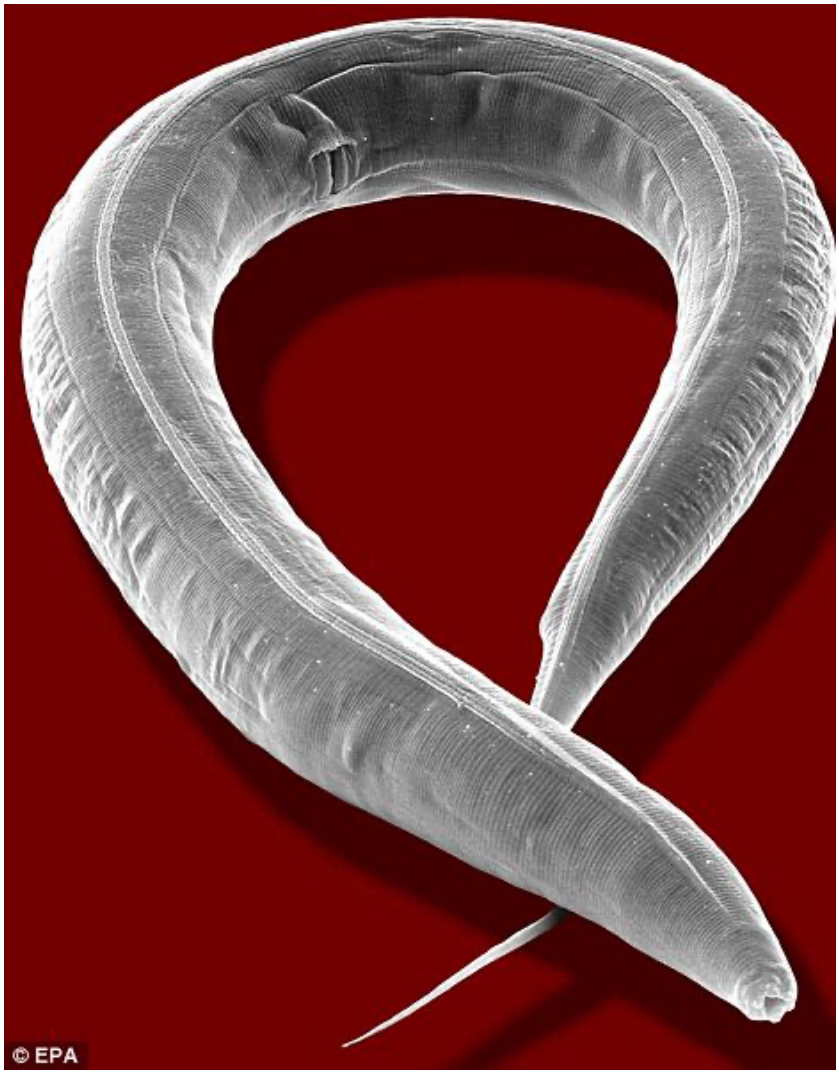
The nematode *Caenorhabditis elegans* as a model organism

Mum: Why do scientists study animals in the lab?



Scanning electron micrograph of an adult *C. elegans* hermaphrodite. This animal is 1 mm in length.

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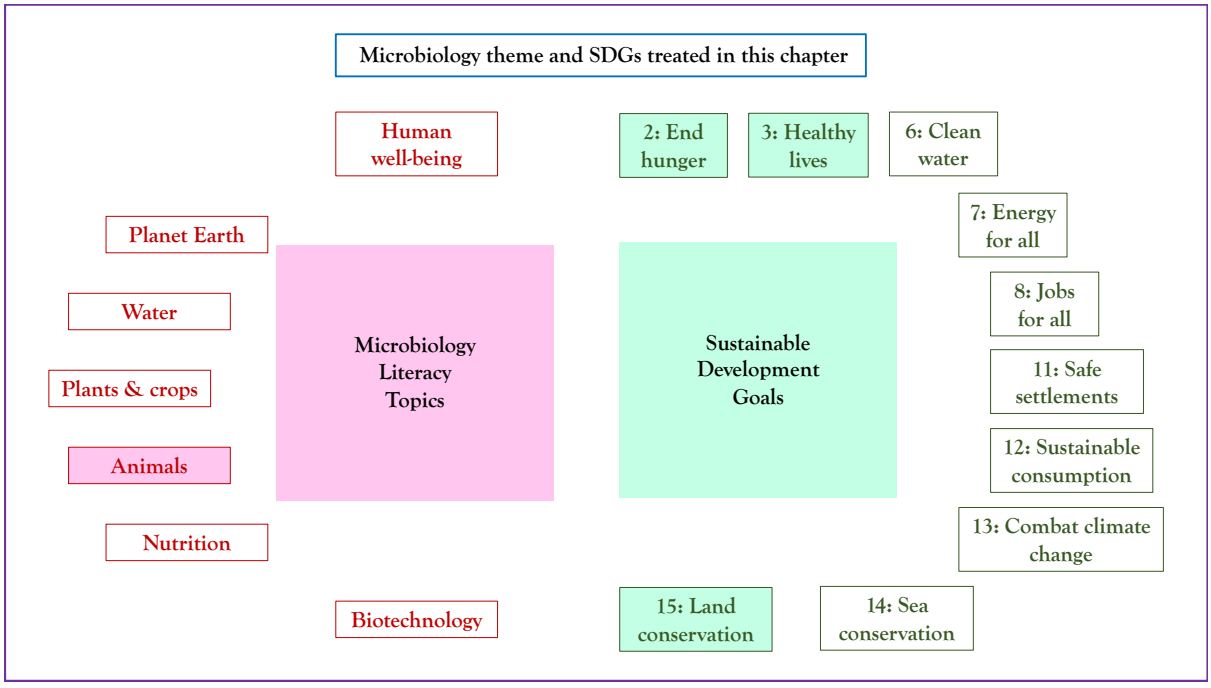
C. elegans as an Experimental Model

Storyline

Biologists seek to understand the basic principles of life: how does life work? How do organisms grow and develop? How do they digest food? What are the principles of sensing light and other environmental factors? How do they interact with other organisms in the environment? What are the principles of evolutionary change over time? Biologists have been tackling these questions for more than a century, but the approaches they use have changed drastically in the last 50 years. While scientists have originally observed wild organisms in nature, they now bring organisms into the laboratory. Selected bacteria, fungi, plants and animals with features that make them easy to grow under constant laboratory conditions, were selected as so-called 'model organisms'. Indeed, modern biology relies on a dozen or so model species. One of these species is the roundworm (nematode) *Caenorhabditis elegans*. Studying this nematode has helped to answer many basic questions in biology. This is not only helpful for biologists, but also for society as a whole because general principles often emerge that can be important for medicine and human health.

The Microbiology and Societal Context

The microbiology: nematodes; microbial diversity; microbial interactions; symbioses; infections of plants and animals; biological control of insect pests. *Sustainability issues:* end hunger; healthy lives; protect terrestrial ecosystems.



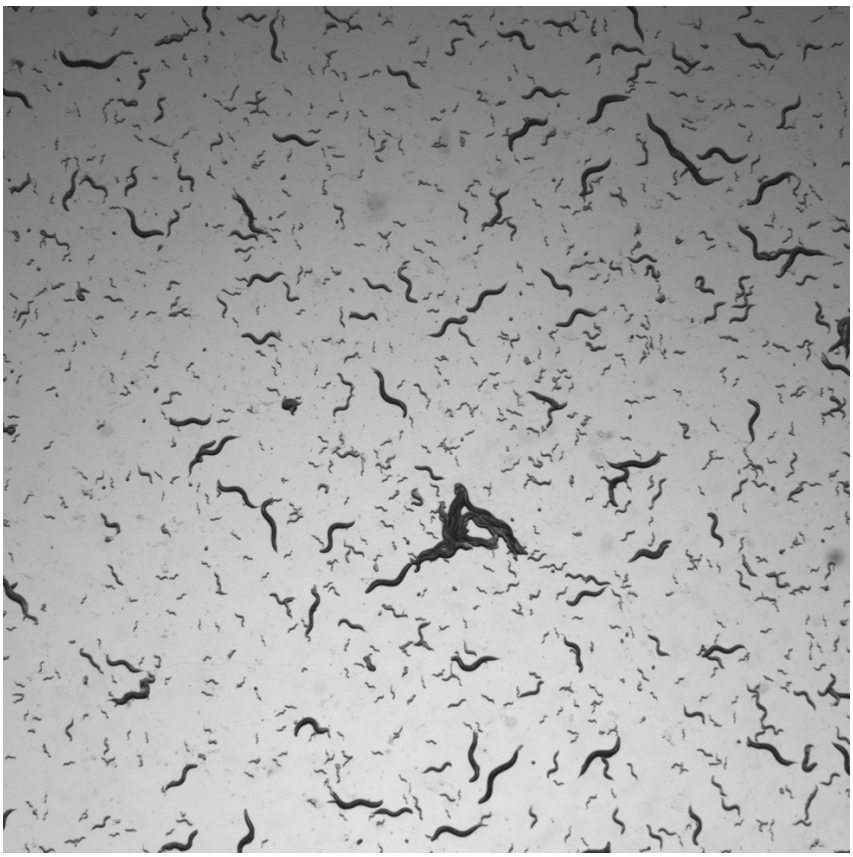
C. elegans as an Experimental Model: the Microbiology

1. ***Caenorhabditis elegans is a roundworm that can be cultured in the laboratory.*** In animals, microscopic multicellular species are found abundantly in soil, marine and fresh-water habitats. One phylum is the most numerous and widespread: the *nematodes* or *roundworms*. Nematodes are thought to represent the dominant group of animals, both with regard to abundance and species numbers, and in all ecosystems. Some of them can easily be cultured in a laboratory setting, one of which, *Caenorhabditis elegans,* was selected as a potential model organism. What no one could foresee in the early days of *C. elegans* research was that in 2022 more than 1000 laboratories around the world would work on all aspects of the biology of this small worm.

2. ***What matters: size, time and money.*** In the 1960s, scientists started to search for simple animal model systems. Several criteria were important. First, reproduction under constant laboratory conditions should be rapid to allow the growth of many generations of animals per year. Second, husbandry should be simple with limited demands for the required food source. Finally, the housing of the organism in the lab should be easy and cheap. The South African biologist Sydney Brenner introduced the nematode *Caenorhabditis elegans* as a potential model fulfilling all these requirements:

   a. *C. elegans* can be grown using the human gut bacterium *Escherichia coli* as its only food source. Eggs are laid and hatch into juvenile worms that undergo molting. After four larval stages (called L1-L4), the adult worm is formed that is only 1 mm in length.

   b. Typical cultures of *C. elegans* are established on 6 cm agar plates, which allows many worms to be cultured on such plates. These plates cost only a few cents, making culture systems easy and cheap.

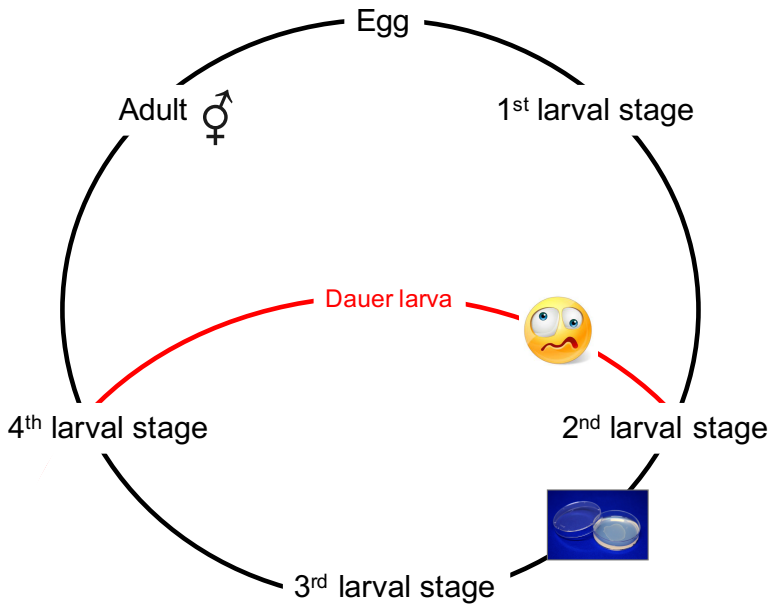


c. C. *elegans* has a generation time of 3,5 days at 20°C and 2,5 days at 25 °C, and thousands of animals can be produced in less than a week.

d. Additionally, worms can be frozen and stored in liquid nitrogen in small vials (glass tubes) indefinitely, and can be 'revived' within minutes after thawing.

3. ***Reproduction makes culture easy***. The features mentioned above made C. *elegans* easy to culture in the lab. But one more feature of this organism was 'mission' critical for attracting large numbers of researchers, to study C. *elegans* biology. While most nematodes have two sexes, males and females, C. *elegans* has another mode of reproduction, namely as a self-fertilizing hermaphrodite. Late C. *elegans* larvae are males that produce a limited number of sperm during postembryonic development. However, when they develop into adults, they become females and use their stored sperm to fertilize their own eggs. This mode of reproduction results in an important advantage: researchers can establish so-called 'isogenic cultures'. In isogenic cultures, all animals are genetically identical because they derive from the same parent; there has been no mixing of genes from different fathers and mothers. This is important for the analysis of mutants as we will see below. Importantly, however, C. *elegans* also forms males as a second sex, and hermaphrodites can outcross to male individuals.

In summary, self-fertilizing C. *elegans* hermaphrodites can be cultured on simple agar plates with *E. coli* as food and they complete their life cycle in less than 4 days. Establishing such a culture system was a great start to study all aspects of the biology of this simple animal.



C. *elegans* life cycle. First stage larvae hatch out of the egg. Under favorable conditions (agar plate with bacterial food), they will go through direct development and reach adulthood after around two days. Under harsh environmental conditions, 2nd stage larvae form dauer larvae. This stage is stress-resistant and long-lived and will go back to development only when the environmental conditions become favorable again.

4. *The model nematode provides insights into all aspects of biology.*

a. Development

At the time when *C. elegans* was identified as a potential model organism, many biologists were becoming interested in the developmental biology of animals. What are the principles of embryonic development? How does the fertilized egg develop into a fully grown organism? How do cells form the different tissues of an adult form? And how do cell types become specified during the development of the individual? Again, nematodes, and in particular *C. elegans,* was one of the selected species for more detailed investigations.

Two additional characteristics of *C. elegans* made it a superb choice for studying developmental biology. First, the animal is transparent, making it possible to follow cell divisions throughout development using high-resolution microscopy. Second, the development of *C. elegans* is highly reproducible with all individuals having the same cell division pattern throughout their complete development. This results in an identical cell number and a 'fixed' cell lineage pattern in all individuals. During embryogenesis, a total of 558 cells are formed from the uncleaved egg. After hatching, many cells in the worm divide further to reach the final number of 959 somatic cells (excluding the germ (reproduction) cells that form the sperm and oocytes). This constancy in cell number and the reproducibility of the cell lineage pattern throughout development are often referred to as 'eutely'. Eutely has allowed researchers to give a name to each cell by following the development of individual animals by microscopy, a research project that was published in 1977 and 1983. Once the cell lineage was completely known, a worm could be put under the microscope at any point of its development and the next cell divisions predicted. There is no other animal system with such a high precision in embryonic and postembryonic development.

Finally, it should be noted that each individual worm first produces 1090 cells, but exactly 131 cells subsequently die in a programmed manner, often shortly after these cells have been born. This observation resulted in the notion of 'programmed cell death'. Indeed, studies revealed that the death of cells requires a specific developmental program. Many genes involved in executing this program are conserved between worms and humans. The discovery of programmed cell death was rewarded with one of the Nobel prizes for *C. elegans* research.

b. The genome

C. elegans was the first metazoan animal (animals whose cells are differentiated into tissues and organs) to have its genome fully sequenced. In 1998, as part of the 'human genome sequencing project', the complete genome sequence of *C. elegans* was released. The genome consists of approximately 100 Megabases, which corresponds to a book with 100 million letters. The alphabet of the genetic information encoded in the DNA has four different letters and the linear sequence of these 100 million letters contains the information for genes and non-coding regions that are of regulatory nature or unknown function.

Already in 1998, the number of genes in the *C. elegans* genome was predicted to be around 20,000, a number that is still valid today. In comparison, the fruit fly *Drosophila melanogaster* has fewer genes (around 14,000) and humans are thought to have 24,000 genes. Thus, organismal complexity is not directly correlated to the number of genes.

But what is the function of all these genes and how do cells contribute to the formation of tissues and organs? One major inroad to answer such questions comes from genetics.

c. Mutagenesis and reverse genetics

Geneticists study the function of genes and their encoded proteins by manipulating genes in an unbiased or biased manner. In unbiased mutagenesis experiments, mutations can be randomly inserted into the genome through chemical or physical treatment of the organism. Geneticists then search for mutant animals with a particular characteristic or phenotype. For example, a developmental geneticist interested in a particular organ of a worm or another model organism would search for mutants in which this organ is no longer properly formed or not even formed at all. Such mutants can be further genetically investigated to identify which genes have been mutated (altered in sequence) to cause a particular phenotype. Such approaches have been called 'forward genetics', because mutations are introduced randomly in the genome and treated animals are searched for altered phenotypes.

In contrast, 'reverse genetic' approaches target individual genes. Such approaches became possible through the elucidation of the genome sequence (see above). In *C. elegans*, two reverse genetic approaches have been used intensively, RNA interference and CRISPR gene knockout. While the former allowed the systematic knockout (inactivation) or knockdown (inhibition of expression) of the genes in the genome, the latter provides the ability to systematically engineer and thereby modify individual genes in the genome. Together, genetic analysis has provided unprecedented tools to identify the molecular mechanisms of many biological processes. Below, we will have two such examples.

d. Neurobiology and behavior

The small and constant cell number of *C. elegans* is also a unique advantage for neurobiology. Of the 959 somatic (non-reproduction) cells of the adult hermaphrodite, exactly 302 are neurons with a total of 118 neuronal cell type classes. Over the years, scientists have learned i) which neurons control locomotion of the worm, ii) to what type of odors the worm responds and which neurons are responsible for this, iii) how males and hermaphrodites search and find each other for mating, and how mating is controlled as a behavioral process.

For these, and several other neurobiological processes, knowledge about the identity of the 302 neurons was just a starting point. Through serial reconstruction of electron microscopic sections throughout the worm body, the synaptic connectivity of all neurons is known. This is another feature that makes *C. elegans* unique because it allows researchers to study which neurons are in communication with one another, and much has been learned about associated molecular mechanisms.

e. Aging

C. elegans has a rapid generation time under laboratory conditions of 2,5 - 4 days depending on temperature. This means that a fertilized egg develops to an adult hermaphrodite, which lays its first eggs in less than 4 days. However, this adult hermaphrodite, if given a chance, will live much longer. Under standard laboratory conditions, *C. elegans* will live for 18-20 days at 20° C. Thus, the lifespan of the worm is much longer than its generation time.

Aged *C. elegans* animals show anatomical and functional degradation at all levels. Many tissues and cell types are affected and whole organism features, such as immunity, learning and motility, decline over time. Structural changes in neurons and muscles are general features of aged worms. Thus, a small nematode shows aging features similar to mammals or humans, indicating that aging is a general feature of many animals.

Geneticists have found mutants that affect lifespan in *C. elegans*. Of particular interest have been mutants in which lifespan is increased and more than 50 genes have been identified to control *C. elegans* lifespan. Some of these genes are highly conserved in evolution with corresponding genes being found in flies or humans. The most prominent group of genes are the Insulin signaling pathway. Insulin is a proteo-hormone or growth factor in humans and the *C. elegans* genome contains multiple genes encoding for insulin-like growth factors. These peptides bind a receptor at the cell surface called insulin receptor. In *C. elegans*, a single insulin receptor is encoded in the genome by the daf-2 gene which, when mutated, has several developmental and physiological phenotypes. Interestingly, daf-2 mutant animals live around 15% longer than wild type worms. Researchers learned that the primary role of the DAF-2 protein, and thus insulin signaling, is to repress the transcription factor DAF-16. When both genes are mutated, daf-2, daf-16 double mutant worms do not live longer than wild type indicating that, under normal conditions, DAF-16 would positively regulate longevity genes, but is hindered to do so by the insulin pathway. These findings allow three important conclusions. First, longevity and aging are at least in part genetically controlled. Second, this genetic control is, again in part, evolutionarily conserved so that studies in a nematode model can provide insight relevant for human biology and health. And finally, researchers working with animal species under laboratory conditions can establish 'models' for important human conditions like longevity. In some cases, researchers working with mice or fish have been able to establish 'disease models', in which the animal displays some or many of the pathological features observed in a human disease.

f. Conclusions

C. elegans is a small roundworm and as such, nearly invisible to the naked eye. But small can be beautiful and powerful. Building on easy culture conditions that allow growing millions of worms in a single laboratory by a small group of scientists, the *C. elegans* research community has systematically studied many biological questions. From a general understanding of developmental progression and adult homeostasis in physiology, research has also provided insight into the general principles important for human health and society. Surprisingly, or not surprisingly, work on 'the worm' found many principles of biology to be highly conserved throughout the animal kingdom, including humans. Nonetheless, *C. elegans* is just one of several million nematode species on our planet, which have diversified into many different ecological niches. Thus, while many basic principles in biology are shared and conserved among species, evolution has created a near endless diversity of form and function, also in nematodes.

The Evidence Base, Further Reading and Teaching Aids

Eds.: I. Glazer, D. Shapiro-Ilan, P. W. Sternberg (2022): Nematodes as model organisms. CABI, Oxfordshire.
WormBook: The online review of Caenorhabditis elegans Biology: dev.wormbook.org
WormBase: Exploring worm biology facilitating insights into nematode biology: https://wormbase.org