In the news:

Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood

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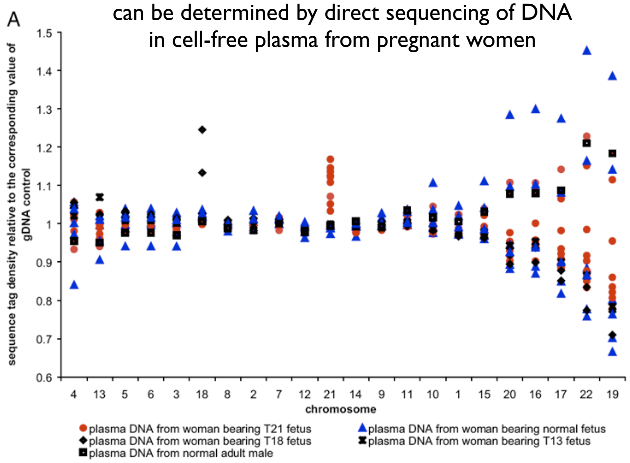
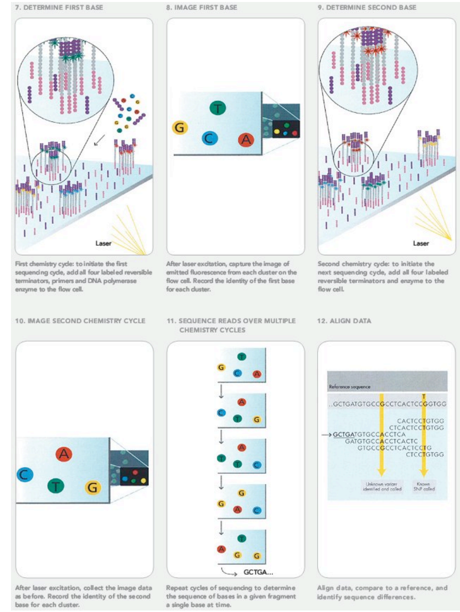
The copy number of each fetal chromosome can be determined by direct sequencing of DNA in cell-free plasma from pregnant women

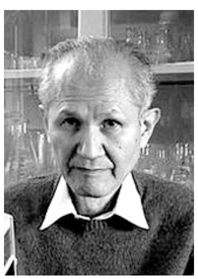




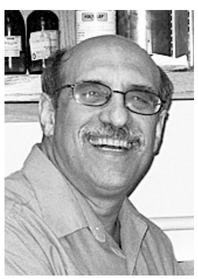
High-throughput sequencing using Solexa/Illumina technology

Confession:
I think I'm one of those people who's happy to use high-throughput sequencing without a complete understanding of how it works.

Today's Nobel Prize in Chemistry



Osamu Shimomura



Marty Chalfie

Roger Tsien

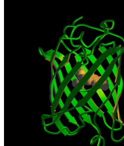
Green Fluorescent Protein (GFP)

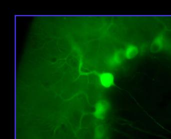
Comes from a jellyfish, *Aequorea victoria*

Gene has been cloned and transferred into a wide variety of "heterologous" expression systems

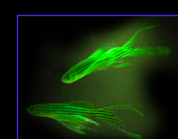
... including Drosophila, mammalian cells, *C. elegans*, yeast, zebrafish etc. etc.

**** Permits dynamic and *in vivo* analysis**** of biological processes





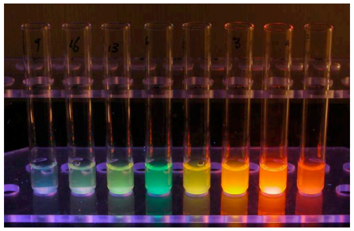
neurons

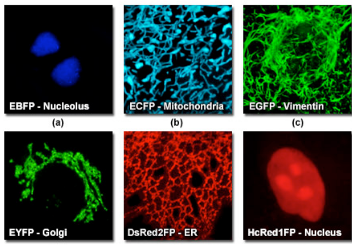


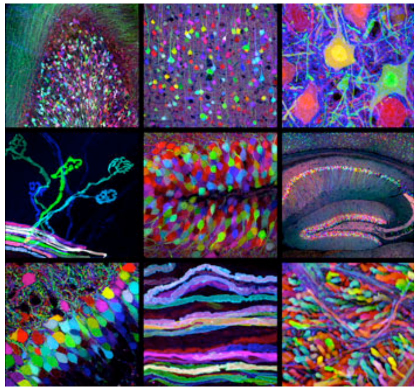
zebrafish

pigs!!!?

Variants of Green Fluorescent Protein and DsRed have been engineered to have different excitation and emission spectra, and other useful properties

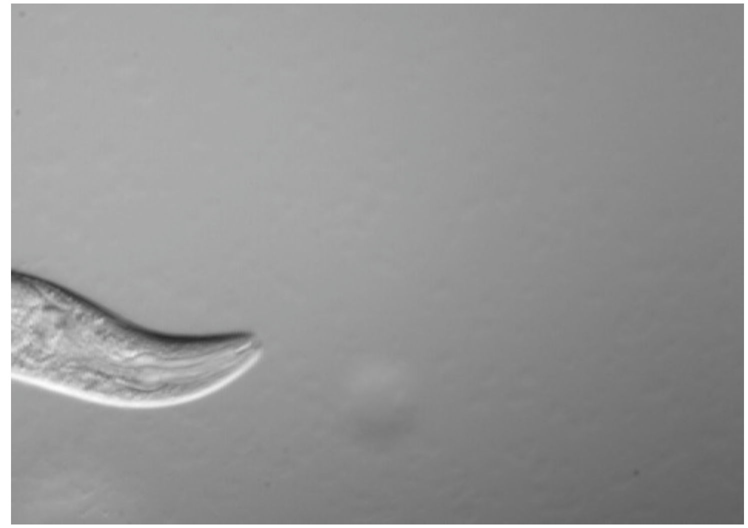






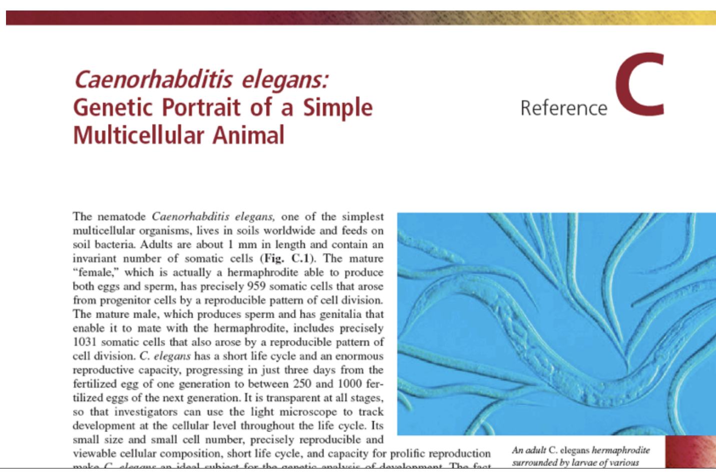
A "Brainbow" of possibilities

It's a bird! It's a plane! It's.... C. *elegans!*



Laboratory of Bob Goldstein, UNC

Reading: the Portrait chapter
(will be posted on the course website today)



Caenorhabditis elegans:
Genetic Portrait of a Simple Multicellular Animal

Reference C

The nematode *Caenorhabditis elegans*, one of the simplest multicellular organisms, lives in soils worldwide and feeds on soil bacteria. Adults are about 1 mm in length and contain an invariant number of somatic cells (**Fig. C.1**). The mature "female," which is actually a hermaphrodite able to produce both eggs and sperm, has precisely 959 somatic cells that arose from progenitor cells by a reproducible pattern of cell division. The mature male, which produces sperm and has genitalia that enable it to mate with the hermaphrodite, includes precisely 1031 somatic cells that also arose by a reproducible pattern of cell division. *C. elegans* has a short life cycle and an enormous reproductive capacity, progressing in just three days from the fertilized egg of one generation to between 250 and 1000 fertilized eggs of the next generation. It is transparent at all stages, so that investigators can use the light microscope to track development at the cellular level throughout the life cycle. Its small size and small cell number, precisely reproducible and viewable cellular composition, short life cycle, and capacity for prolific reproduction

An adult C. elegans hermaphrodite surrounded by larvae of various

Sidney Brenner

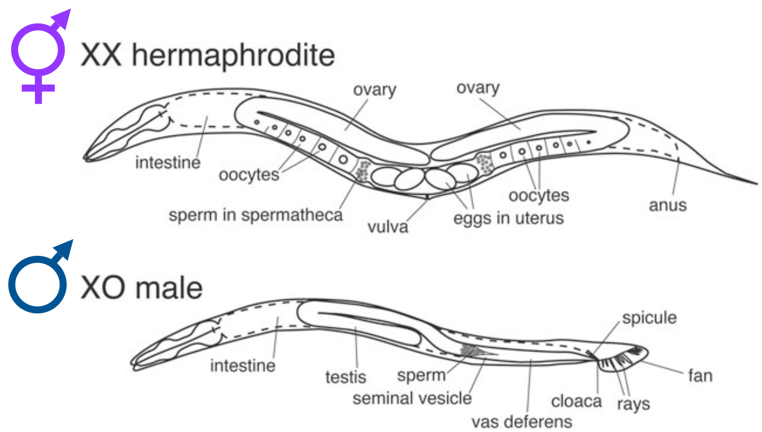
Using *C. elegans* as a genetic model system was this guy's idea

John Sulston

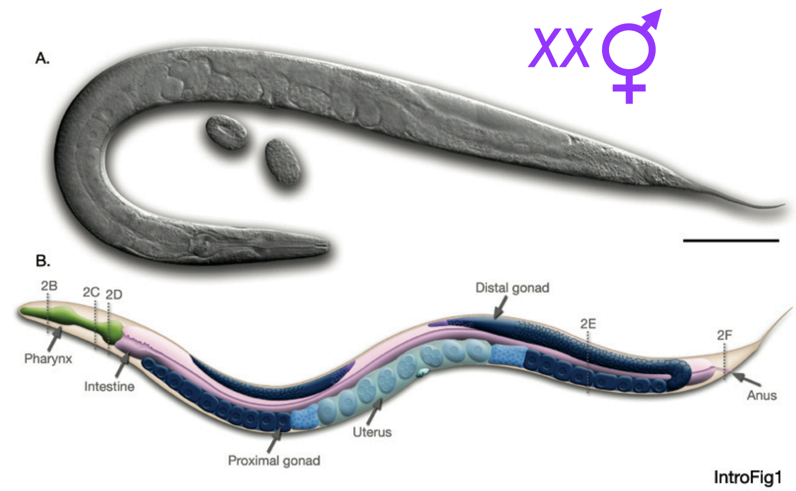
He shared the 2002 Nobel prize with these guys for working out the cell lineage and apoptosis

Bob Horvitz

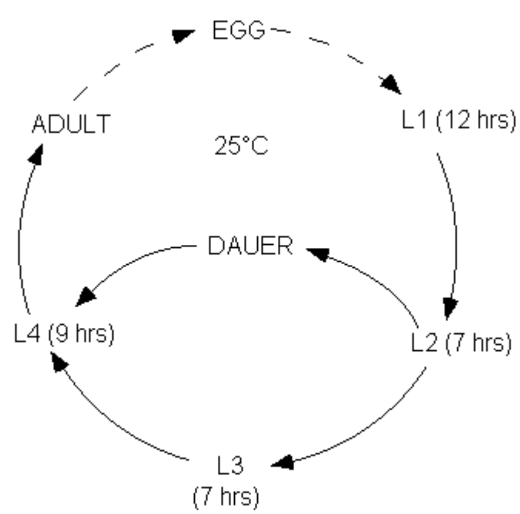
Anatomy of the worm



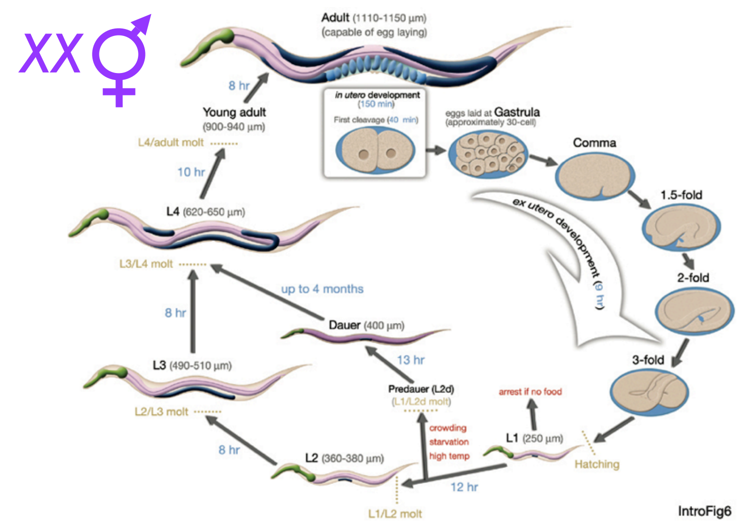
Anatomy of the worm



C. elegans Development



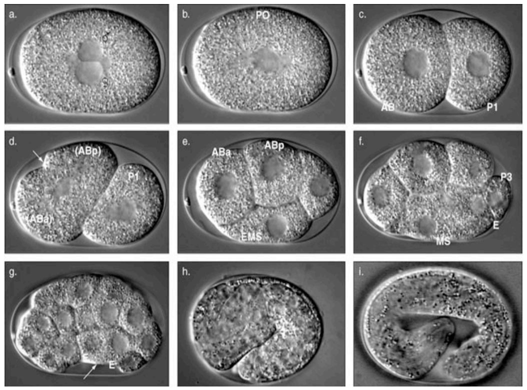
The worm life cycle: 3.5 days



Embryonic development

(takes ~24 hrs at 20˚C)

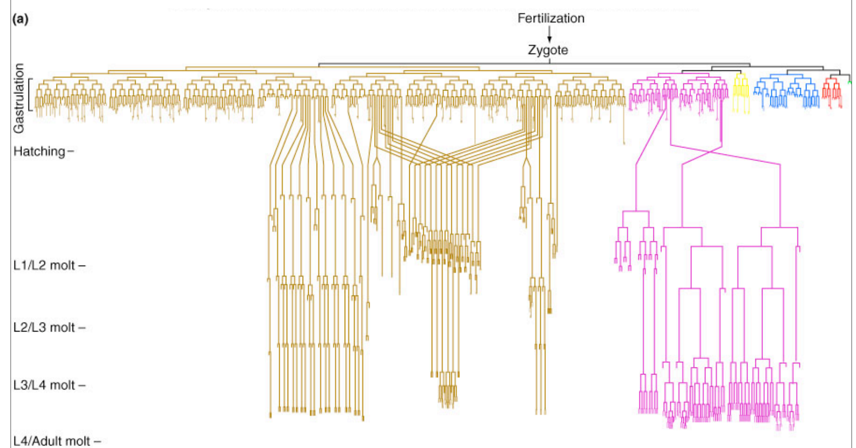
fusion of sperm and egg nuclei (karyogamy) | I[rst] mitotic division



"comma stage" embryo

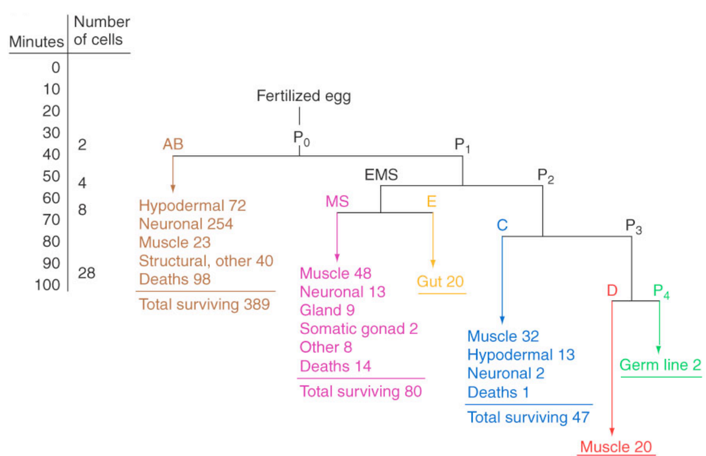
"pretzel stage" embryo (about to hatch as L1 larva)

C. elegans has an "invariant" cell lineage*

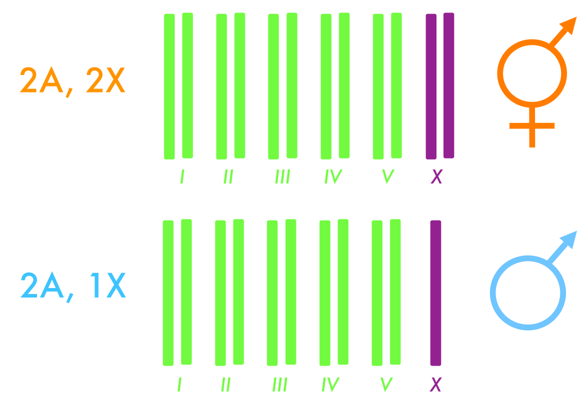


*No, you do not have to memorize it.

The earliest divisions give rise to many different tissues



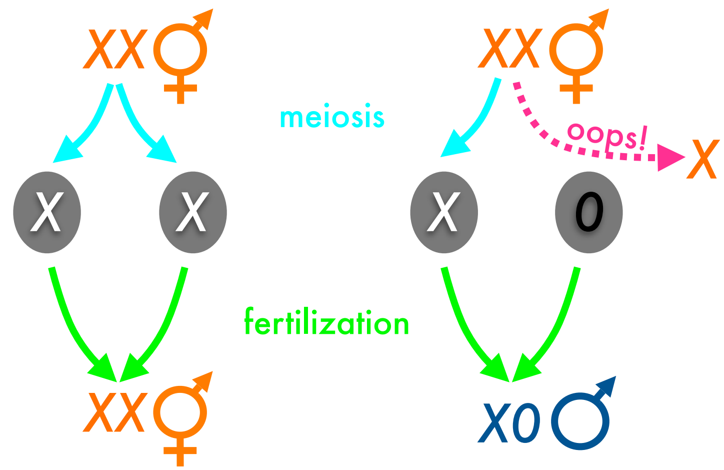
Sex determination in C. *elegans*: *XX* and *XO*



The chromosomes are drawn this way because they are "holocentric" (centromeres are distributed throughout).

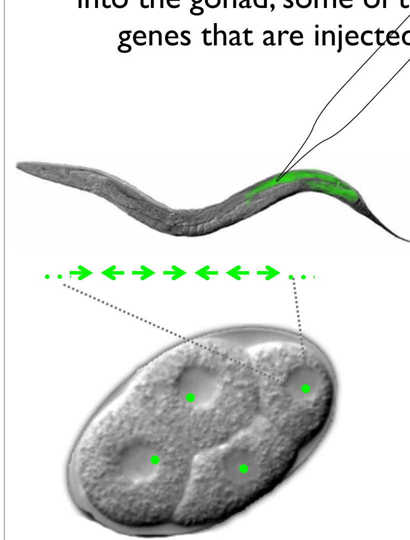
This is confusing at first when you think about meiosis, but you get used to it.

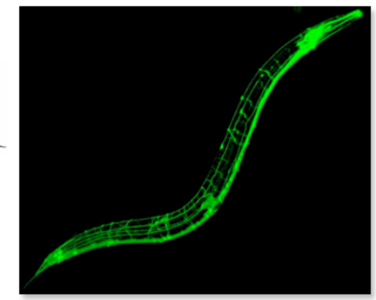
Where do *C. elegans* males come from?



Hodgkin, Horvitz, and Brenner (1979), *Genetics* **91**:67-94

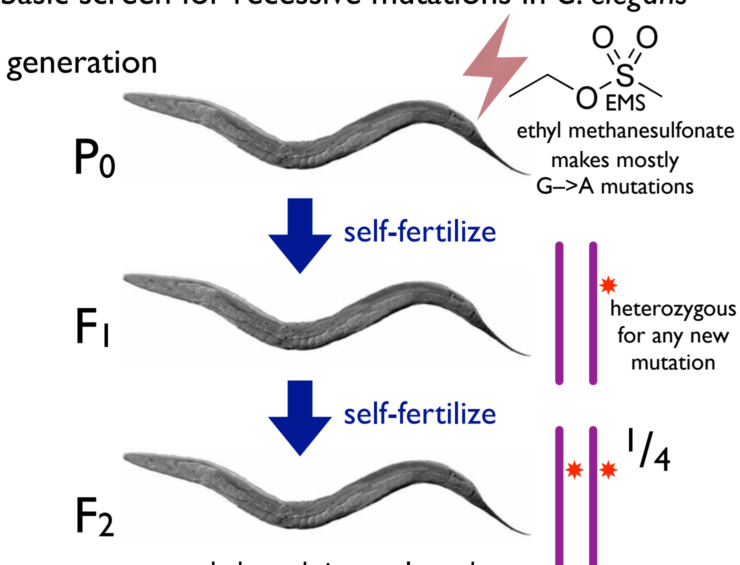
Transgenic C. elegans can be made by injecting DNA into the gonad; some of the progeny will carry the genes that are injected, in high copy number





worm expressing GFP in all cells of the nervous system

A basic screen for recessive mutations in *C. elegans*



total elapsed time: ~1 week.

Worms are simple creatures, and so many mutations cause the same general phenotype

Unc = Uncoordinated (aberrant or absent movement)

Dpy = Dumpy (short and/or fat)
(can result from hyperexpression of the *X* chromosome)

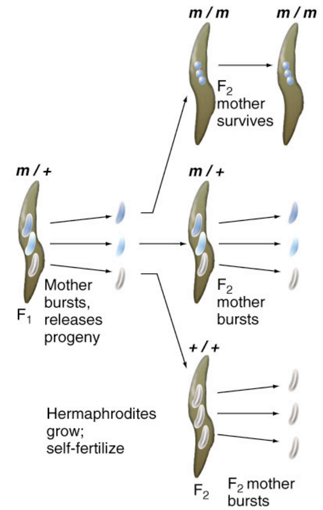
Let = Lethal

Emb = Embryonic lethal (also Zyg, for zygotic lethal)

Lon = Long and thin

Phenotypes are Capitalized (Unc), genes are *lower-case and italicised*, with 3 letters, a hyphen, and a number (*unc-51*), and the encoded proteins are ALL CAPS (UNC-51)

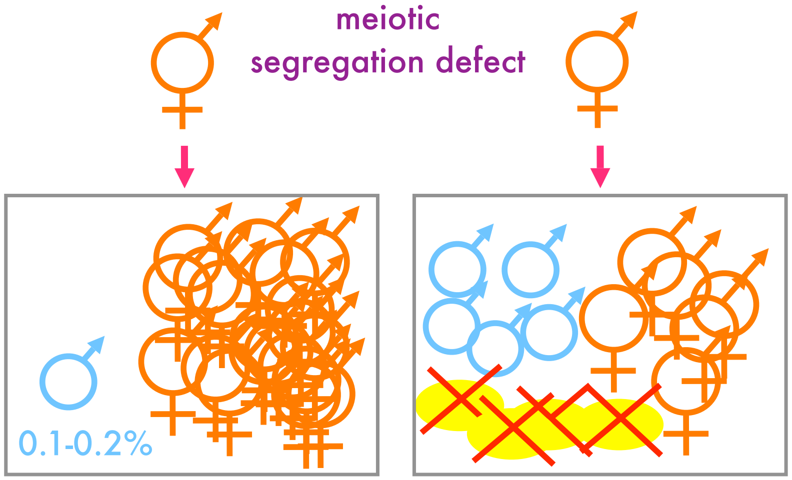
The "bag-of-worms" phenotype results from an inability to lay eggs (Egl - egg laying defective or Vul - vulvaless)



This (admittedly gross) phenomenon can be used to screen for "maternal effect lethals" (Mel mutants: homozygous mothers are o.k., but their embyros die.

One class of Mel mutants are severely defective in meiosis - they produce aneuploid embyros, which die.

The Him phenotype indicates a meiotic segregation defect



normal hermaphrodite

High incidence of males (Him)

Hodgkin, Horvitz, and Brenner (1979) *Genetics* 91: 67-94

A screen for recessive meiotic mutations in *C. elegans*

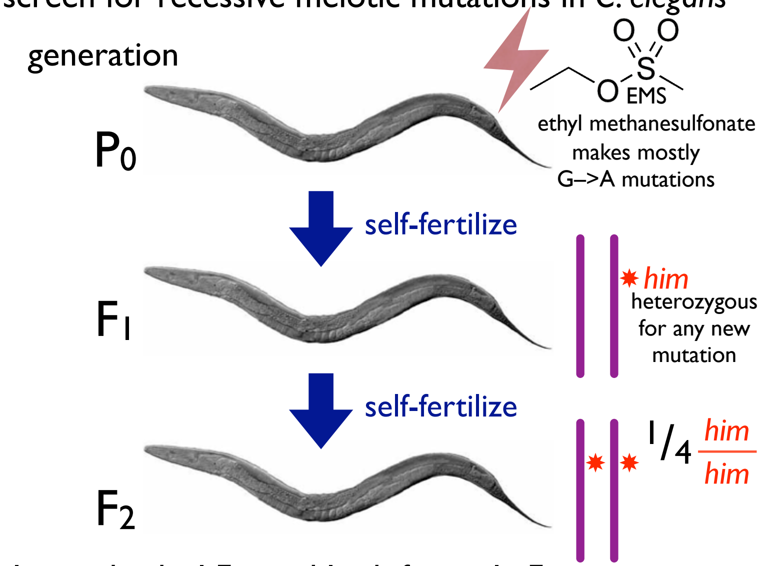
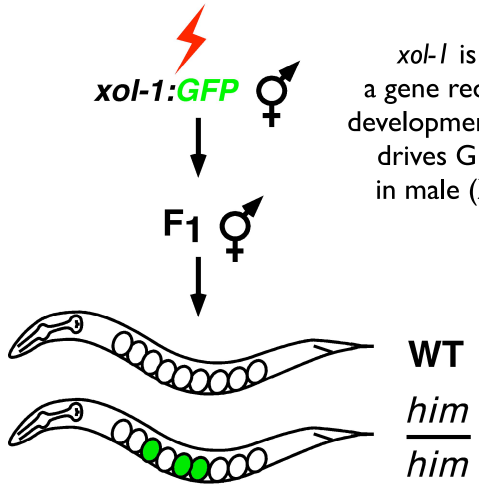
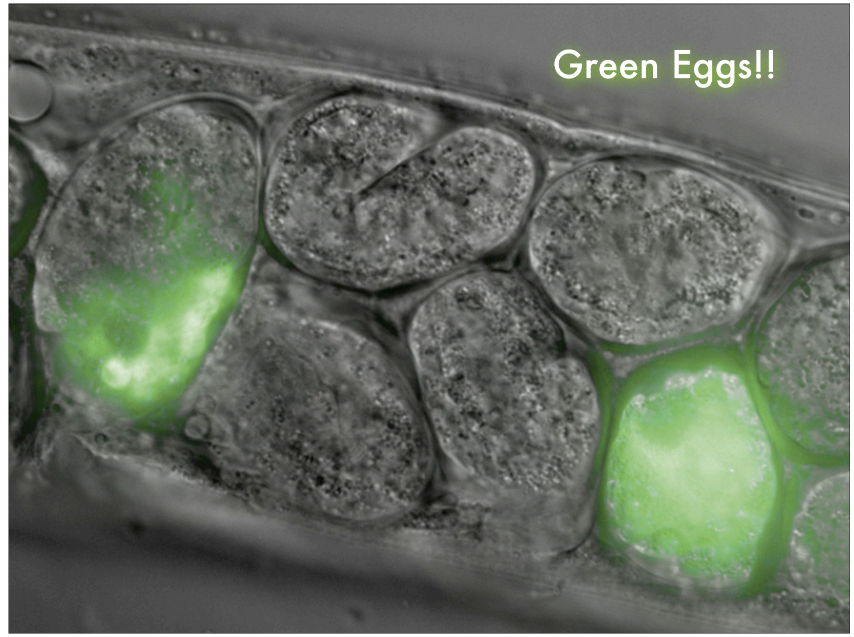


plate individual F2s and look for male F3 progeny

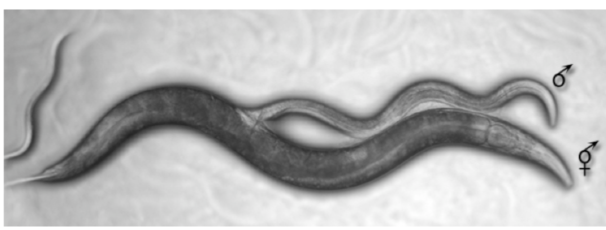
A simpler way to screen for meiotic mutants: look for the Him phenotype using the "Green Eggs and Him" trick



xol-1 is "*XO* lethal";
a gene required for male development. Its promoter drives GFP expression in male (XO) embryos



Anatomy of the worm



mating

Note: Mating requires a lot of activity on the part of the male, but is essentially a passive process from the perspective of the hermaphrodite... this means that some mutations (like Unc mutations, which compromise mobility) cannot be homozygous/hemizygous in the male.

So how do you tell the difference between self and cross progeny?

Dpy (*dpy-5 I*) ⚥ x WT ♂

↓

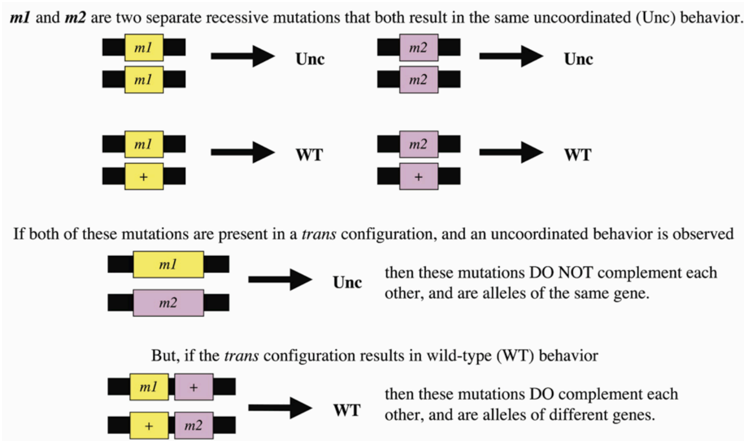
self progeny
Dpy ⚥ (plus the rare ♂)

OR

cross-progeny
50% nonDpy ⚥; 50% nonDpy ♂

Note: this example uses an autosomal *dpy* mutation. What would you expect if the *dpy* gene were on the *X* chromosome?

Complementation tests in *C. elegans* are straightforward



Note: failure to complement usually, *but not always* means that mutations affect the same gene. Conversely, complementation *usually but not always* means that mutations are in different genes.

Next lecture (Friday):
mapping genes in *C. elegans*
pathway analysis
how we sort out what meiotic genes do