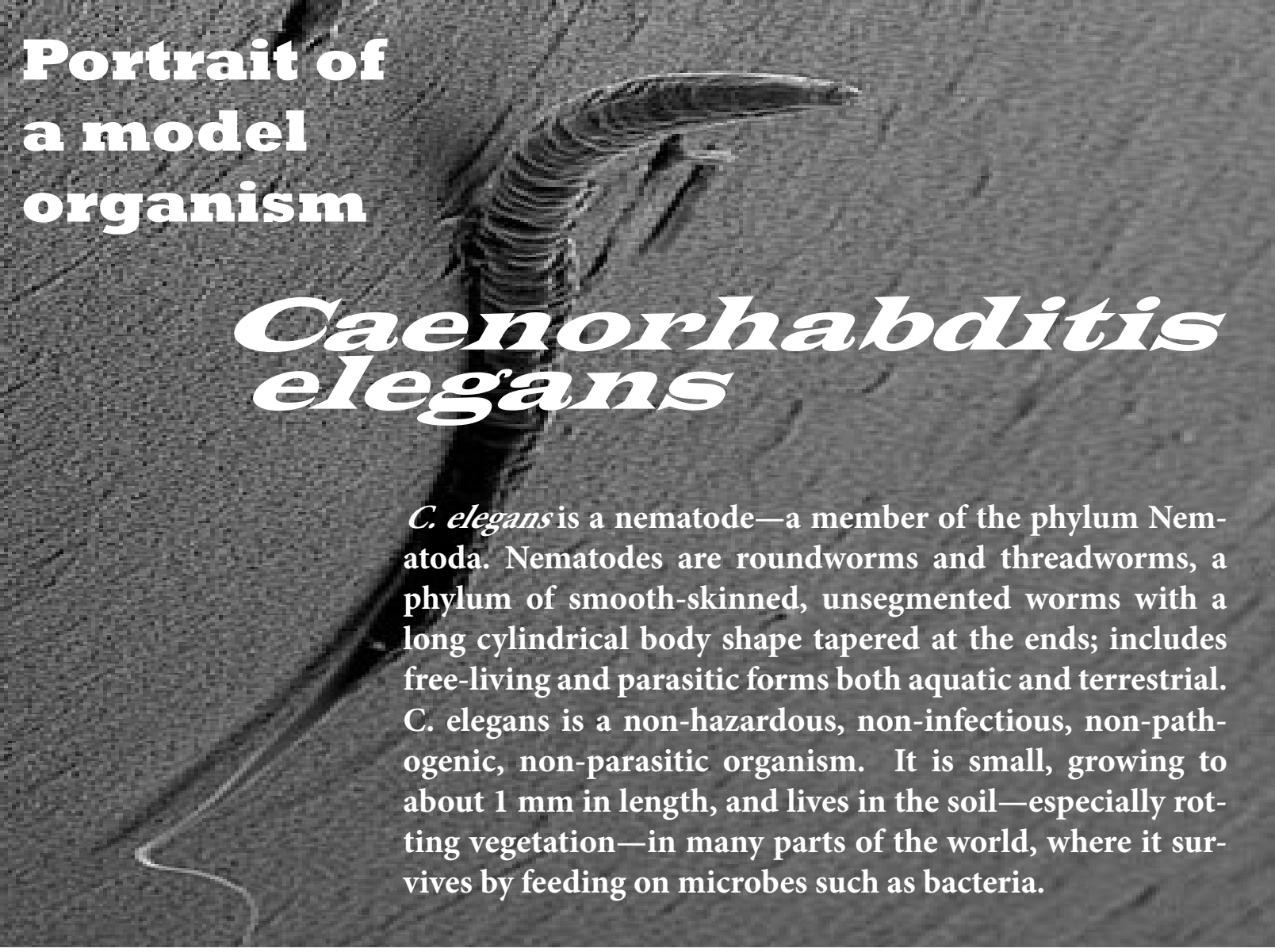
Portrait of a model organism



Caenorhabditis elegans

***C. elegans* is a nematode—a member of the phylum Nematoda. Nematodes are roundworms and threadworms, a phylum of smooth-skinned, unsegmented worms with a long cylindrical body shape tapered at the ends; includes free-living and parasitic forms both aquatic and terrestrial. C. elegans is a non-hazardous, non-infectious, non-pathogenic, non-parasitic organism. It is small, growing to about 1 mm in length, and lives in the soil—especially rotting vegetation—in many parts of the world, where it survives by feeding on microbes such as bacteria.**

What makes *C. elegans* best model for biomedical research?

Simple anatomy
Easy to grow in lab
Short life cycle: 3 days per generation
Powerful genetics (both self-fertile and cross-fertile)
Transparent body
Small size
Fully described anatomy and development
Completely sequenced genome
Post-genomic tools -- RNAi

There are several other attractive features that make Caenorhabditis elegans (C. elegans) an ideal organism for the study of gene regulation and function:

- C. elegans is a eukaryote, which means that it shares cellular and molecular structures (membrane bound organelles; DNA complexed into chromatin and organized into discreet chromosomes, etc.) and control pathways with higher organisms.
- C. elegans is a multicellular organism, which means that it goes through a complex developmental process, including embryogenesis, morphogenesis, and growth to an adult.
- C. elegans genome size is relatively small (9.7 x 107 base pairs or 97 Megabases), when compared to the human genome which is estimated to consist of 3 billion base pairs (3 X 109 bp or 3000 Megabases).

Life cycle of C. elegans

Similar to other nematodes, the life cycle of C. elegans is comprised of the embryonic stage, four larval stages (L1-L4) and adulthood. The end of each larval stage is marked with a molt where a new, stage-specific cuticle is synthesized and the old one is shed .

Embryogenesis in C. elegans is roughly divided into two stages: (i) proliferation and (ii) organogenesis/morphogenesis (i) Proliferation (0 to 330-350 min post-fertilization at 22oC): This stage involves cell divisions from a single cell to 558 essentially undifferentiated cells by the end of "16 E stage"

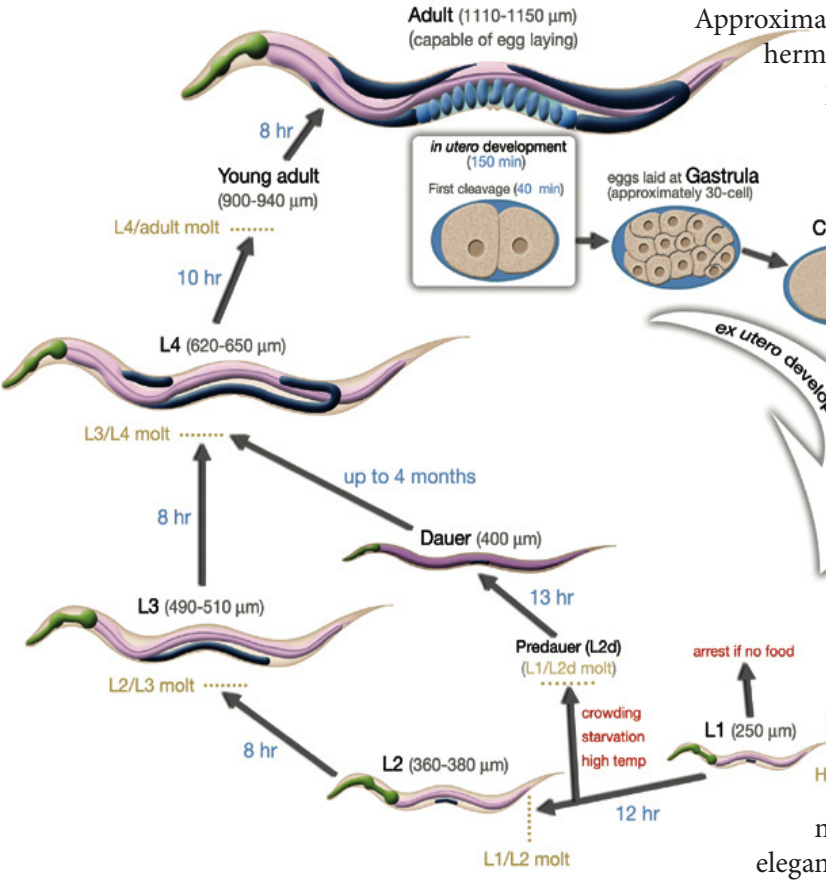
Organogenesis/morphogenesis (5.5-6 hr to 12-14 hr): During this stage terminal differentiation of cells occurs without additional cell divisions, the embryo elongates threefold and takes form as an animal with fully differentiated tissues and organs.

Postembryonic development- A Postembryonic development is triggered by feeding of the larva after hatching. In the presence of food, cell divisions resume and postembryonic developmental program begins 3 hours after hatching. The animal normally passes through four larval stages to reach adulthood.

The Larval Stages and development of adult body

L1 Larva - Nervous System, Reproductive System, Coelomocyte system
L2 Larva - Nervous System, Reproductive System
L3 Larva - Reproductive System
L4 Larva - Reproductive System

Adult



Approximately at 45-50 hrs posthatch at 22-25 C, a newly matured hermaphrodite lays its first cells, hence completing its 3-day reproductive life cycle. The adult hermaphrodite produces oocytes for about 4 days and after this fertile period of 3-4 days, the mature adult lives for an additional 10-15 days. A hermaphrodite that self-fertilizes will produce about 300 progeny because of the limited number of sperm, whereas if mating with a male occurs, the progeny number can increase to 1200-1400. Males can successfully mate with a hermaphrodite for 6 days after their last larval molt and can father approximately 3000 progeny. Out of 1090 somatic cells generated during hermaphrodite development, 131 undergo programmed cell death at characteristic times. Hence, the adult hermaphrodite has 959 somatic nuclei, 302 of which are neurons and 95 are body wall muscle cells. The adult male, on the other hand, has 1031 somatic nuclei and 381 of these are neurons (extra neurons are mostly dedicated to male mating behavior). Although it has more cells, the adult C. elegans male is more slender than the hermaphrodite and slightly shorter (approx. 0.8 mm).

Sex and the single nematode.

C. elegans exists either as a hermaphrodite or a male. The predominant sexual form of C. elegans is the hermaphrodite — this animal produces both sperm and eggs. Thus, it can self-fertilize. When it does, each animal produces about 300 progeny. The standard lab strain of C. elegans has beem propagated by self-fertilization for many generations. Self-fertilization leads to homozygosity of alleles; therefore, individual worms are considered to be genetically identical (as long as mutations have not occurred).

Note that in C. elegans hermaphrodite development there is a developmental switch — first sperm are produced and stored in the spermatheca and then oocytes are produced.

C. elegans development.

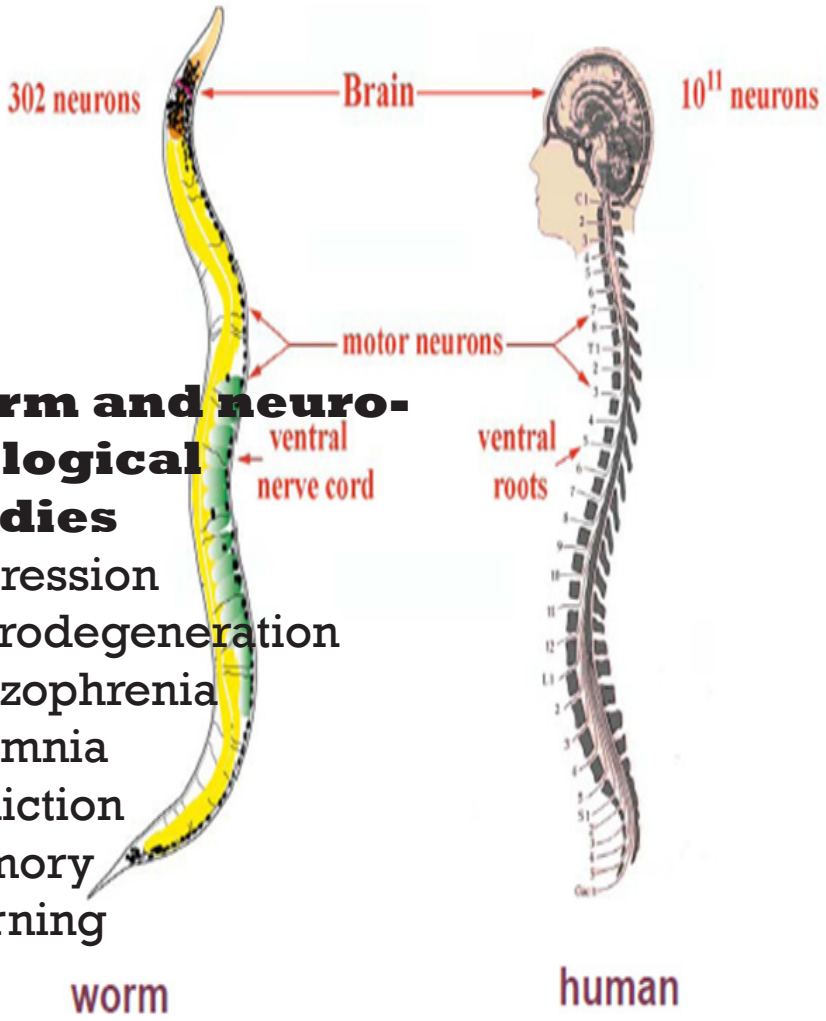
C. elegans development is characterized better than any multicellular organism the complete cell lineage of the animal has been recorded. A cell lineage is a description of all the cell divisions that occur to generate a specific group of differentiated cells (in the case of C. elegans, the entire animal!). In other words, the developmental pattern of each somatic cell is known, from the zygote to the adult worm. Thus, a scientist can identify any cell at any point in development, and know the fate of that particular cell.

Nobel Prizes for C. elegans research

Nobel Prize for Physiology or Medicine,2002
H. Robert Horvitz, John Sulston, Sydney Brenner
"for their discoveries concerning genetic regulation of organ development and programmed cell death"

Nobel Prize for Physiology or Medicine, 2006
Andrew Fire, Craig Mello

Nobel Prize for Chemistry, 2008
Martin Chalfie (with Osamu Shimamura and Roger Tsien)



Worm and neuro-biological studies

Depression
Neurodegeneration
Schizophrenia
Insomnia
Addiction
Memory
Learning
etc.

Dauer larva

Dauer (German for "permanent") describes an alternative developmental stage of nematode worms, particularly *Caenorhabditis elegans* whereby the larva goes into a type of stasis and can survive harsh conditions.It may also be equivalent to the infective stage of parasitic nematode larvae. The Dauer state is given other names in the various types of nematodes such as 'diapause', 'hyperbobis', 'spring rise', but since the C. elegans nematode has become the most studied nematode, the term 'dauer stage' or 'dauer larvae' is becoming universally recognised when referring to this state in other nematodes.

Under environmental conditions that are favorable for reproduction, larvae develop through 4 stages or molts which are designated as L1, L2, L3 and L4. After L4, animals moult to the reproductive adult stage. However, when the environment is unfavorable, L1 and L2 animals have the option to divert their development from reproduction to dauer formation. Signals such as temperature, food supply, and a dauer-inducing pheromone (population density cue) influence this dauer decision. Dauer larvae are thus considered an alternative L3 stage larva, and this stage is sometimes referred to as L2d. L2d animals are also considered pre-dauer and are characterised by delayed development and dark intestines produced by storage of fat.

Dauer larvae are extensively studied by biologists because of their ability to survive harsh environments and live for extended periods of time.

The C. elegans Lifespan Machine

No amount of best-practice protocols and clever software can replace the attention of a careful and thoughtful experimenter. Just as in manual survival assays, conclusions drawn from automated assays should be evaluated in context of the quality of data and assumptions made in its collection.

The Lifespan Machine was developed as part of Nicholas Stroustrup's Ph.D thesis in Walter Fontana's Lab in theDepartment of Systems Biology at Harvard Medical School. It combines modified flatbed scanners with custom image processing and data validation software to automate the collection of Caenorhabditis lifespan data (Stroustrup et. al 2013). This method allows the observation of thousands of nematodes once an hour over the course of several weeks. Like the manual technique, it utilizes agar plates and bacterial lawns as food stock. The time-lapse nature of the approach does not permit longitudinal analysis when individuals are young and change location on the plate. However, it becomes longitudinal once individuals are old and their motion is confined.

Our automated methods can be combined with many existing genetic approaches to quantitatively characterize the statistical consequences at the population level of molecular perturbations to aging. We found our approach especially useful for obtaining high-resolution estimates of time-dependent hazard rate functions. We also deploy our method for performing stress resistance assays, such as exposure to temperature and oxidants, when lifespans can be as short as a dozen hours.

Many aspects of hardware and software interact with experimental design to determine the interpretability of Lifespan-machine derived survival data. Our 2013 methods paper, especially the supplementary information, represents a good starting point for understanding these issues. This website is intended as a clearing house to provide resources, such as links to evolving software and documentation, as well as the development of "best practice" techniques via our mailing list.

The Lifespan Machine is a research tool that applies objective criteria to identify death times from image data. To do this at all, several assumptions must be made with regard to nematode morphology and movement. Most importantly, individuals should look approximately like wildtype adult animals and should move occasionally throughout their life, since death is defined as the persistent cessation of spontaneous changes in posture. The Lifespan Machine performs well, for example, when characterizing late L4 larvae, thin strains like glp-1(2141), other Caenorhabditis species (such as C. briggsae and C. brenneri), uncoordinated mutants including unc-64(e246), and behavioral mutants like rol-6(su1006). However, some strains and environmental conditions may violate the machine's assumptions in less obvious ways, decreasing the interpretability of automated death times. For this reason, the Lifespan Machine integrates human validation as a rapid but necessary step during analysis ("Worm browser" software).