insects (e.g. [40,41]), *C. elegans* presents an ideal opportunity to study synergy on the molecular level and to discover how multiple signals converge into a specific behavior.

Ascaroside Biosynthesis

Butcher and coworkers used *daf-22* animals, which lack the dauer pheromone [42], to characterize some of the steps in ascaroside biosynthesis [39]. They showed that the *daf-22* encodes a homolog of human sterol carrier protein, SCPx, an enzyme that catalyzes the final step in peroxisomal fatty acid β-oxidation reactions [39]. Based on this information, they also examined a *dhs-28* mutant *C. elegans*. The *dhs-28* gene encodes a protein homolog of human D-bifunctional protein, which biosynthetically acts just upstream of SCPx. They showed that these proteins are expressed in the intestine and suggest that this is the site of ascaroside biosynthesis in *C. elegans* [39]. This pathway degrades short-, medium-, and long-chain fatty acids into acetyl-CoA and bile acid intermediates. Butcher et al. grew liquid cultures of *daf-22* and *dhs-28* animals for both 10 and 20 days, conducted dauer formation assays, and identified ascarosides in the long-term *dhs-28* cultures. Interestingly, both mutants were relatively inactive in dauer formation at 10 days but had significant dauer-forming activity at 20 days. They identified several longer-chain ascarosides up to 15 carbons in length in the 20-day *dhs-28* culture, suggesting that these were intermediates ascaroside pheromone biosynthesis. Many steps in ascaroside biosynthesis remain to be elucidated, both in the initial formation of long-chain ascarosides and in details regarding specific functional groups, especially the aromatic amide in ascr#8 [35] or the indole in ascr#9 [37].

Common themes between *C. elegans* and *Ascaris*

Many years before the contemporary work in *C. elegans* pheromone biology, ascarosides were identified in the eggs of *Ascaris* species, which are parasitic nematodes commonly known as large intestinal roundworms. A variety of long-chain ascarosides were initially discovered in the horse parasitic nematode *Parascaris equorum* [43] and subsequently characterized and identified in *Ascaris lumbricoides* (the human parasite) and *Ascaris suum* (the pig parasite) (Fig 1B) [44-46]. *Ascaris* eggs are amazingly resilient, can survive for several years in damp soil, and resist degradation by many chemicals [47]. This remarkable stability contributes to the infection by *Ascaris lumbricoides* of approximately 1.5 billion people in the world in 2002 (http://www.cdc.gov/ncidod/dpd/parasites/ascaris/factsht_ascaris.htm). *Ascaris* eggs have three layers: a protein coat, a chitin middle layer, and an inner layer of mixture of long-chain ascarosides, which have been shown to be responsible for the unusual impermeability [48]. Although both *A. lumbricoides* and *C. elegans* are in the phylum Nematoda, the orders

Ascaridida and Rhabditida are significantly diverged; however, nematodes appear to be the only eukaryotes from which the sugar ascarylose has been isolated [46]. The chemical properties of the very long-chain ascarosides identified from *Ascaris* (Fig 1B) and *Parascaris* differ from those of the *C. elegans* pheromone components (Table 1), but long-chained ascarosides very similar to those identified from *Ascaris* and *Parascaris* have now also been found in *C. elegans* [35,39].

Are there any similarities in the functions of ascarosides in *Ascaris* spp. and *C. elegans* ? Whereas *C. elegans* hatches from its egg as an L1 larvae, *A. suum* undergoes two molts in the egg. The *Ascaris* L3 stage emerges from the hatched egg when the egg is ingested into a new host, and the *Ascaris* L3 appears homologous to *C. elegans* dauer larvae [49]. It is likely no coincidence that ascarosides line *Ascaris* eggs and are in direct contact with the larval animals, so compounds may play a similar dauer-forming (or at least dauer-maintaining) role in *Ascaris* as they do in *C. elegans*. Investigations of the functions of ascarosides in *Ascaris* and *Parascaris* thus may provide new insights into the control of parasitic nematode infections.

More Pheromone Functions

De Bono and colleagues have discovered that mutations in the neuropeptide Y-like receptor (NPR-1) are responsible for different naturally occurring strains of *C. elegans* to exhibit either solitary or social feeding behavior [50]. Wild-type N2 animals are solitary feeders, but several natural isolates form aggregates at the edges of bacterial lawns. A null mutation of *npr-1* can convert solitary into social feeders. Recent work by Macosko and coworkers has shown that the RMG interneuron is the primary site of action of the *npr-1* gene [51]. They found that RMG forms gap junctions with several other neurons, most notably the ASK sensory neuron, which Srinivasan, Kaplan et al. had shown to be responsible for male-specific attraction to ascr#3 [30]. Srinivasan, Kaplan etal. also showed that N2 hermaphrodites were repelled by ascarosides, and Macosko verified these results. Interestingly, Macosko also found that *npr-1* loss-of-function hermaphrodites were attracted to ascarosides. They went on to show that calcium levels in ASK neurons decrease in response to a blend of ascr#2, ascr#3, and ascr#5 and that ascaroside-generated signals in ASK can propagate to other cells [51]. High RMG activity promotes social aggregating behavior and also enhances the ASK response to ascarosides, and high NPR-1 activity diminishes the response [51]. Thus, ascarosides appear to modulate aggregation behavior in a strain-specific manner, adding to the dauer and mating functions previously established.

Dauer Conditions Increase Males

Morran and coworkers recently published an interesting study analyzing the frequency of males in three different *C. elegans* strains exposed to dauer conditions [52]. They observed that male frequency increases following exposure to dauer conditions for two naturally occurring strains (CB4856 and JU440) but not for the common laboratory strain N2. The increase in male frequency in CB4856 extended to several generations following exposure to dauer conditions. The authors identified two mechanisms that accounted for the increased males: differential male survival during dauer and an increase in male mating (outcrossing) rates following exposure to dauer [52]. Several other potential mechanisms including sexual conversion, differential migration, and increases in X chromosome nondisjunction rates were explored but ruled out as contributing to increased males [52]. It must be pointed out that this study did not explicitly identify the dauer pheromone or any other chemical cue as the mechanism responsible for increased males, but as discussed below, an increase in male frequency in response to dauer conditions reinforces the dual roles of ascarosides in dauer and mating.

General Model of Ascaroside Function

It took 25 years to identify the first dauer pheromone [21], and in the past three years a tremendous amount has been discovered: there are currently ten known ascarosides produced by *C. elegans* [21,30,31,35-37], the dauer pheromone is a mixture of ascarosides [31,36], the mating pheromone is based on the same chemical compounds as the dauer pheromone, but at much lower concentrations and perhaps with different ascaroside components [30], environmental conditions influence ascaroside expression [35,36], solitary hermaphrodites are repelled by ascarosides [30,51], and social hermaphrodites are attracted to ascarosides [51]. We know that CEM [27,30] and ASK [30,51] cells mediate the response to ascarosides, that ascarosides are biosynthesized through the peroxisomal β-oxidation pathway [39], and that male frequency increases with dauer conditions [52].

However, much remains unknown: we have no ascaroside receptors, we know relatively little about how environmental factors influence ascaroside expression, we know nothing about the mechanisms of ascaroside release (constitutive secretion, regulated secretion, defecation, and other possible mechanisms), and we know relatively little about the mechanisms of synergy of this large family of signaling molecules. It is no small task to unravel the overlapping functions of ten or more ascarosides that act synergistically and in a concentration-dependent manner with modulation from several additional cellular and environmental inputs. Zwaal and coworkers have identified two G-protein alpha subunits, GPA-2 and GPA-3, that are involved in the signal transduction by dauer pheromone [53], but neither dauer nor mating receptors havebeen reported. The physiological function of the original daumone [21] (a.k.a ascr#1 and C7) remains a mystery. Many details of ascaroside biosynthesis and its regulation in response to environmental cues remain unknown. We also know very little about the role of ascarosides in other nematode species, despite the fact that they were discovered in *Ascaris*. Significant gaps remain in our knowledge, but it is possible to piece together a general model that unifies the role of ascarosides in the life history of *C. elegans*.

Figure 2 illustrates several key points in an ecological cycle of *C. elegans*. It is necessarily simplified, and each panel could last from one to several generations depending on environmental factors, especially food supply. The bottom wedge of the circle represents abundant food and little or no pheromone. Pheromone levels increase and food decreases clockwise from the bottom, which specifies a single hermaphrodite in this region as a limiting case of dispersal into a new microenvironment. Under conditions of abundant food and low worm population, hermaphrodites would self-fertilize and thus produce primarily hermaphrodites as offspring, represented by the second wedge clockwise from the bottom. Morran and coworkers found that male frequency increased after exposure to dauer conditions [52]. In the model in Fig 2, I assume that ascaroside pheromones are responsible for the increased male frequency and that the effect will increase as overall local pheromone levels increase, even at levels too low to induce dauer. This assumption clearly needs to be tested experimentally. Males are attracted to a synergistic mixture of ascarosides [30,35]. A second untested assumption of this model is that out of the known ascarosides (Table 1), some will be constitutively expressed to monitor population density and others will be regulated according to environmental or developmental factors. For example, ascr#4 is produced at high levels when worms are incubated in water or media withno food [30], but in standard liquid bacterial co-cultures, ascr#4 levels are extremely low [35]. Moreover, when Srinivasan, Kaplan et al. tested material generated from worms at distinct developmental stages, only exudates from L4, young adult, and adult animals caused male-specific attraction [30]. Thus, the existing data support the idea that males will be potently attracted to hermaphrodites when the right combination of ascarosides is produced. The strong synergy observed in male attraction allows for population-sensing (e.g. dauer-forming) ascaroside(s) to accumulate in the microenvironment without leading to male attraction. Then, small amounts of different

ascaroside(s) could be released by a hermaphrodite that is developmentally ready to mate, and this additional cue would provide directionality, allowing the male to find the fertile hermaphrodite in a relatively uniform level of dauer pheromone.

Since it appears that some ascarosides, most notably ascr#4, are regulated by food, this provides a mechanism for *C. elegans* hermaphrodites to outcross with males as the environmental conditions worsen. This would increase the number of offspring from about 300 for a sperm-limited self-fertilizing hermaphrodite to about 1000 for a hermaphrodite who mates with males [54]. Importantly, it would also provide a mechanism for genetic variability as conditions deteriorate. As the levels of pheromone increase and the food decreases, the offspring that arise from outcrossing will become dauer. As shown by Morran et al., the dauer state will lead to increased males [52]. The dauer state will allow these offspring to survive for several months and disperse to a fresh microenvironment with abundant food, thus completing the cycle (Fig. 2). I have illustrated the limiting case of a single hermaphrodite dispersing to new food, but depending on the density of dispersed animals, outcrossing with males could still be significant in the initial fresh microenvironment [52].

Clearly, as we discover new ascarosides, receptors, regulators, and functions, the general model proposed in Figure 2 will be refined (or replaced all together!). However, the accumulated knowledge over the past three years is nicely consistent and points to a critical role of pheromones in nematode ecology.

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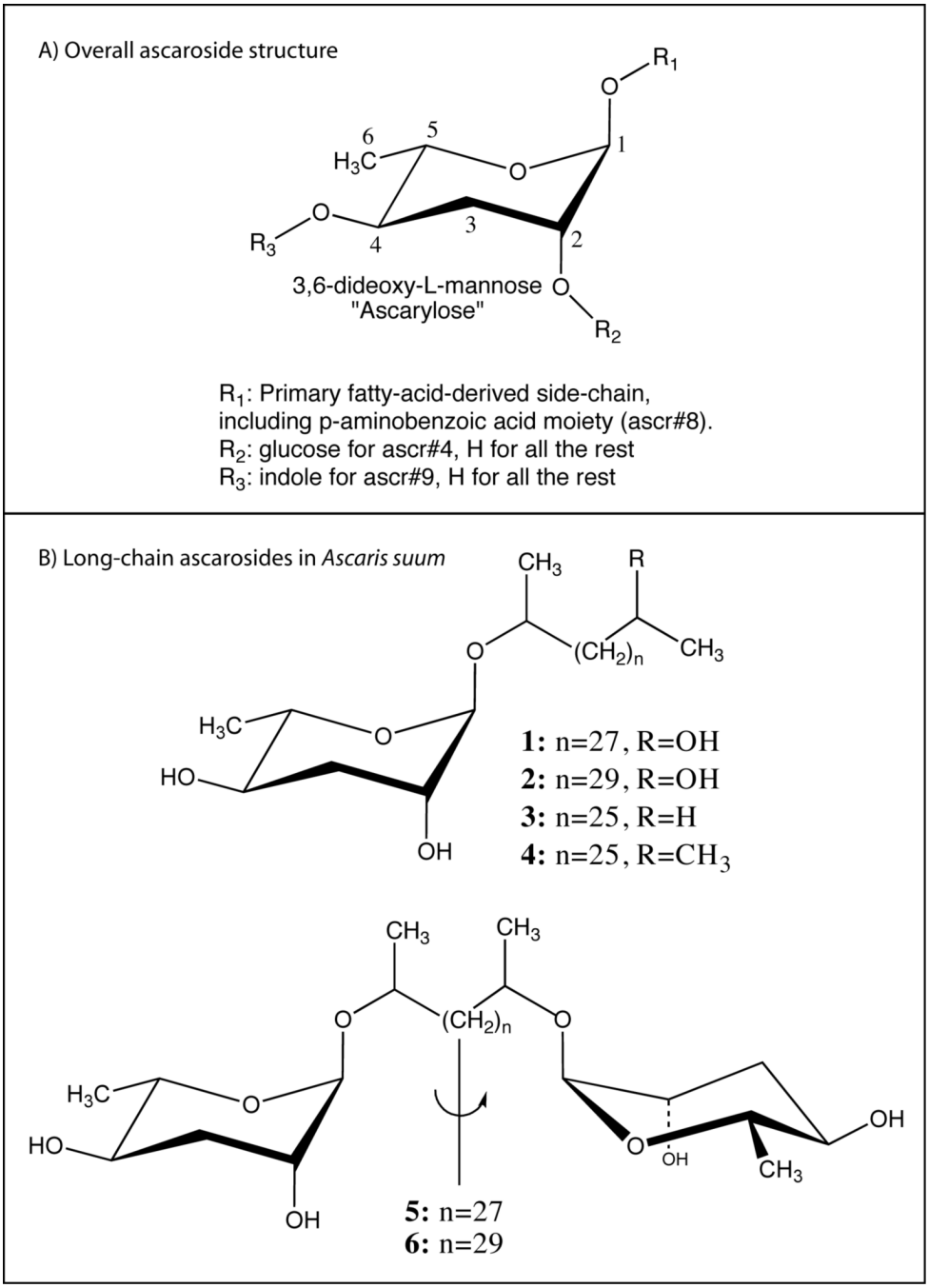


Figure 1. Ascaroside structures

A) Overall structure of ascarosides. The ascarylose sugar is numbered and locations of known modifications are indicated. The known *C. elegans* ascaroside pheromones are shown in Table 1. B) Six long-chain ascarosides from the eggs of the parasitic nematode *Ascaris suum* [46]. Structures **4**and **5**are symmetric—indicated by the vertical line and arrow— and contain two ascarylose sugars [46]. Similar long-chain structures have been reported in both N2 (wild type) and *daf-22 C. elegans* [35].

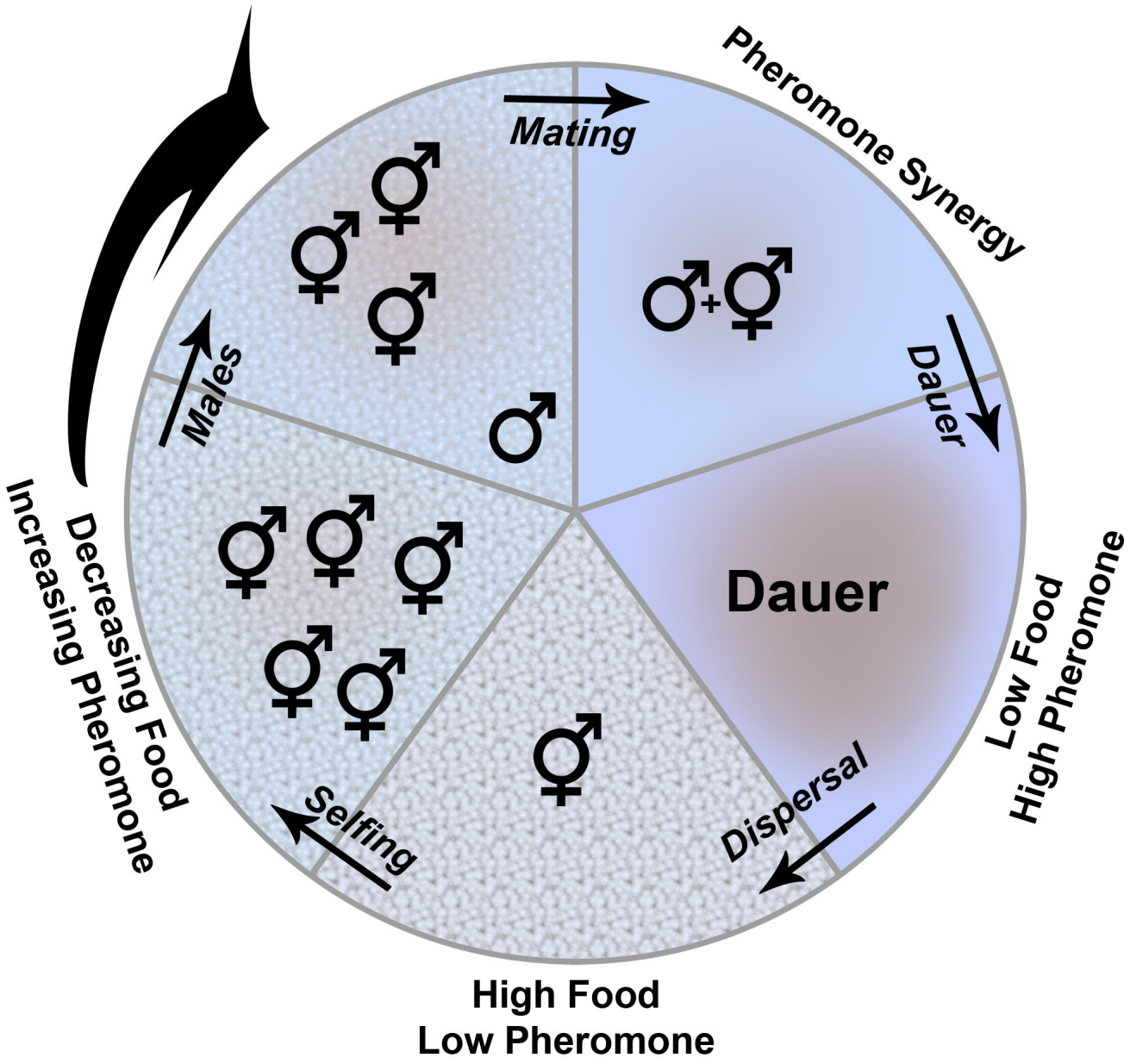
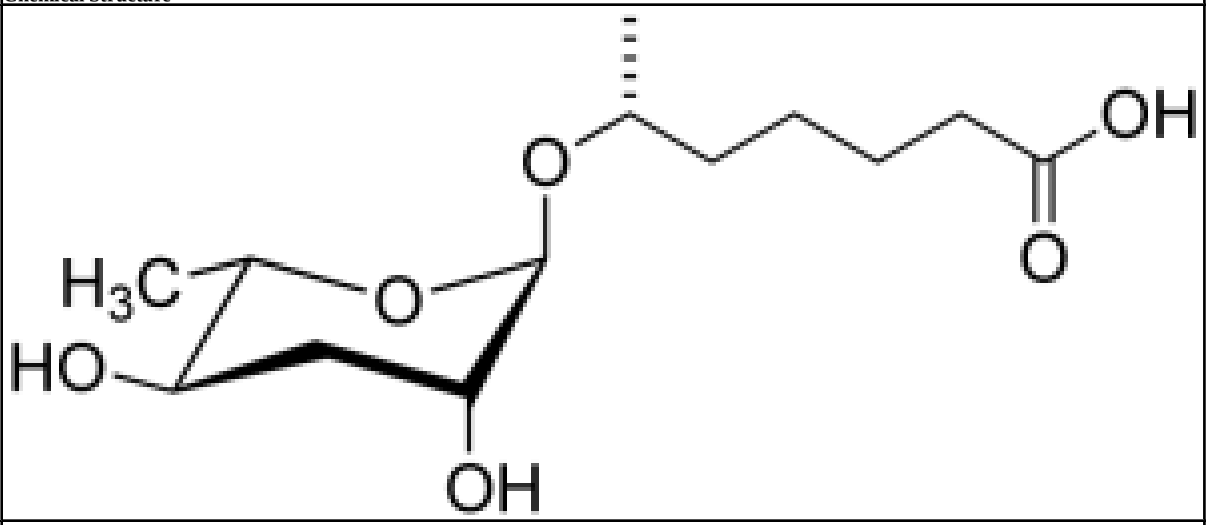
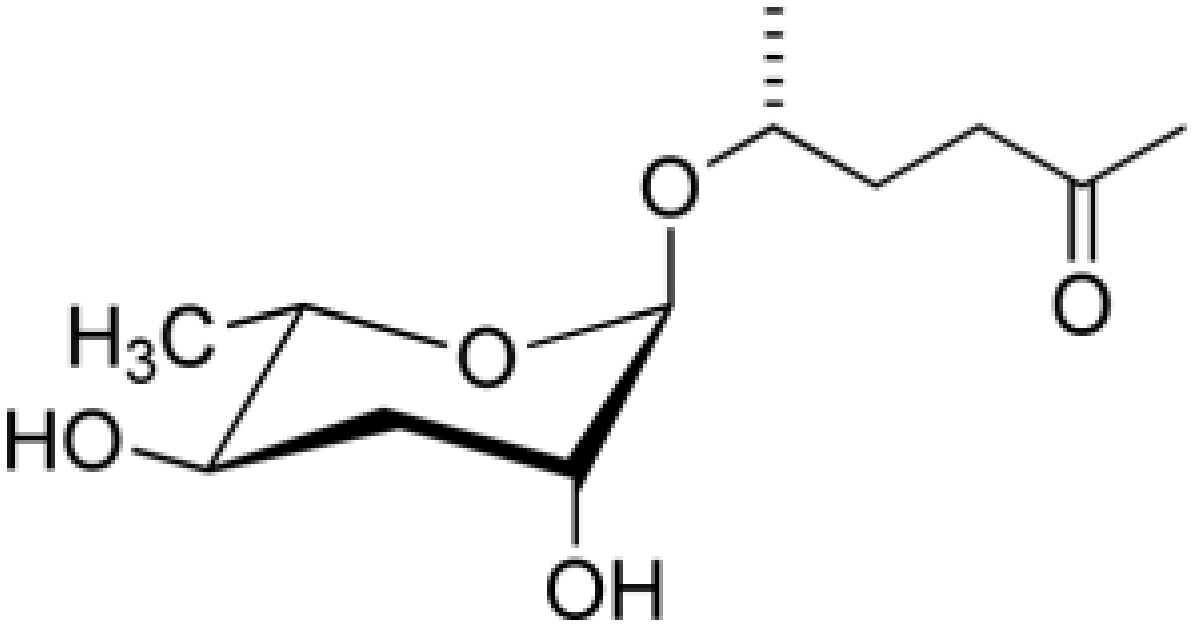


Figure 2. General model for *C. elegans* ascaroside pheromone function

Starting with the bottom wedge in the circle that represents a microenvironment with high food and low pheromone, food levels decrease and pheromone increases going clockwise around the circle. Food is represented by the pattern on the surface and the colors represent pheromone. Discussion and overall rationale for each stage is given in the text.

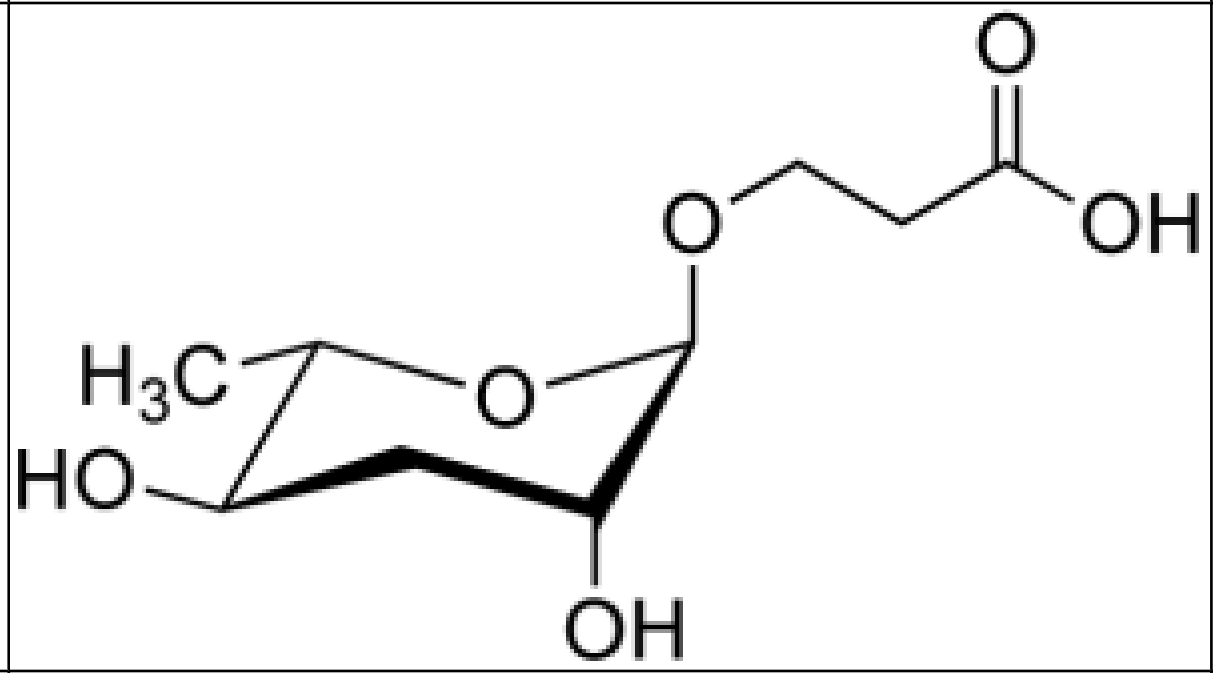
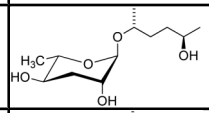
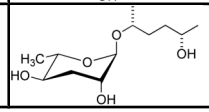
Table 1

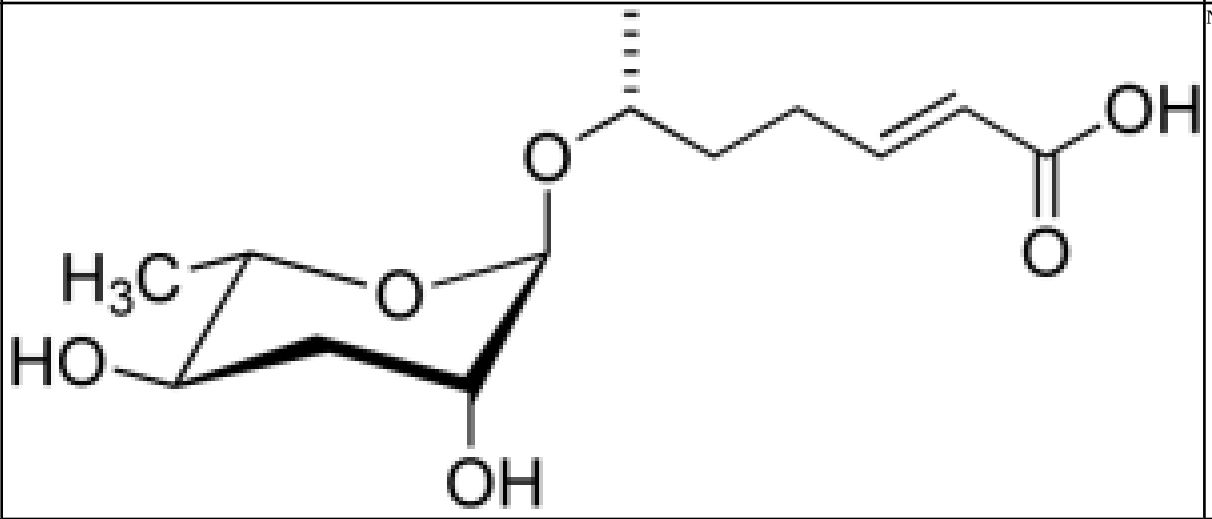
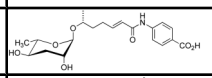
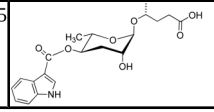
***C. elegans* ascarosides**

e	Alternate Name(s)	Chemical Structure	Dauer Activity	Mating Activity	Reference
1	Daumone C7		Very Low	None	[21]
2	C6		High	Moderate, Synergy with ascr#3 and ascr#4	[31]

Name	Alternate Name(s)	Chemical Structure	Dauer Activity	Mating Activity	Reference
ascr#3	C9		High	High, Synergy with ascr#2 and ascr#4	[31]

Alternate Name(s)	Chemical Structure	Dauer Activity	Mating Activity	Reference
		Low	None alone, Synergy with ascr#2, ascr#3	[30]

e	Alternate Name(s)	Chemical Structure	Dauer Activity	Mating Activity	Reference
5	C3		High, some synergy with ascr#2 and ascr#3	None [35]	[36]
6.1			None	Moderate	[35]
6.2			Not tested	Not tested	[35]

e	Alternate Name(s)	Chemical Structure	Dauer Activity	Mating Activity	Reference
7			None	None	[35]
8			Moderate	High, synergy with ascr#2 and ascr#3	[35]
9	indolecarboxyl-ascaroside C5		Moderate, Bell-shaped response	Not tested	[37]