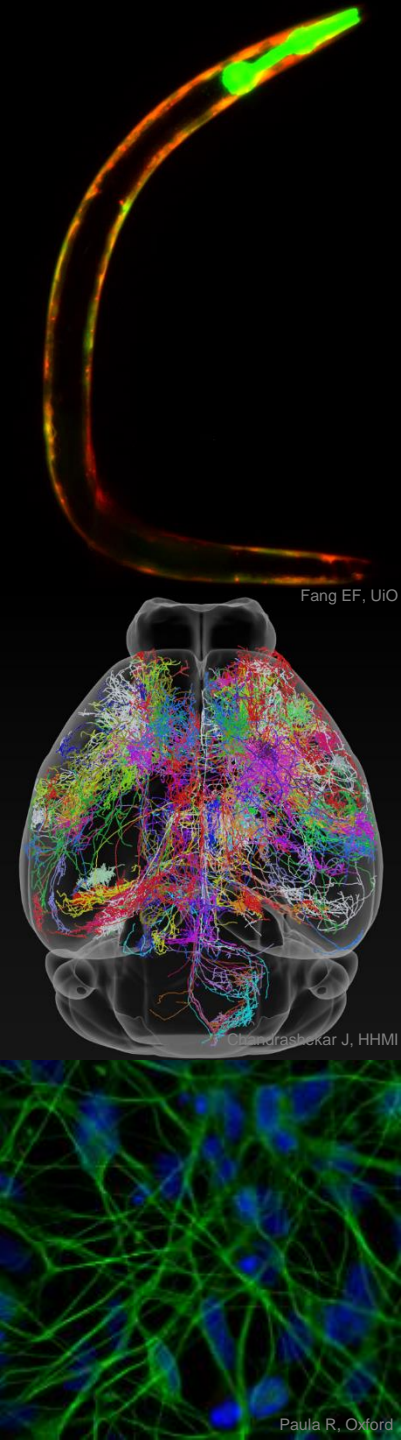


Introduction of the round worm *C. elegans*

Evandro F. Fang, Ph.D.
University of Oslo, Norway
08th July 2021

Website: evandrofanglab.com



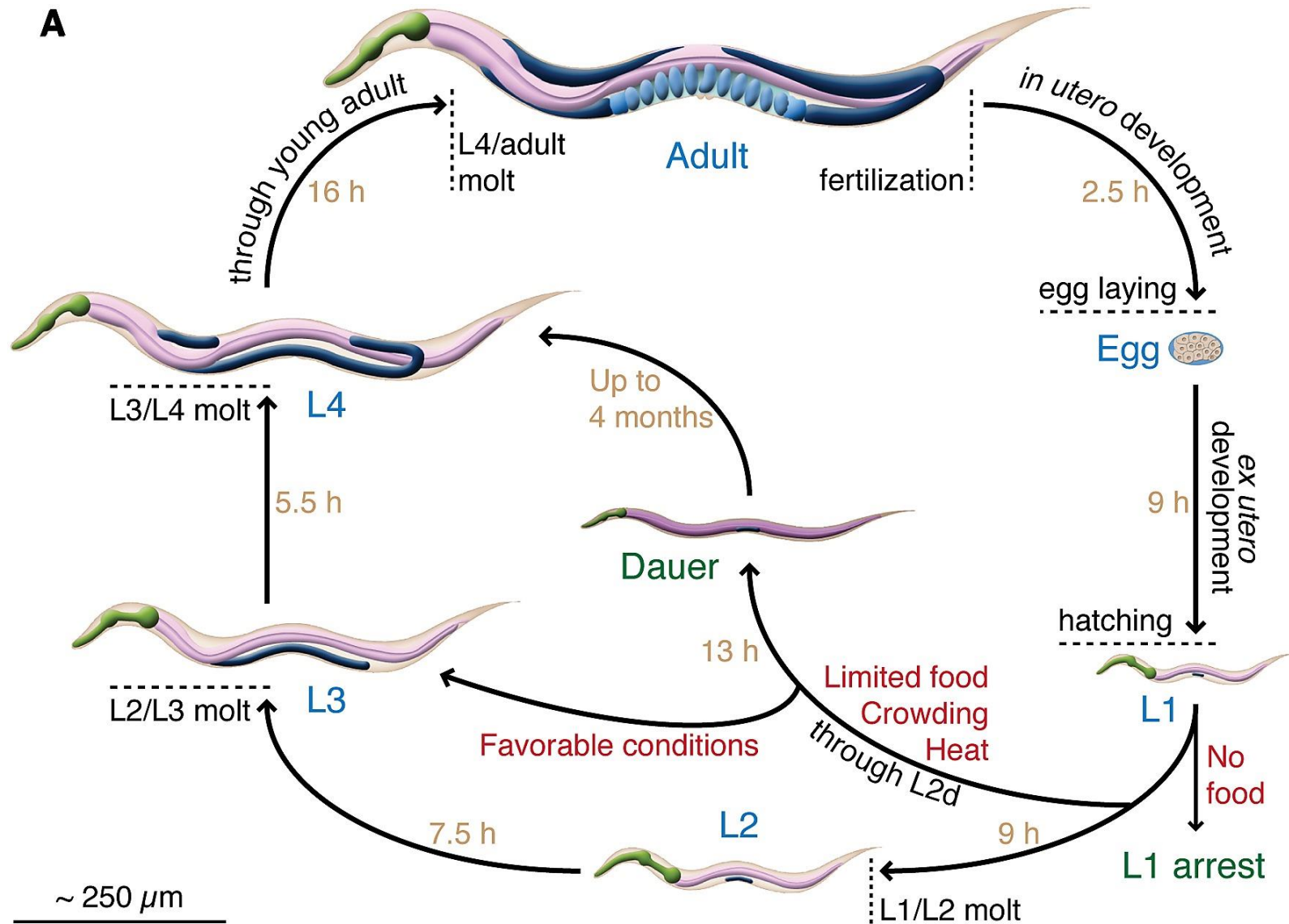
AKERSHUS UNIVERSITETSSYKEHUS

2021-07-08 edition EFF

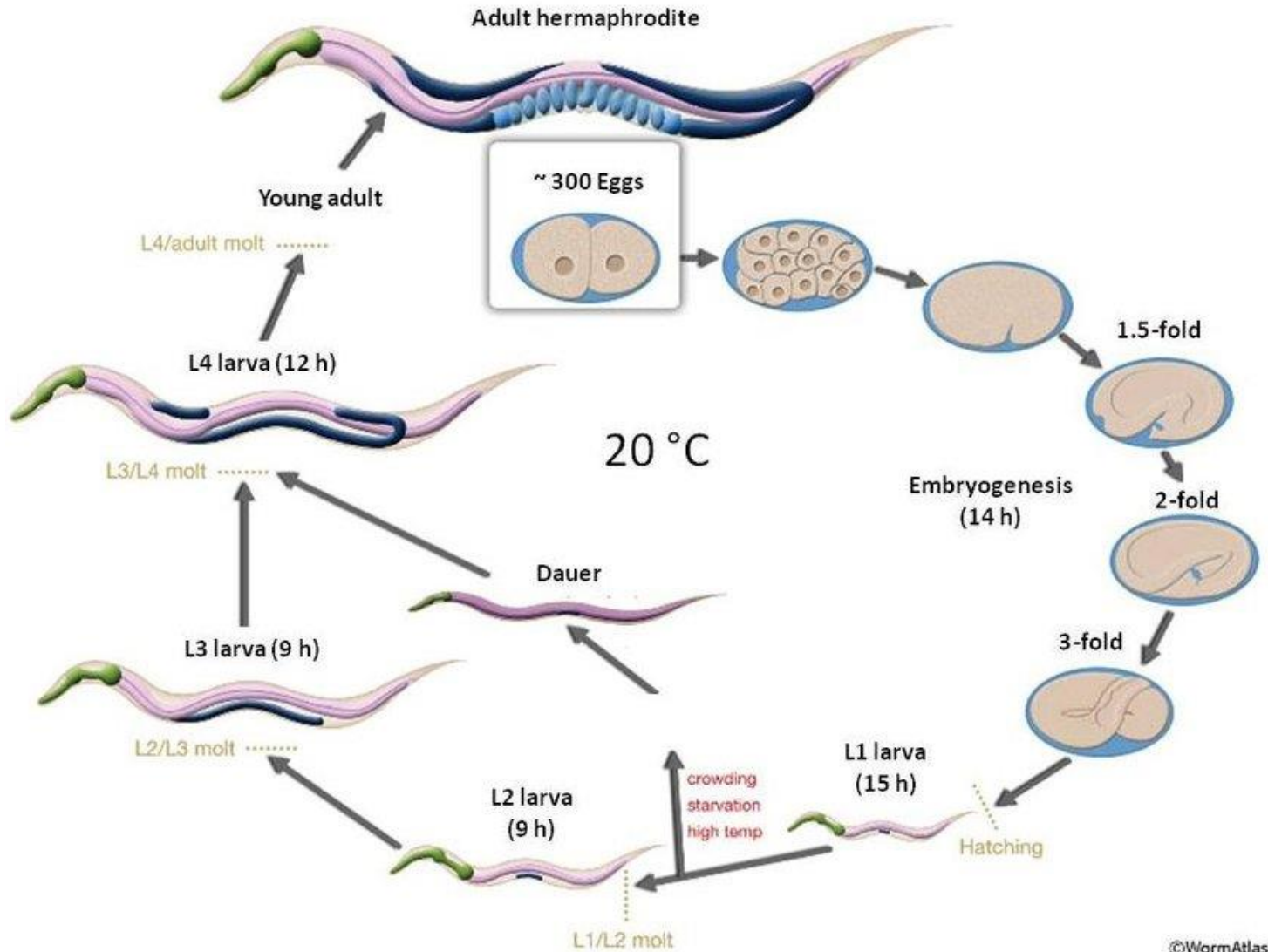
An adult *C. elegans* under light microscope



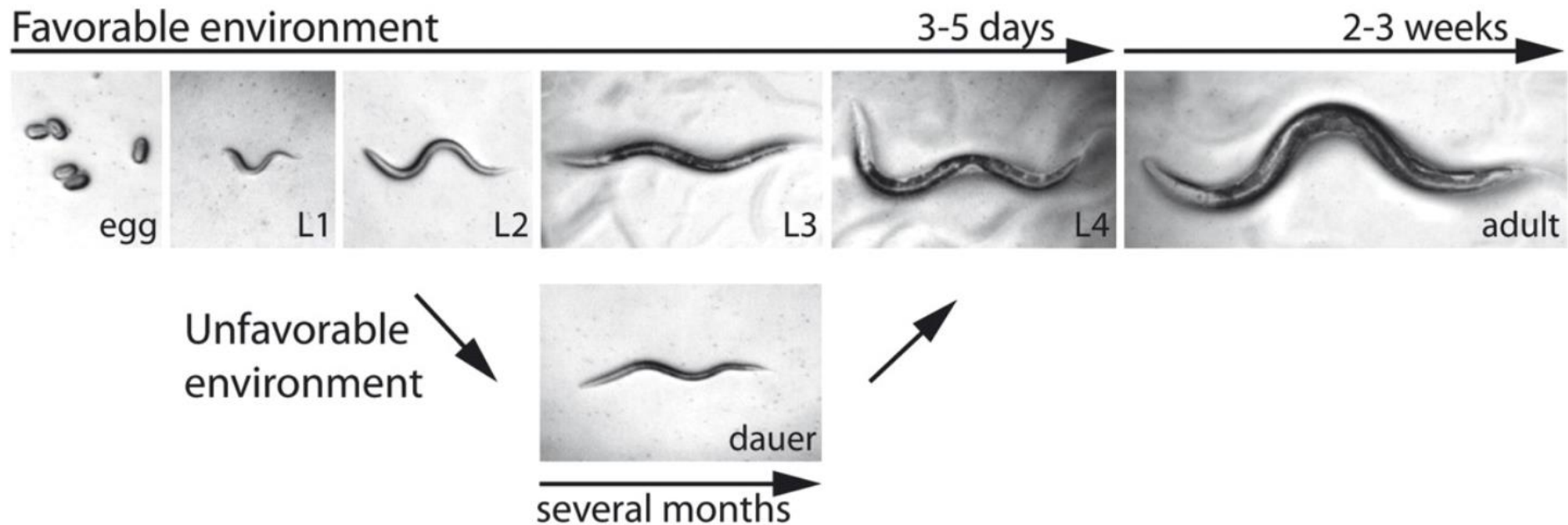
C. elegans life cycle with dauer branch (25 C) From egg to reaching L4 (31 hours)



C. elegans life cycle with dauer branch (20 °C) From egg to reaching L4 (47 hours)



C. elegans life cycle with dauer branch



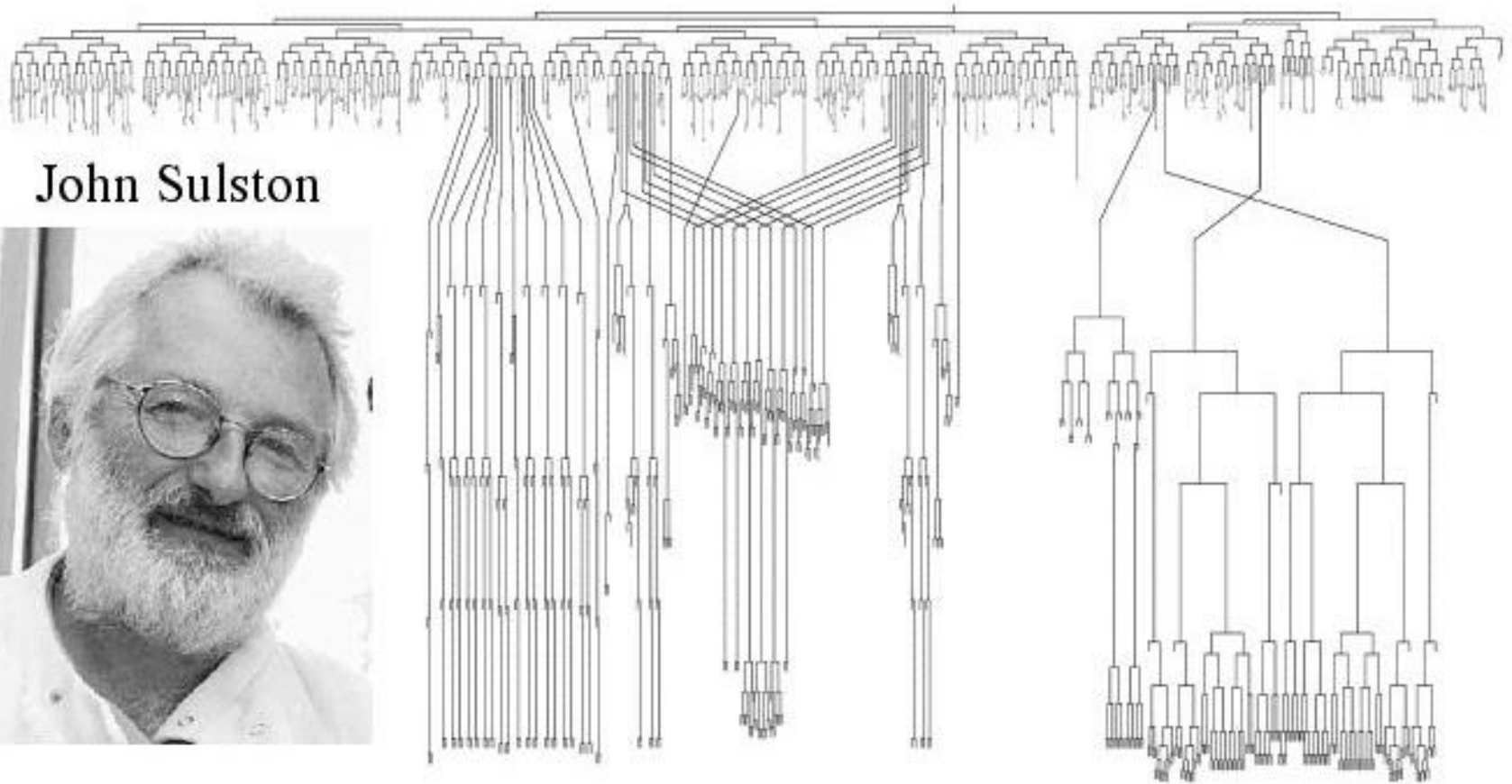
© Cold Spring Harbor Laboratory Press. Fielenbach, N., and A. Antebi. "C. *elegans* Dauer Formation and the Molecular Basis of Plasticity." *Genes & Development* 22 (2008): 2149-65. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

A molting worm

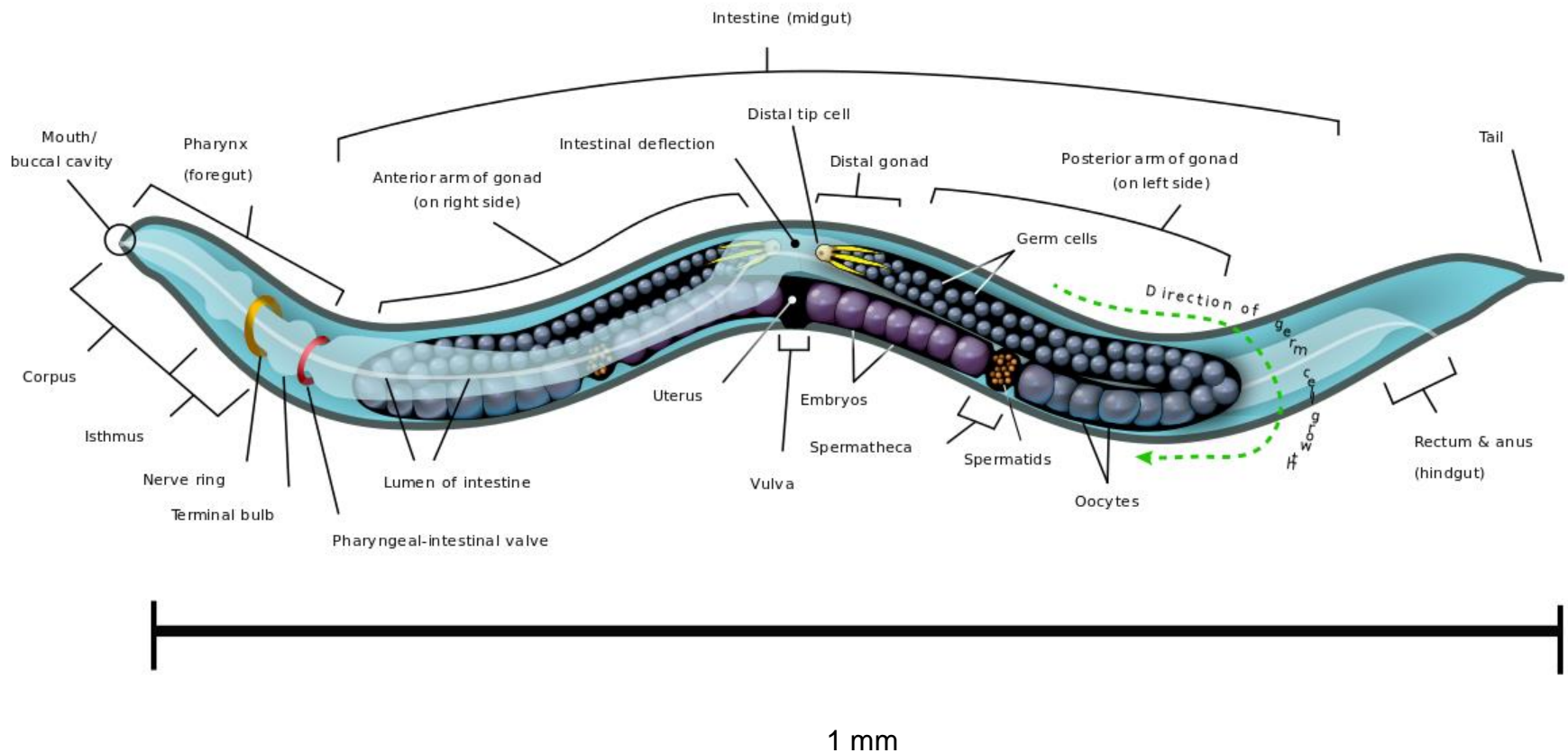
<https://evandrofanglab.com/experimental-models/>

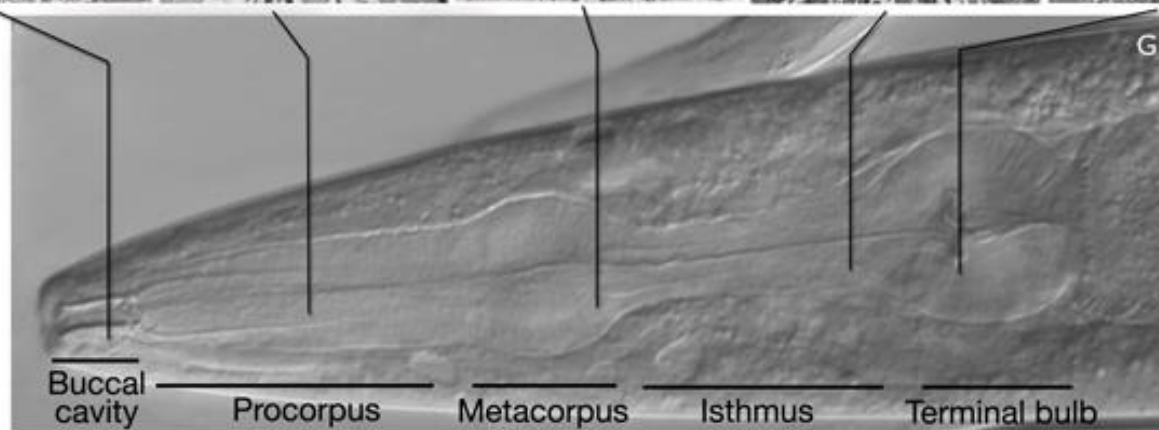
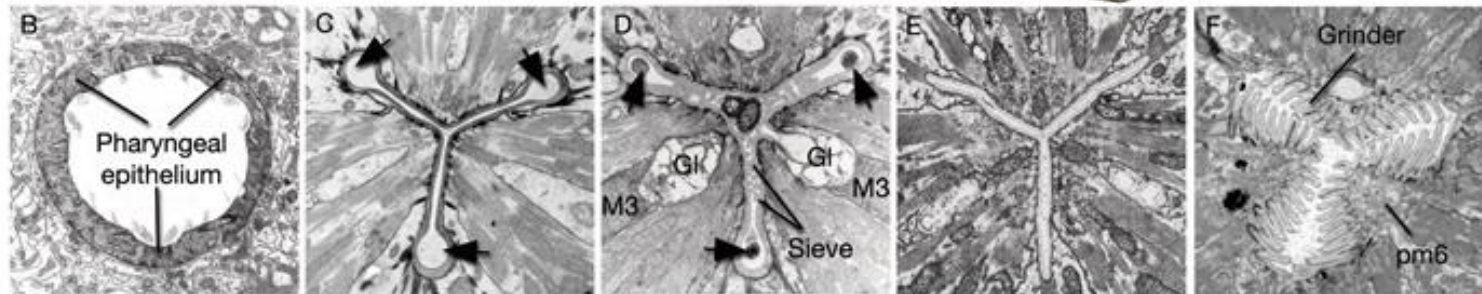
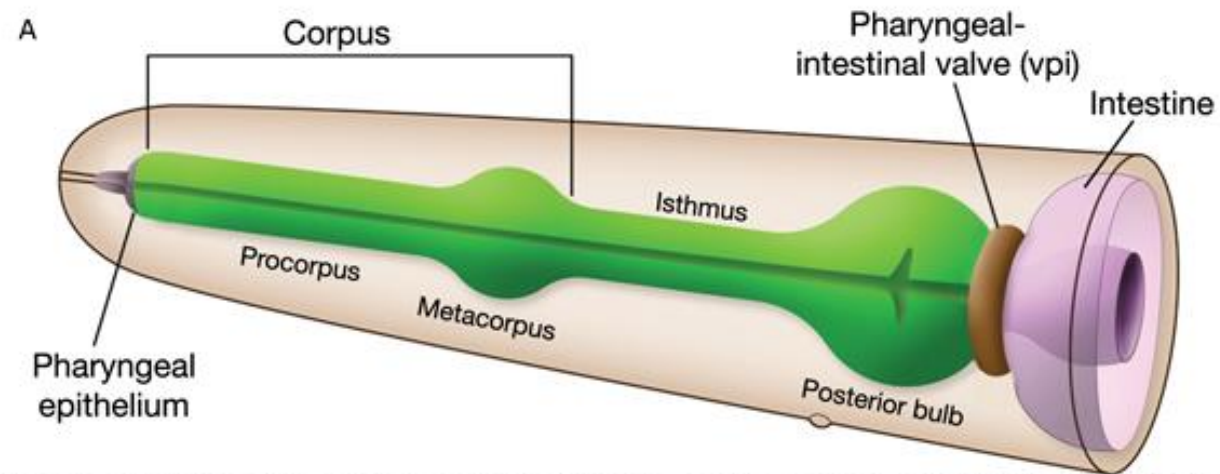
The adult *C. elegans* hermaphrodite contains exactly 959 somatic cells + gonad cells

John Sulston



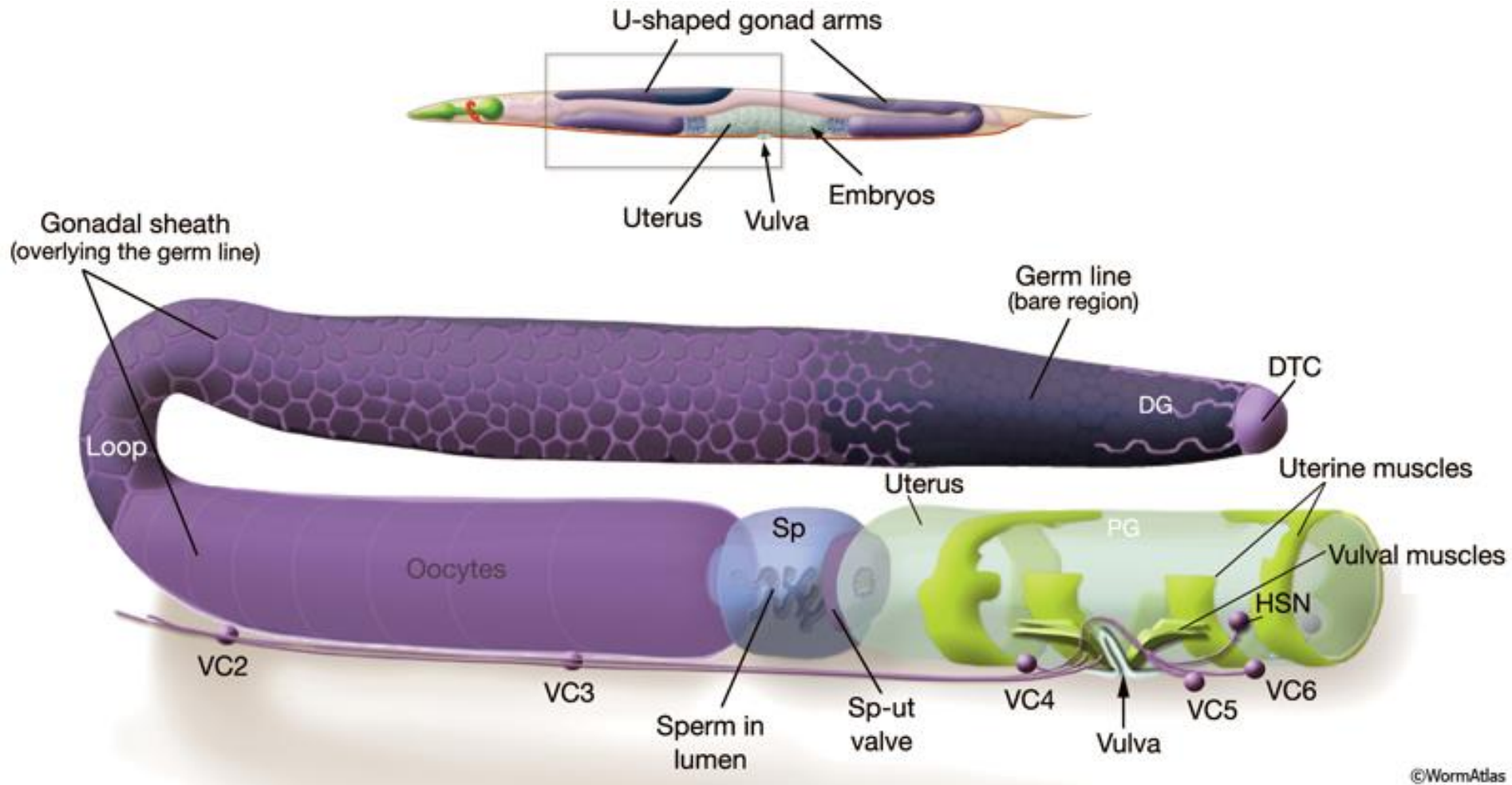
C. elegans anatomy



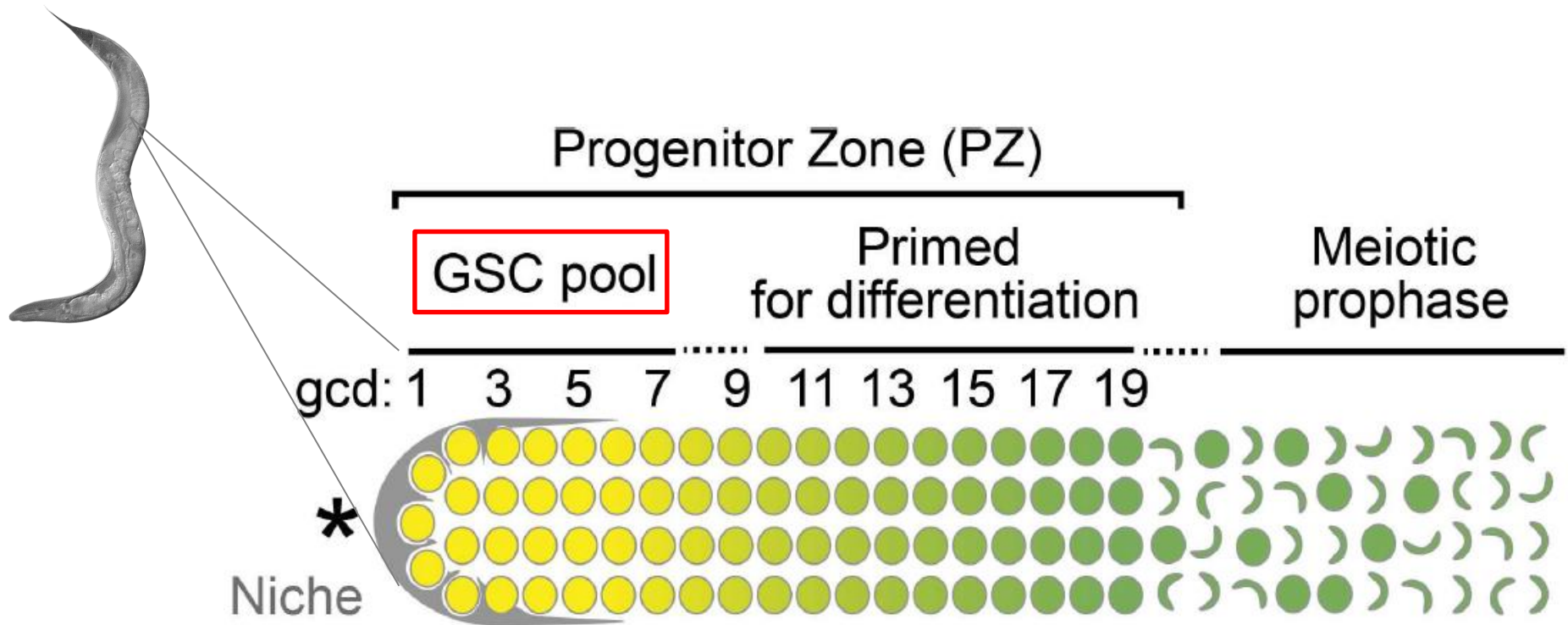


©WormAtlas

The adult hermaphrodite reproductive system

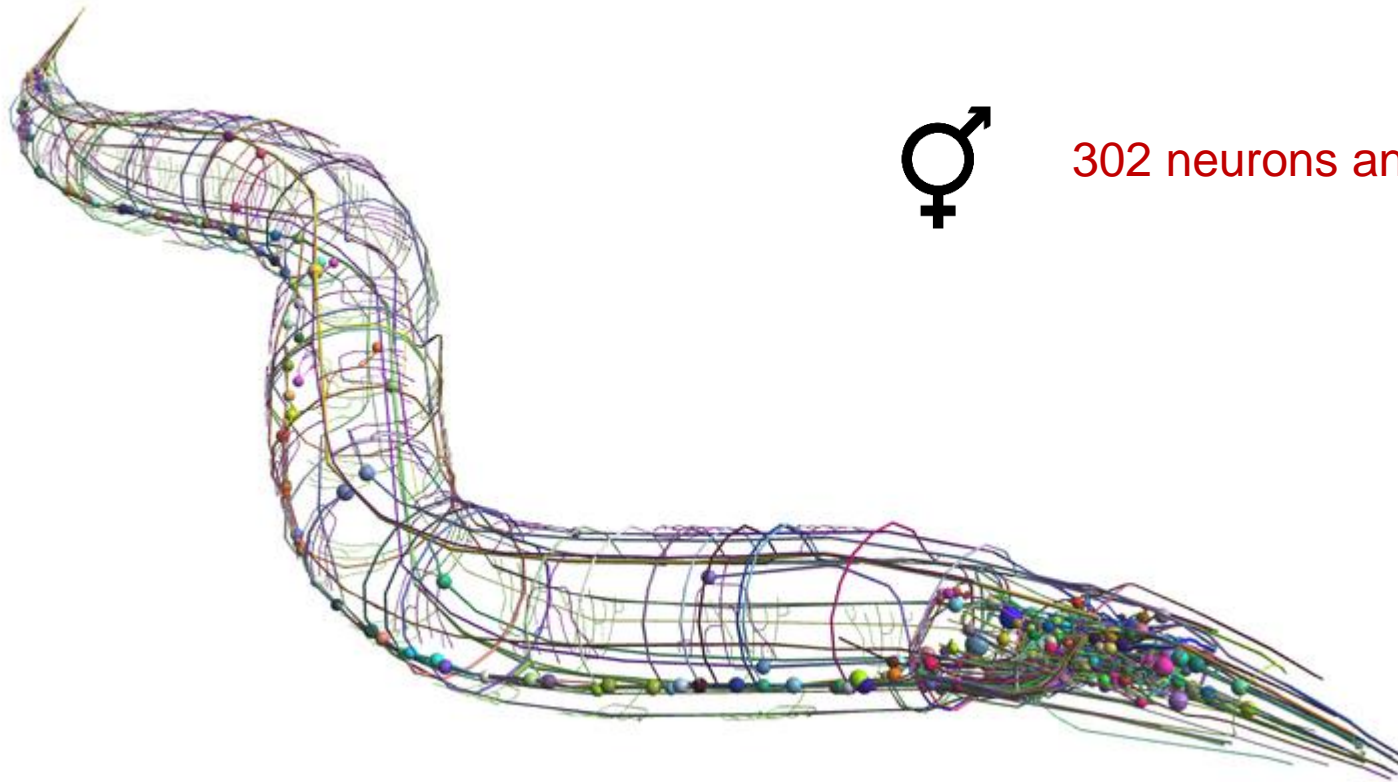


A germline stem cell population in *C. elegans*, but not well accepted



Schematic of adult distal gonad. The progenitor zone (PZ) includes a distal pool of **germline stem cells (GSC)** and a proximal pool of cells primed to differentiate. The conventional metric for axis position is number of germ cell diameters from the distal end (gcd). Somatic niche for GSCs (gray); naïve stem cell state (yellow circles); early meiotic prophase (green crescents); primed transiting state (yellow to green gradient). Asterisk marks distal end.

***C. elegans*, a good model to study neuroscience (no brain, but a brain-like sophisticated neuronal system)**



The nervous system represents the most complex tissue of *C. elegans* both in terms of numbers (302 neurons and 56 glial cells = 37% of the somatic cells in a hermaphrodite) and diversity (118 morphologically distinct neuron classes). (From Wormbook). Image: Neuroinformatics 2012

Basic information of *C. elegans*

- Wild type (N2): 1 mm size; 3 weeks lifespan (20, 25 °C)
- Fertilized egg>4 larval stages>adult *C. elegans* begins life as a single cell, the fertilized egg, which gives rise, through repeated cell divisions, to 558 cells that form a small worm inside the egg shell. After hatching, four successive larval stages separated by molts. The entire developmental sequence, from egg to egg, takes only about three days.
- An adult *C. elegans* consists of
 - a) exactly 959 somatic cell nuclei plus about 2000 germ cells are counted in hermaphrodites;
 - b) exactly 1031 somatic cell nuclei plus about 1000 germ cells in the other)
- Two sexes A hermaphrodite and a male. The hermaphrodite can be viewed most simply as a female that produces a limited number of sperm: she can reproduce either by self-fertilization, using her own sperm, or by cross-fertilization after transfer of male sperm by mating. Self-fertilization allows a single heterozygous worm to produce homozygous progeny. This is an important feature that helps to make *C. elegans* an exceptionally convenient organism for genetic studies.

How to identify L4 worms

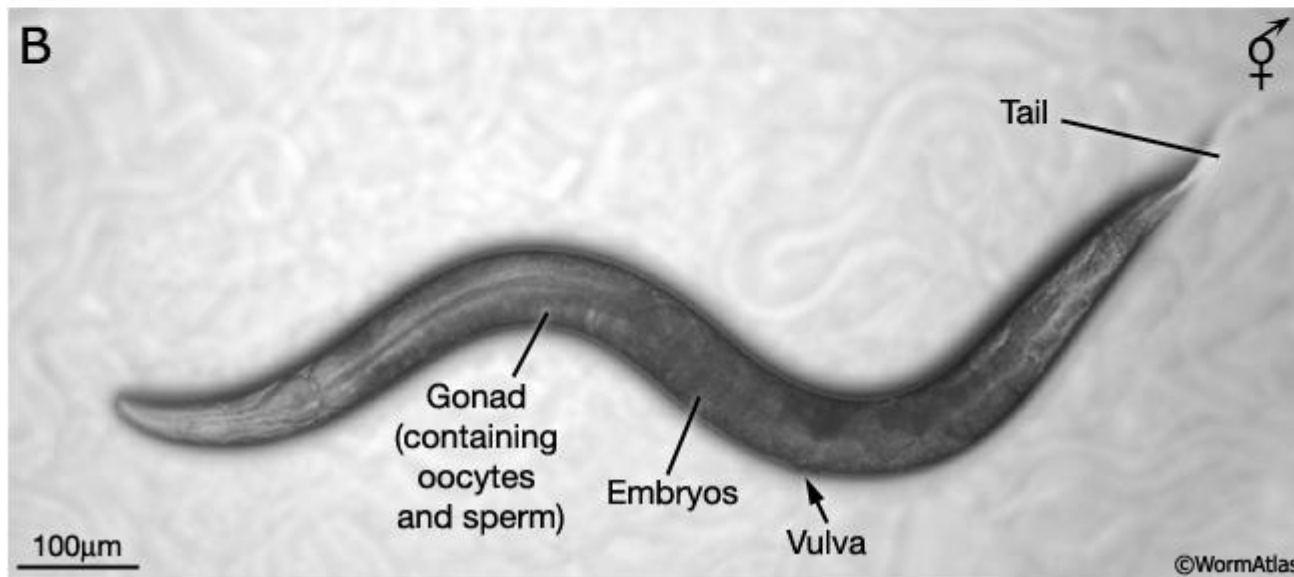
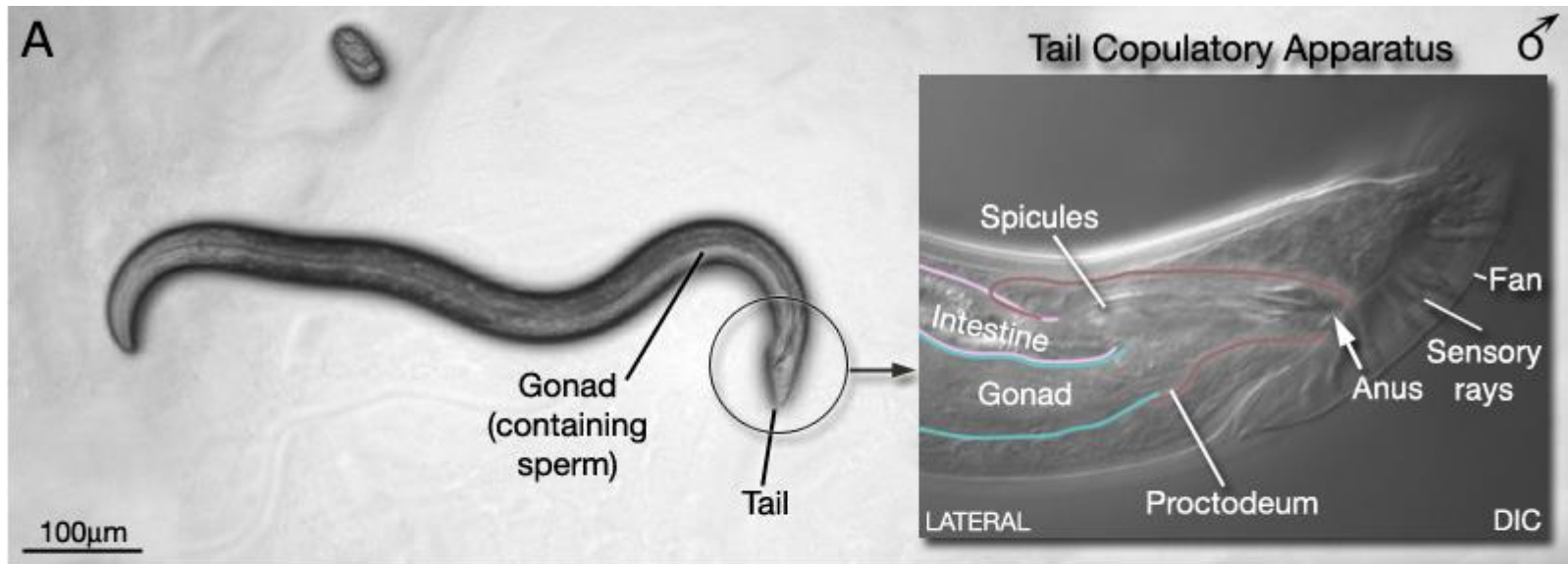


Key: white crescent

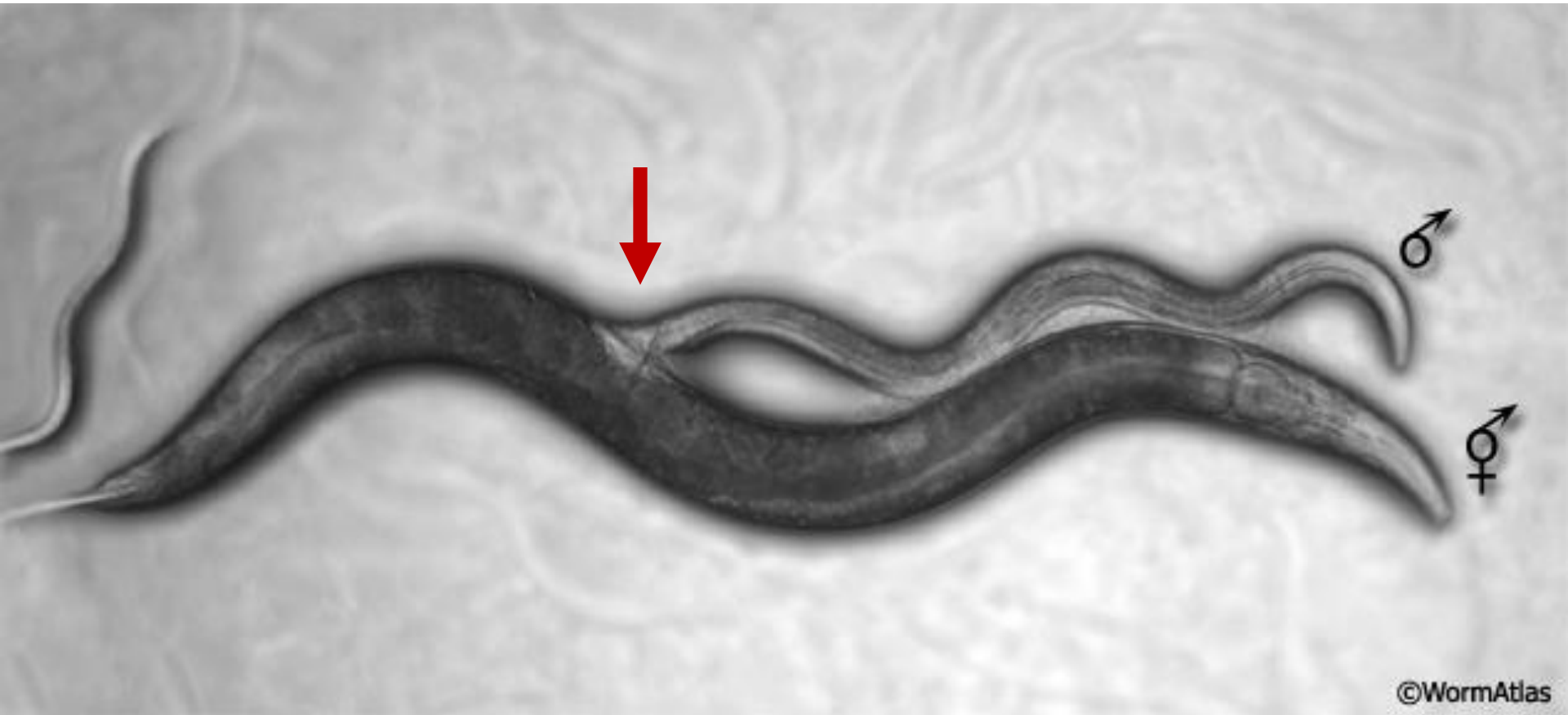
© Cold Spring Harbor Laboratory Press. Fielenbach, N., and A. Antebi. "C. *elegans* Dauer Formation and the Molecular Basis of Plasticity." *Genes & Development* 22 (2008): 2149-65. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

L4 hermaphrodites can be distinguished by the presence of a small white half-circle patch in the worm midsection. This patch corresponds to where the vulva will eventually develop.

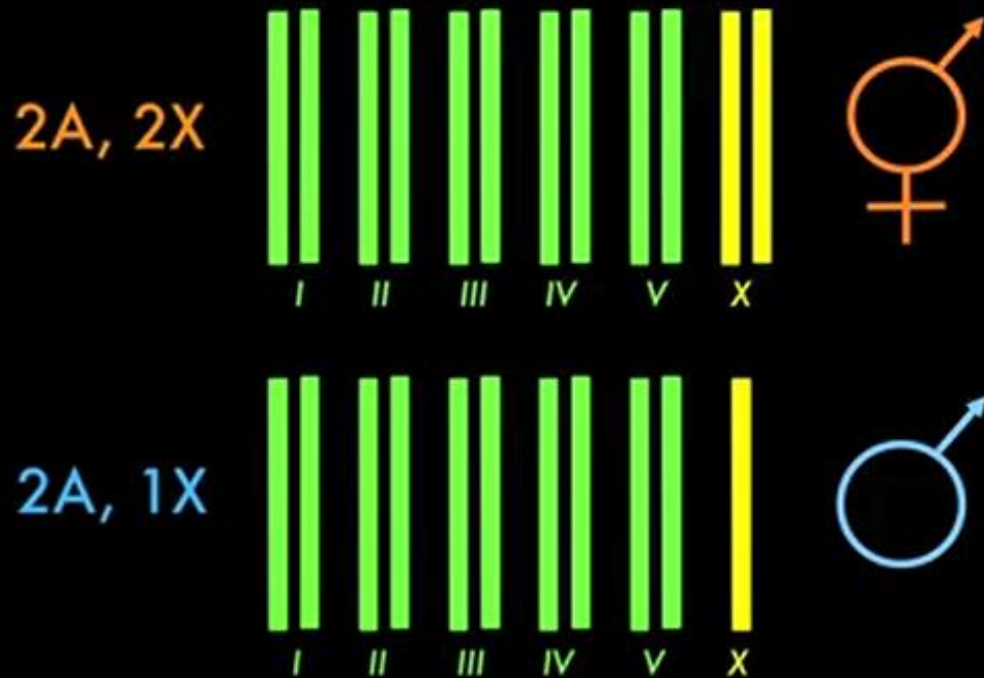
How to identify male worms



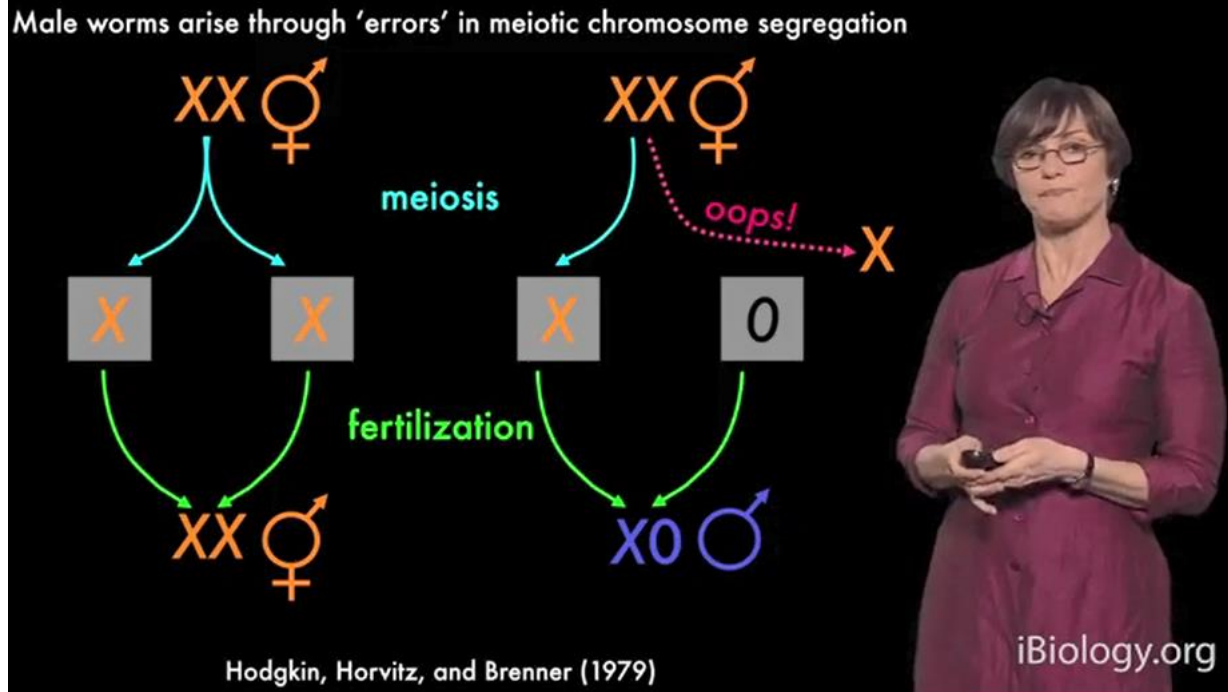
Worm mating



The mechanism of sex determination in *C. elegans* provides a simple genetic assay for meiotic chromosome missegregation



iBiology.org



<https://www.youtube.com/watch?v=Z1A7bOPfcpk>

X0(O) sex-determination system

In this system, there is only one sex chromosome, referred to as X. Males only have one X chromosome (X0), while females have two (XX). **The zero (sometimes, the letter O) signifies the lack of a second X.** Maternal gametes always contain an X chromosome, so the sex of the animals' offspring depends on whether a sex chromosome is present in the male gamete. **Its sperm normally contain either one X chromosome or no sex chromosomes at all.**

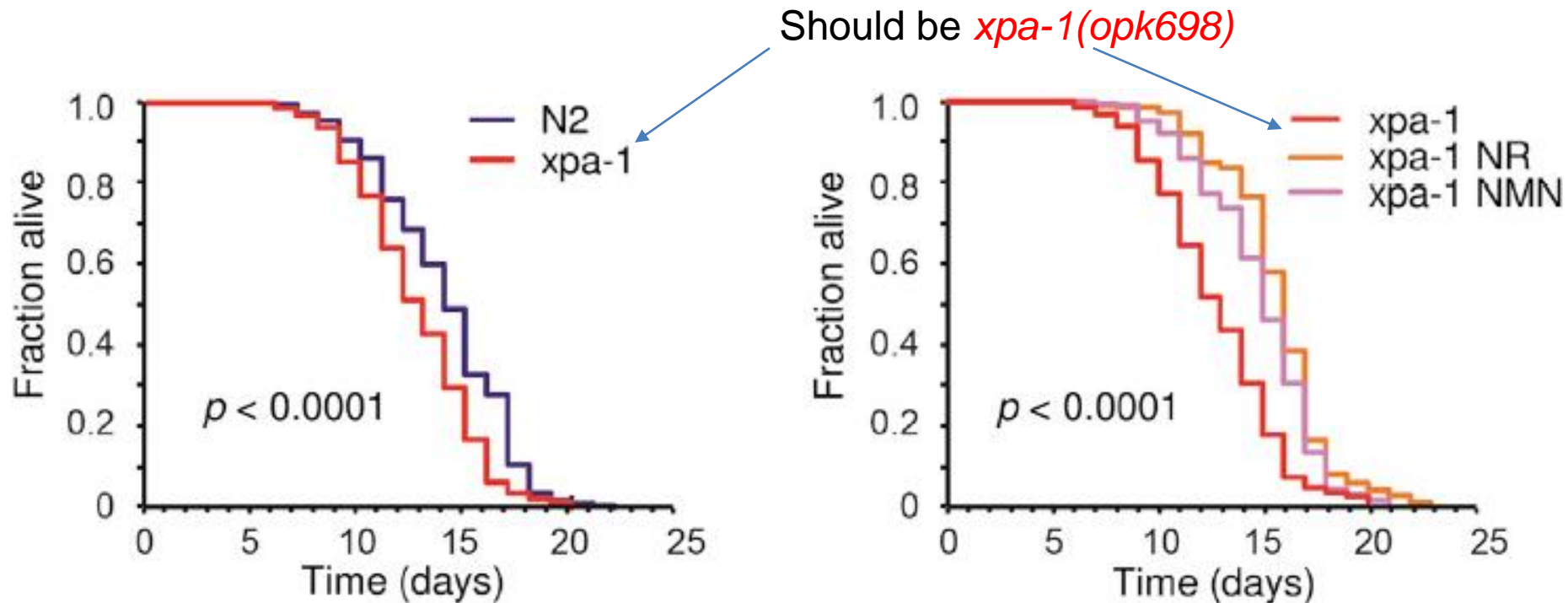
In a variant of this system, most individuals have two sex chromosomes (XX) and are hermaphroditic, producing both eggs and sperm with which they can fertilize themselves, while rare individuals are male and have only one sex chromosome (X0). The model organism *C. elegans*—a nematode frequently used in biological research—is one such organism.

The frequency of males (5AA; X0) among the self-progeny of wild-type *C. elegans* hermaphrodites (5AA; XX) is about one in 500.

***C. elegans*: an exceptional model for aging research**

- **Lifespan:**
Wild type (N2): 1 mm size; 3 weeks lifespan, 25 °C
- **Healthspan:**
Normal aging features: neurodegeneration, muscle atrophy; frailty
- **Conserved longevity pathways:**
SIR2.1 (SIRT1); DAF-16 (FOXO3); autophagy; mitophagy

An example: lifespan studies in a premature aging disease



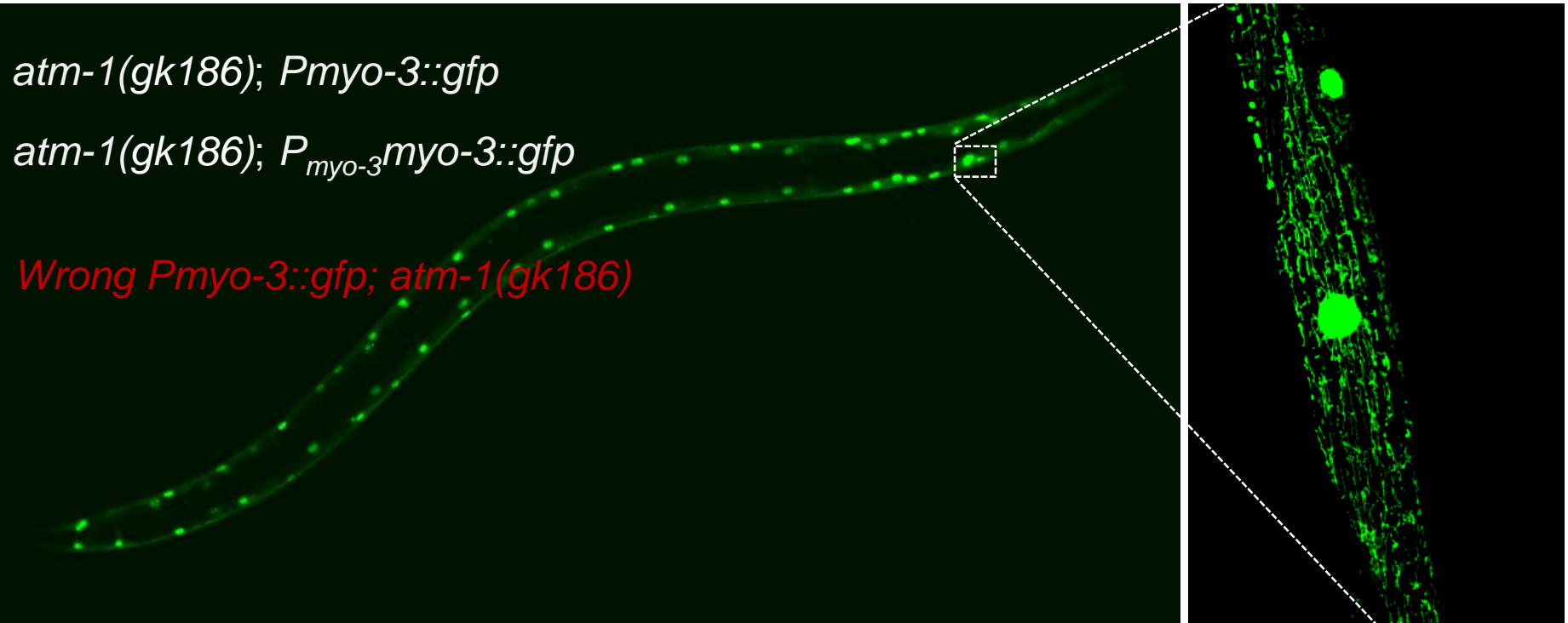
How to perform lifespan:

<https://www.jove.com/video/1152/measuring-caenorhabditis-elegans-life-span-on-solid-media>

To perform healthspan studies in *C. elegans*

- **Pumping**
- **Swimming**
- **Maximum velocity**
- **Mitochondrial muscle network**
- **ATP production**
- **Stress resistance**
- **Others**

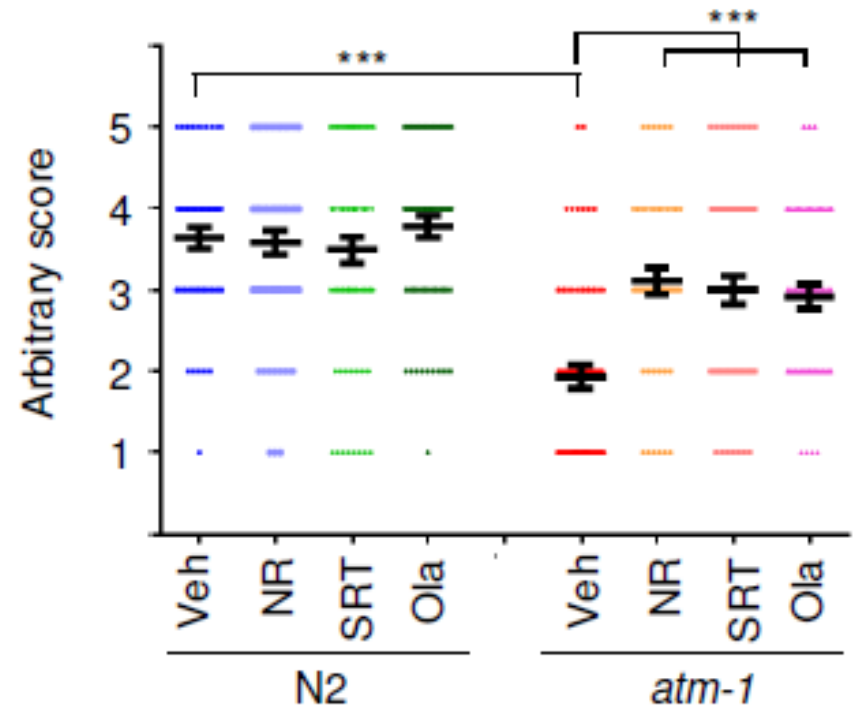
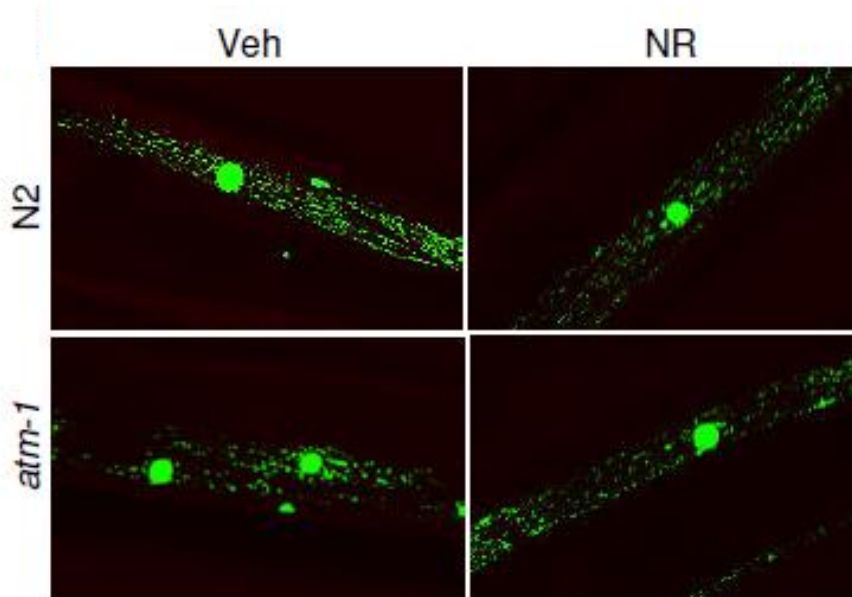
A *C. elegans* strain with GFP expressed in the Muscle cells



myo-3 encodes the minor isoform of myosin heavy chain which is muscle-specific

Image: Fang EF/Kassahun H

Muscle mitochondrial network: a mark of aging research



The NAD⁺/Sir-2.1 signaling reverts impaired mitochondrial networking in *atm-1* muscle cells (Fang EF et al., Cell Metabolism 2016)

The *atm-1* worms are less active than N2

atm-1 control



atm-1 NR



N2 control



N2 NR



Genetic Nomenclature

<https://www.ncbi.nlm.nih.gov/books/NBK20203/>

WRN human

Wrn^{-/-} mouse

wrn-1 (gene) C. elegans (WRN-1 protein)

Worm nomenclature (very very important)

The Fang group

Laboratory/strain prefix (**EFF**)
allele prefix (*efg*)

Details in the summarized 'nomenclature' document

Also online resource: <https://wormbase.org//about/userguide/nomenclature#4bfij9063gedh7m5a2c81kl--10>

Questions

- **Does *C. elegans* have a brain?**
- **Blood?**

- **Does it go sleep?**

E.g., Developmentally timed sleep (DTS), or termed lethargus

- **Are the genomes of the progeny from one hermaphrodite 100% the same?**

Erik Jorgensen: These progenies are not true clones; they are not genetically identical to the mother because the germ cells go through meiosis and the chromosomes recombine (EFF:

https://en.wikipedia.org/wiki/Meiosis#/media/File:Meiosis_Stages.svg). Thus, the progeny may be homozygous for mutations which were heterozygous in the cloned adult.

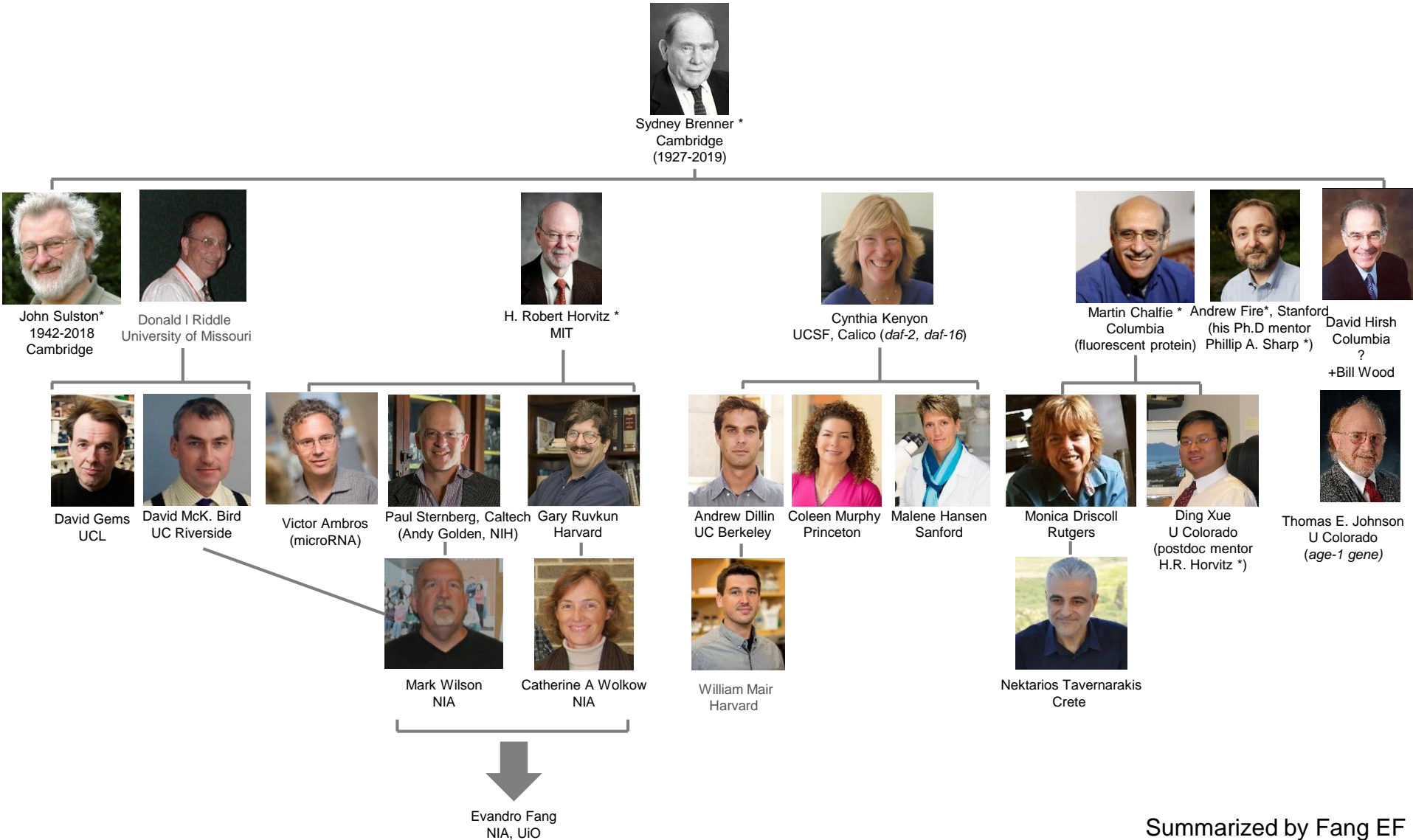
History of the *C. elegans* research

Key timelines in the *C. elegans* research history (with three nobel prizes)

- Developed by Sydney Brenner (1963)
- Mutants published by Brenner (1974)
- 1976 postembryonic cell lineages determined (Sulston and Horwitz)
- 1982 "Programmed cell death" (Horwitz et al.) (Nobel Prize Brenner, Sulston, Horwitz 2002)
- 1983 complete embryonic cell lineages determined (Deppe et al., Sulston et al.)
- 1986 Complete connectivity of nervous system established (White et al.) "The mind of a worm"
- 1991-98 RNAi and miRNA discovered in worms. (Nobel Prize: Fire, Mello 2006)
- 1994 - First use of GFP in animals (Nobel Prize: Chalfie, 2008)
- 1998 First animal genome sequenced (97Mb, now 100.3Mb)
 - This is about 1/30 the size of the human genome (3 Gb). *C. elegans* have about 20,000 coding sequence genes, more than half that of humans (30,000-40,000 genes). First animal to be sequenced! Knowing the sequence allows genes of interest to be easily cloned. Also it opened opportunity for reverse genetic approach.

Human: 20,000 genes, updated

The *C. elegans* pedigree trees (incomplete)



Summarized by Fang EF
*Nobel Laureates

Further readings

- **Thomas E. Johnson** 'the father of genetic research in aging'
<http://dose-response.org/2016-outstanding-career-achievement-thomas-e-johnson/>
- **Germline, meiosis, why 0.1% of males (Abby Dernburg)**
<https://www.youtube.com/watch?v=Z1A7bOPfcpk>

Quality control of *C. elegans* research: 101

1. When receive a new strain from CGC (if not back crossed), backcross with your N2 wild type worms 4-5 times (or at least 1 time, to be discussed with EFF). After careful genotyping and phenotype verification, freeze down **5-15** vials (depends on how often you use the worms). After 2-15 days, thaw one vial and see survival (this is to make sure the frozen worms can be **recovered—only the L1 population can survival**);
---alternatively, ask the N2 from the original lab which provides the mutant(s).
2. **Every 4-6 months, discard the worm plates you are culturing, thaw a new vial.**
3. Temperature control. Make sure to choose the best temperature for the maintenance of your worms
4. Avoid contamination among the many strains you are working daily.
5. IMPORTANT: if the worms are re-thawed, or are starved, you need to maintain them with sufficient food for at least 2-3 generations before you use for your experiments (to normalize starvation/stress-induced genetic changes). Please do not use the starvation-recovered worms to lay eggs and set up experiments right away; need 2-3 generations to normalize genetic changes/deacetylation+methylation.
6. Avoid strong external influences: e.g, no perfume and no loud music (earphones are recommended) when doing worm experiments
(EFF 2018-06-20)

Shipping worms: Declaration

I only had to declare what was in the package. Not the most ideal/honest declaration, but I usually just tend to declare the contents as "**plastic**" and it seemed to work fine so far.

- To declare as 'plastic', or you can also declare as 'NON-TOXIC, NON-PATHOGENIC, NEMATODE (C. ELEGANS)'
- To include a Letter for product clearance: non commercial product, research use only, 5 NOK. You can use the attached template as I used recently.

- **Mailing address:**

Tomas Schmauck (Evandro Fang lab)

Epi-Gen, B203Akershus University Hospital, 1478

Lørenskog, Norway

Office: +47-67963923; Cell: +47-92230968

FedEX account: 849781472

Questions to **Aili Nitteberg**

Clearance Broker

FedEx Express Europe

Group Tel +47-63870466

Customer Service Tel +47-63940300