# Worm Sex 101: How *Caenorhabditis elegans* males find their mates

Written by Arthur S. Edison on behalf of the *C. elegans* mating team:

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## SUMMARY

This story describes the identification of a chemical mating signal produced by a small worm that was facilitated by the new technology of the Magnet Lab 1-mm HTS probe. The probe allowed us to collect much smaller samples than would be required with conventional technology. As a result, we were able to efficiently examine the composition and activity of the material as a function of development (e.g. time) of the organism and discover new biology.

## BACKGROUND

*Caenorhabditis elegans* is a nematode that reaches about 1-mm in length as an adult. This small worm is one of the best-studied laboratory animals in the world. The adult worms have fewer than 1000 cells, and the fate of every cell division has been mapped from a single fertilized egg to adult. The nervous system of this animal is very simple with just about 300 neurons. The worm's entire anatomy has been studied by thin-section electron microscopy. And, it is very easy to grow and manipulate in the lab using molecular genetic techniques. Many major biological discoveries have been made using *C. elegans* including apoptosis (programmed cell death) and RNA interference, both of which resulted in Nobel Prizes. Despite all of this research, very little has been known about the chemistry that *C. elegans* uses to communicate with its environment.

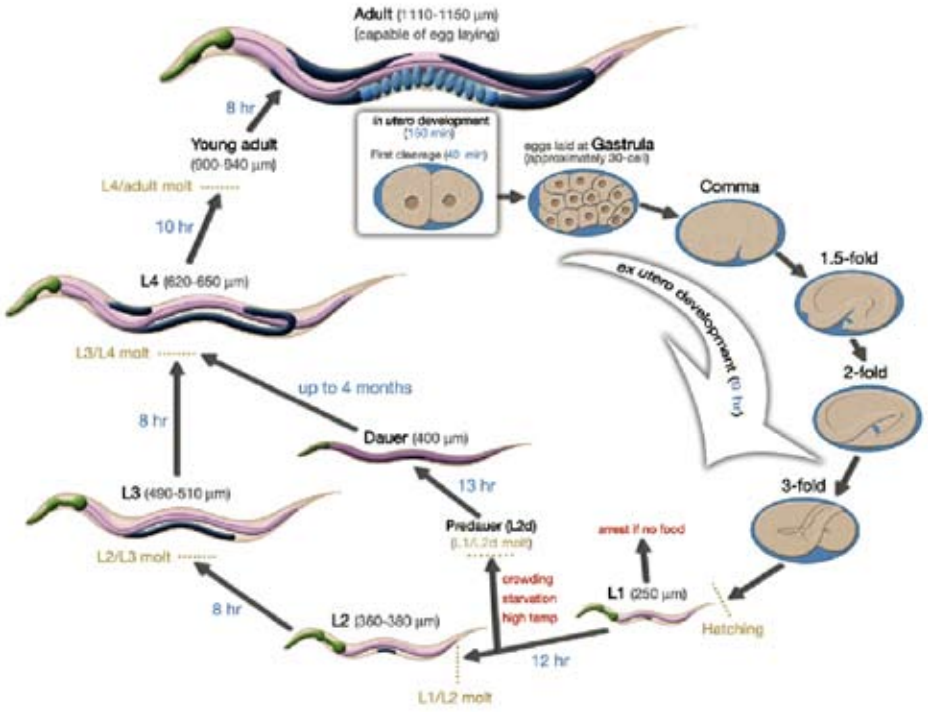


**Figure 1:**
Developmental life stages of *C. elegans*. Figure from http://www.wormatlas.org/

*C. elegans* grow in just 3.5 days from a fertilized egg to a sexually mature adult by going through four larval stages (L1-L4). The worms come in two sexes: male and hermaphrodite. Hermaphrodites make both eggs and sperm and can self-fertilize. Hermaphrodites can mate with males to produce over 1000 progeny

with an increase of genetic diversity. In contrast, if males are scarce, hermaphrodites will self fertilize, but the number of progeny is limited to a few hundred by the amount of sperm. Under conditions of low food and high worm density, *C. elegans* enter an alternate developmental stage called dauer (Figure 1). For several decades, researchers have known that worms secrete a dauer pheromone to sense their population density[1], and the chemical identities of 3 different dauer pheromones were recently published[2,3].

In 2002 Paul Sternberg's group at Caltech and HHMI also showed that hermaphrodites produce a chemical signal that attracts males[4], and the goal of our study was to discover the *C. elegans* mating pheromone. The complete scientific story of this work has been recently published[5] and are just summarized below.

## RESULTS/DISCUSSION

*C. elegans* eats bacteria (*Escherichia coli*), and large amounts of *C. elegans* can be grown in liquid culture by adding both worms and *E. coli*. Furthermore, starting the culture with fertilized eggs can synchronize the worms' growth cycle. Because we wanted to isolate a chemical that was secreted by worms, we began our study by growing standard liquid cultures of worms and bacteria and analyzing the liquid supernatant. This preparation was difficult to use, because the bacteria produce many small molecules, making the "haystack" too big to find the "needle". Therefore, we developed a way to separate worms from bacteria and to incubate the hermaphrodites in water to isolate their released chemicals. This "worm water" was collected at each of the defined developmental stages (Figure 1) and tested for its ability to attract males. In collaboration with Jagan Srinivasan in the Sternberg laboratory, we found that worm water from sexually mature young adult and adult hermaphrodites attracted males, but at earlier developmental stages there was no response.

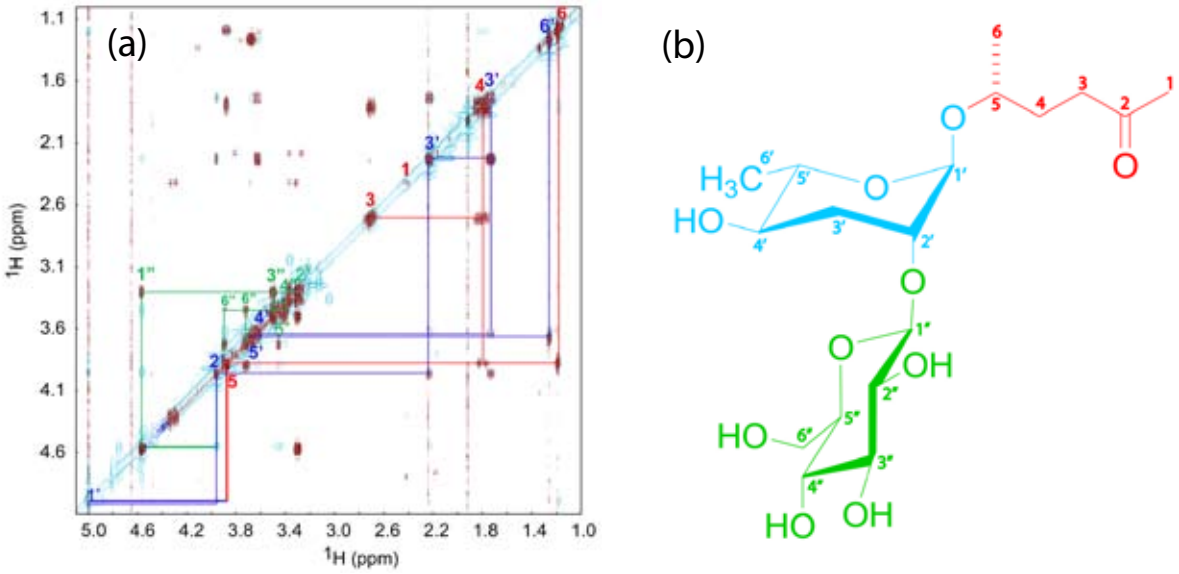


**Figure 2:**
The NHMFL 1-mm HTS probe was important for this study. We used about 0.4 L of culture to obtain the material for the COSY and NOESY spectra (A) that led to the identification of one of the mating pheromones, ascr#4 (B). Previous studies utilized over 300 L to identify the first dauer pheromone.

Using young adult worm water, Fatman Kaplan and Ramadan Ajredini in the Edison lab then conducted a series of chemical fractionations, followed by bioassays. Male *C. elegans* were essentially the "detector" in this purification. At one stage in the purification, we lost all the activity but could regain it by adding all the fractions together. This showed that there was more than one chemical involved in attracting males.

The Mag Lab 1-mm HTS probe[6] was very important for this project, because we did not need to produce large amounts of sample. Previous studies to identify the first dauer pheromone required several 300 L liquid cultures[2]. Because of the extremely high sensitivity of the 1-mm HTS probe, we were able to start with just 0.4 L of liquid culture. We analyzed chemical fraction "A" that that was necessary but not sufficient for activity; if we took fraction "A" away, all activity was lost, but it alone was not active.

Figure 2A shows an expansion and overlay of 2D NOESY and COSY NMR data of fraction "A" from the 1-mm HTS probe. The structure of the major chemical in this fraction is shown in Figure 2B and is called ascr#4. The "ascr" stands for an ascaroside sugar (the cyclic part of the structure), and the "#4" indicates that it is the

fourth new chemical discovered in this group; the preceding three are the known dauer pheromones. Just when the Fatma and Cherian in the Edison laboratory had collected the NMR data from fraction "A", Frank Schroeder (Cornell University) came to give a seminar at UF. Frank was part of the team that identified two of the *C. elegans* dauer pheromones called ascr#2 and ascr#3[3], and we realized at that point that the mating pheromone we were finding was very similar to the dauer pheromones.

The Schroeder lab synthesized all of the different known ascarosides, and Jagan in the Sternberg lab tested them for male specific attraction. We were surprised to find that the known dauer pheromones, ascr#2 and ascr#3, were both active in attracting males. Ascr#3, in particular, is active at about 1 pM concentrations, so it is very potent. Both of these compounds attract males with a bell-shaped curve response: at low or high concentrations the effect is gone. We also tested for and found synergy, because of the observation that more than two fractions in our purification were required for activity.

We then tested for all the known ascarosides by using liquid chromatography-mass spectrometry with Peter Teal and Hans Alborn in the USDA laboratory in Gainesville. Hans found that all known ascarosides were present in very low concentrations in many of the fractions from worm water. When we combined synthetic compounds in the approximate ratios found by mass spec, we were able to reproduce the male mating response with natural material.

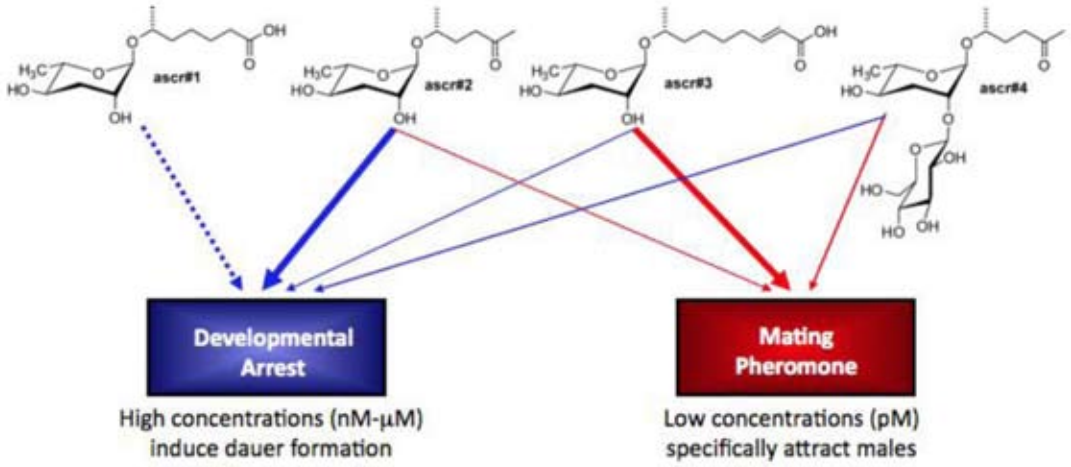


**Figure 3:**
*C. elegans* ascarosides have dual roles in male mating response and dauer formation.

This study was the first to link—via small molecules—two major behavioral traits in *C. elegans:* dauer formation and mating. At very low concentrations (picomolar), ascarosides tell males that there are hermaphrodites nearby and that they are ready to mate. Incidentally, hermaphrodites are not attracted to the signal at these low concentrations. At higher concentrations, hermaphrodites are repelled (but males are indifferent). At the highest concentrations (high nanomolar to low micromolar), the worms enter dauer, because the signal indicates that there are too many worms for the available resources (Figure 3).

This study also nicely demonstrates the synergy that is possible when new technology (e.g. HTS NMR probes) is coupled with important biological problems.

## REFERENCES

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