A LOOK INTO A WORMY FRIEND: THE MODEL OF *CAENORHABDITIS ELEGANS*

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ABSTRACT

This mini-review aims to be a glimpse into the widely used model *Caenorhabditis elegans,* a free-living nematode found in nature. As the information around this worm is vast and it cannot be summarized in these few lines we will try to introduce its main features, the interest to work with it, and how to begin the journey to incorporate it into your laboratory.

Keywords: Caenorhabditis elegans; nematode; approach model; physiology.

RESUMEN

Esta mini revisión pretende ser un vistazo al modelo ampliamente utilizado *Caenorhabditis elegans*, un nematodo de vida libre que se encuentra en la naturaleza. Como la información en torno a este gusano es vasta y no se puede resumir en estas pocas líneas, intentaremos introducir sus principales características, el interés por trabajar con él y cómo iniciar el viaje para incorporarlo a tu propio laboratorio.

Palabras clave: Caenorhabditis elegans; nematodo; modelo de abordaje; fisiología.

Introduction

It was Sydney Brenner, in 1965, who saw the potentiality of using nematodes as models for the future of molecular biology, especially for the study of development and the nervous system (Brenner, 1988; Brenner, 2003). Nematodes were already an interesting target as they were seen as simple eukaryotic organisms, with a manageable size and number of cells, and Brenner saw *Caenorhabditis briggsae* as an ideal system (Corsi et al., 2015). Later, he switched to *C. elegans* because the strain grew better than the *briggsae* isolate; which was for the better as *C. elegans* proved to be an even better model than what Brenner could have predicted. *C. elegans* is so far the only hermaphroditic free-living species in which external application of dsRNAs inactivates gene expression, which proved to be a great tool that rocketed genetic studies to the sky. Second, its syncytial germ line makes for easy transgenesis, as injected DNA recombines and forms an additional chromosome that is frequently passed onto later generations. Third, chemical mutagenesis proved to be efficient due to a balance between toxicity and mutagenic effect (Félix, 2008). Finally, in 1969 John Sulston successfully froze *C. elegans*, which meant that strains could be stored in labs for several years. From then, the *C. elegans* community started to grow rapidly and nowadays there are over a thousand laboratories registered worldwide inwww.wormbase.org, with over 1700 research articles published each year for the last 5 years (Corsi et al., 2015).

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A worm's life

C. elegans is a free-living nematode found worldwide growing in rich soil or compost with decomposing plant material, which provides bacterial food. In nature, *C. elegans* colonizes food sources and goes through four larval stages (L1, L2, L3, and L4) after hatching. When food starts to scarce or the population crowds, individuals can shift during L1 stage to an alternative developmental path, delaying its molt in a pre-dauer stage (L2d) and afterward enters a nonfeeding diapause stage called "Dauer". This stage is crucial for the nematode population as it can endure long periods without feeding, changing its metabolic pathways and stress resistance. It also changes its behavior as it moves faster and stands on its tail (called nictation), making these larvae more susceptible to be carried to another niche, where upon reaching a food source it can escape the dauer stage and reassume the development at L4 stage(Golden & Riddle, 1984). The alternation between life-cycles in the wilderness, coupled with environmental changes through seasons, gives a boom-and-bust behavior to the *C. elegans* population (Frézal & Félix, 2015).

In the laboratory, the boom-and-bust cycle is lost as worms are grown with plenty of food and are transferred to new plates once food has been depleted or the population starts to crowd. The worms are grown between 15 and 25°C and go through the four larval stages, reaching fertile adulthood in three days at 22°C (Fig. 1). Its size, ranging from 0.25mm at the first larval stage to 1mm at the adult stage, means that worms are usually observed in Petri dishes with solid agar under dissecting microscope as they move, eat, develop and lay eggs. Compound or confocal microscopes are used for much finer resolutions and address observation at the cellular level. As *C. elegans* is transparent, many of its structures can be directly seen. Moreover, its constant number of cells (956 cells) meant working with individual cells was easy to achieve and even individual cells and subcellular details can be visualized. Details can be enhanced using fluorescent proteins as tags, allowing the study of developmental processes, easy screen for mutants, isolating cells, and characterizing protein interactions in vivo (Boulin et al., 2006; Chalfie et al., 1994; Feinberg et al., 2008; Husson et al., 2013; Kerr & Schafer, 2006). One of the main goals of Brenner was to identify individual neurons and their synaptic connections. Thanks to electron microscopy and new technology tools, something not found in any other organism was achieved, mapping the neuronal connections for the 302 neurons found in the hermaphrodite, making it the first connectome. This was also recently achieved for the male worms (White, 1986; Cook et al., 2019; Jarrell et al., 2012).

The male population is scarce (0.1-0.2%) and rises under unfavorable conditions, increasing offspring and generating different genetic compositions. Therefore, hermaphrodites reproduce mainly by self-fertilization (often referred to as "selfing") and not by mating with other hermaphrodites; as Sydney Brenner said "the animals are driven to homozygosity" (Brenner, 1974), making mutant strains isogenic. Selfing reduces the effort needed to find a mutant and simplifies maintaining stocks, as a single animal can give rise to an entire population (Corsi et al., 2015).

C. elegans was the protagonist of another research milestone, as it was the first eukaryotic multicellular organism to have its genome fully sequenced, unlocking new interesting findings and genetic tools (C. elegans Sequencing Consortium, 1998).

The discovery of RNA interference in 1998 was achieved by injecting double-stranded RNA into worms (Fire et al., 1998). Afterward, other techniques were developed as soaking worms in dsRNA and feeding worms bacteria engineered to produce dsRNA also could induce a robust RNAi response (Tabara et al., 1998; Timmons and Fire, 1998). Feeding proved to be an easy and effective method that is commonly used in genetic studies and two libraries of RNAi bacteria

strains were developed in mass silencing experiments, the library of Ahringer lab and the Vidal lab (Kamath et al., 2003; Rual et al., 2004).

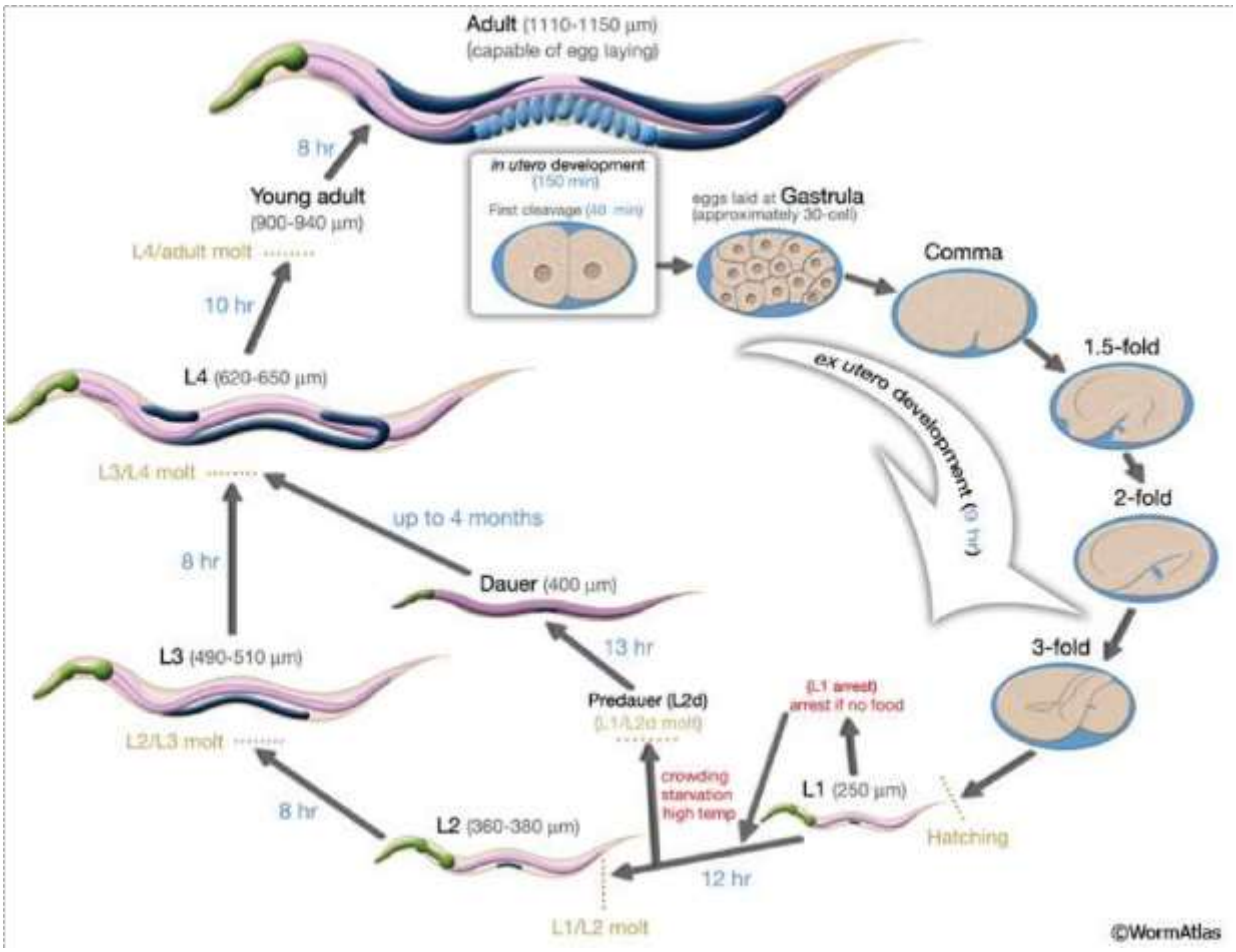

Figure 1. The life cycle of C. elegans at 22°C. 0 min is fertilization. Numbers in blue along the arrows indicate the length of time the animal spends at a certain stage. The length of the animal at each stage is marked next to the stage name in micrometers (µm).

The many fields of *C. elegans* (*C. elegans* community)

Although the *C. elegans* research started with neurology and development, many other fields have expanded with the help of this nematode. For example, we have parasitology, evolution, and ecology, cell biology, biochemistry and metabolism, aging, stress, model of human diseases, drug discovery, and behavior. The first critics of the model were that its behavior was simplistic for a neurobiological study, that it only knew how to move forward and backward. But *C. elegans* proofed them wrong, as it has many sensorial features and new are being discovered.

Although work in *C. elegans* covers many different fields, with lots of laboratories across the globe, it is still a friendly and close community. With International Worm Meetings every two years a good flow of homebrew methods, research data, and exchange of strains is guaranteed. There are also region-specific meetings in Latin American, Europe, India, Asia-Pacific (https://wormmeetings.weebly.com/). This welcoming and open spirit is also seen in the early beginnings of *C. elegans* work, with the "Worm breeders Gazzette" being published in 1975, whose intention was to publish the different methods that were being developed for the work in

C. elegans, months before the publication was finished; making them rapidly available to other labs (The et al., 2009).

The worm in the web

With the advancement of technology, not only new techniques were available to study the biology of *C. elegans*, but new ways of registering and reaching data rose with the coming of the World Wide Web; and this worm was also caught in it. Many platforms were developed to spread the information or make available specific tools to analyze the work done in *C. elegans*, but they can be classified into two types, "portals" and "knowledge environments", where the first organizes links relevant to the domain of interest while the second offers direct information and services. The main and most used sites are the Wormbook, the Wormbase, the Wormatlas, the Caenorhabditis Genetics Center (CGC), and the "Worm breeders Gazzette" previously mentioned, but there are many others. (Lee, 2005)

Wormbook is an open-access collection of original, peer-reviewed chapters that provides basic information about the biology of *C. elegans* and other nematodes. It also has WormMethods, with a collection of protocols, and WormHistory, which is a compilation of personal perspectives, lectures, journals, and ideas. (http://www.wormbook.org/index.html)

WormBase is an international consortium of biologists and computer scientists providing the research community with accurate, current, and accessible encyclopedic information concerning the genetics, genomics, and biology of *C. elegans* and related nematodes. It also provides useful bioinformatics tools for genetic analysis and browsing, providing easy to access curated information about anatomy, genes, papers, phenotypes proteins, mutant strains, etc. Moreover, it presents a register of the *C. elegans* lab community, making it easier to contact other researchers. Founded in 2000, the WormBase Consortium is led by Paul Sternberg (CalTech), Matt Berriman (The Wellcome Trust Sanger Institute), Kevin Howe (EBI), and Lincoln Stein (The Ontario Institute for Cancer Research). WormBase is a founding member of the Alliance of Genome Resources Project. (https://wormbase.org)

WormAtlas is a database featuring behavioral and structural anatomy of *C. elegans,* linking to handbooks, images, and movies, slideable anatomical cuts of the worm, a map of the neuronal wiring, and more available tools to visualize and comprehend the biology of the model. It is a great source of visual content and information. (https://wormatlas.org/)

From the University of Minnesota – USA, the Caenorhabditis Genetics Center (CGC) gathers the nomenclature, strain collection, purchase and distribution, and the genetic map construction. Here mutant strains of *C. elegans*, wild isolates, and bacterial strains for feeding can be purchased for a fee. The C. elegans Gene Knockout Consortium has created a knockout of approximately 21000 individual genes in the *C. elegans* genome, all available for purchase and study. (https://cbs.umn.edu/cgc/home)

There are many more cites that address specific fields of research, different projects, tools, and resources that are not presented here but can be easily accessed by the ones mentioned.

How to start your own *C. elegans* laboratory

All the methods, techniques, and recipes needed to start working in *C. elegans* are available at the Wormbook site, in the section "Worm Methods", Maintenance of *C. elegans* (Stiernagle, 2006). Nevertheless, from a protocol to the lab bench there will always be a distance to jump, and working with this model comes with the day-to-day struggles of a biologic organism. Here we will summarize the basic infrastructure and supplies needed, adding a few suggestions for beginners. To start a laboratory in *C. elegans*, you will need:

- An incubator capable of reaching temperatures between 15-25°C. The usual temperature for growing is 20°C, as the worms grow comfortably and reaches adulthood in three days. Although worms can be maintained at room temperature, an incubator gives a controlled environment suited for many experiments. Apart from this, having an incubator at 15°C is useful for medium-term storage, as worms grow slower and food lasts longer; this also gives flexibility to accelerate or slow the worms cycle, for example, to obtain a certain stage of development for an experiment.
- Stereoscopic microscope for checking plates, seeing how the worms are growing, and detecting any contamination. Every stage of development can be seen with an approximate magnification of 20X and easy observation analysis can be done to detect deficiencies in movement, morphology, behavior, and development.
- Petri dishes and NGM media. 60mm Petri dishes are the most commonly used but bigger ones can be used if larger populations are required; 30mm Petri dishes are frequently used in experiments that use expensive reagents and are easier for working with smaller populations like is the case of RNAi experiments. NGM is the basic solid media for growing worms, easy to make, with a few extra saline sterile buffers and cholesterol that has to be added after the media is autoclaved. After plates are poured they can be dried under a hood cabin, which reduces humidity in the plates diminishing the possibility of contamination. Plates can be stored either at room temperature or at 4°C and NGM media can be stored after sterilization but not after adding the buffers, as it cannot be reheated.
- Food. OP50 bacteria is the most widely used *E. coli* strain for feeding the worms and it has a mutation that limits its growth, which facilitates the observation under the microscope. Bacteria can be grown in LB media with antibiotics at 37°C and then put a drop in each plate, letting it dry afterward (Fig. 2). This will leave a spot of bacteria (preferably in the center of the plates) and clear agar around it, facilitating the visualization of the worms. Apart from OP50, there are many other strains used for food, but it is important to read how it affects worm growth and to be consistent and use the same bacteria through experiments.

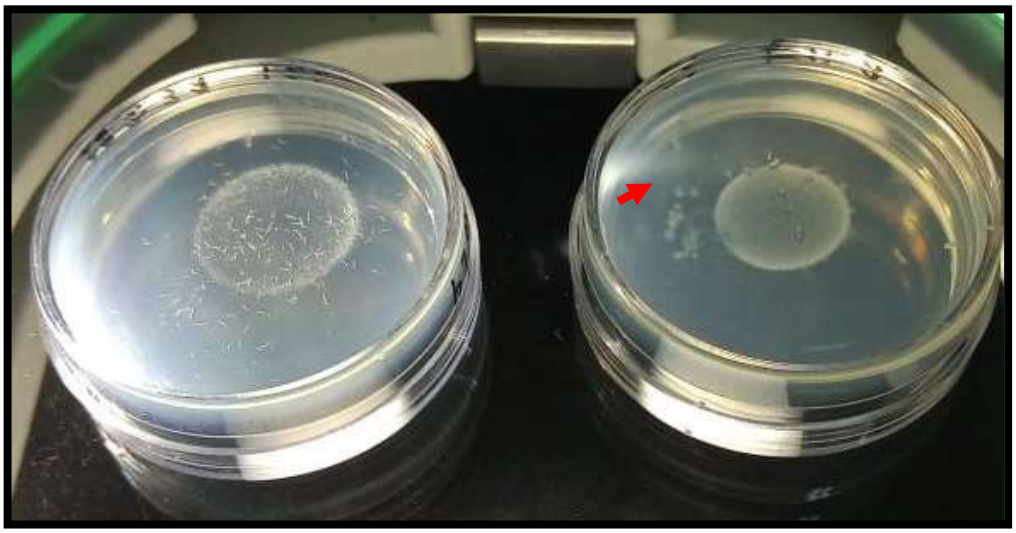

Figure 2. Two plates with NGM and a spot of OP50 bacteria (red arrow). In the left one, adults canbe seen in plain sight mainly in the spot of food, but there is no adult on the right one.

- Extensive workspace is not a must, but certain protocols require sterile environments like under a Bunsen burner or biosecurity hood cabin. Glass alcohol burners are a practical option to use next to the microscope.
- Ultrafreezer at -80° or liquid Nitrogen. As each generation of worms passes, mutations could be accumulated. It is important to remember that these worms are hermaphrodites with self-fertilization. This would affect the original strain genome and, in consequence, the results of experiments and observations carried. To avoid this, it is important to store any strain received, which can be done by collecting early larval stages and freezing them with the proper Freezing Buffer.
- Spatula and worm picker. Transferring worms from one plate to another is a crucial dayto-day practice for maintaining and expanding the worm population. There are two simple methods to achieve this, Chunking and Picking. Chunking is done from a plate, usually a starved or nearly starved plate with crowded worms, cutting a small square of agar using a sterilized small spatula and placing it in a fresh plate; this method allows moving bulk of worms from different stages and getting a decent population in a few days. The other method consists of moving one worm at a time using the worm-picker, a thin flattened platinum wire attached to a Pasteur pipette; touching a worm and pinking it up, and then moving it to a new plate and allowing it to leave the pick.

On a side note, contaminated plates are the main issue when maintaining *C. elegans* strains. Measurements to work properly, on a clean bench with sterile supplies, must be taken. Nevertheless, fungus and other bacteria will grow on plates eventually. However, they can be saved if contamination is not excessive. Worms that are not close to the contamination can be picked and placed in new plates. Contamination can be chunked out and removed from the plate with a spatula. As a last resource, plates can be bleached (See bleaching protocol in the WormBase site) killing contamination and all worm stages except for the eggs that can then be placed in a fresh plate; this can be also used as a method to synchronize the population (Stiernagle, 2014).

Concluding remarks

The work in *C. elegans* is an ever-expanding world with many different lines of research, new techniques being developed, and novel discoveries; with still many questions to be answered, despite the abundance of investigation done and achieved. Nevertheless, *C. elegans* remains to be humble and easy to approach model, with cheap maintenance, many resources available and flexible techniques that can be adapted to each researcher´s need.

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