

**CAENORHABDITIS ELEGANS AND MENDEL'S SECOND LAW**

*Independent assortment of gene pairs during gamete formation.*

**Reading assignment:** In your genetics text, review basic Mendel, probability and  $\chi^2$  (chi square) analysis.

**INTRODUCTION TO CAENORHABDITIS ELEGANS:** Over the past few decades, the free-living roundworm *Caenorhabditis elegans* has become a favorite model organism for the genetic analysis of biological processes.

*C. elegans* is an excellent organism for doing genetic analysis:

- (i) It has a very short generation time of 2.5 days at 25°C. The life cycle includes a period of embryogenesis and four larval stages.
- (ii) *C. elegans* adults are only about 1mm in length. Hundreds or thousands of them can be grown up on a single small Petri dish. (Their food source in the laboratory is the bacterium *Escherichia coli*.)
- (iii) *C. elegans* has a male sex and a hermaphrodite sex. The *C. elegans* hermaphrodite is essentially a female animal that also has the capability to make sperm and to fertilize its own eggs. Thus reproduction can occur by hermaphrodite self-fertilization (called self-progeny) or hermaphrodite-male mating (called cross-progeny). Mutant strains can easily be propagated by culturing hermaphrodites. Genetic crosses and experiments are performed by mating males and hermaphrodites.

A line drawing of the two sexes is shown on the next page. The adult hermaphrodite is larger than the male (it will be the largest animal on the plate) and will be full of brownish-colored eggs. Note it has a tapered whip-like tail. Adult males are thinner than hermaphrodites and have a very elaborate fan-like tail which functions in mating. One easy way to differentiate the two sexes is to look at their behavior. *C. elegans* males show a very distinctive mating behavior. (You will know it when you see it.)

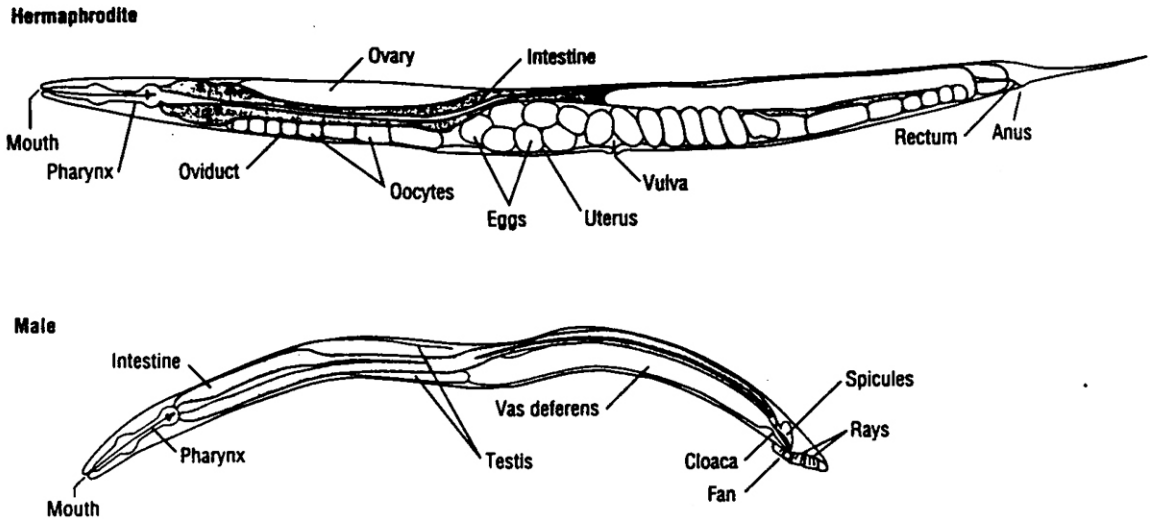
The general body plan of the nematode is in the form of two concentric tubes separated by a space called the pseudocoelom. The inner tube is the intestine; the outer tube consists of cuticle, hypodermis (essentially equivalent to epidermis), musculature and nerve cells. In the adult, the pseudocoelomic space also contains the tubular gonad. The shape of the worm is maintained by internal hydrostatic pressure controlled by an osmoregulatory system. *C. elegans* feeds through a muscular bilobed pharynx, which pumps fluid into the intestine and crushes ingested bacterial cells as they pass through the second lobe.

- (iv) *C. elegans* has a small genome size of 100,000kb ( $10^8$  base pairs per genome).

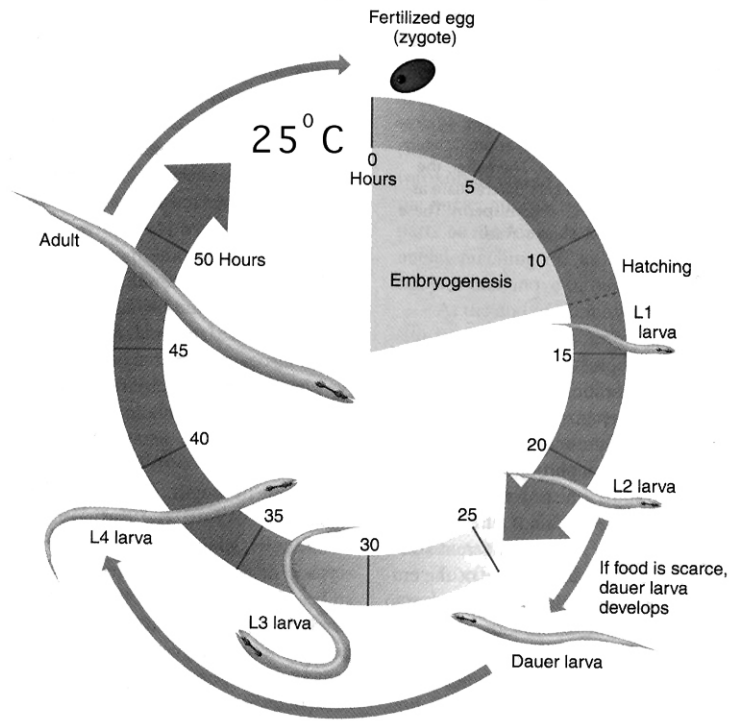
*The complete DNA sequence of the worm genome has been determined.*

For more information: <http://elegans.swmed.edu/>

# C. elegans General Anatomy.



## The C. elegans Life Cycle.



Growth Parameters of the *Caenorhabditis elegans* Life Cycle\*

Temperature (°C)	Embryogenesis (h)	Molts (h posthatch)				First eggs laid (h posthatch)
		L1-L2	L2-L3	L3-L4	L4 adult	
15	25 <sup>b</sup>	24	39	54.5	74.5	94-97
20	18 <sup>b</sup>	15	24	34	46	59-60
25	14	11.5	18.5	26	35.5	45-46

## Introduction to Mendel and *C. elegans*

When a new mutant strain is identified, the initial genetic analysis of such a strain always includes experiments designed to locate the mutation on the genetic map. Such an analysis is done by looking at the genetic behavior of the mutation relative to standard genetic markers on each linkage group (i.e. chromosome). If the new mutation and the particular genetic marker show independent assortment, then the genes are unlinked and the mutation is probably on a different chromosome from the marker. If the mutation and the marker do not show independent assortment, then the two are linked and the mutation is located on the same chromosome as the genetic marker. Further experiments would then be done to map the mutation more precisely relative to other genes on the same chromosome.

The wild-type *C. elegans* strain N2 is a diploid with  $n=5$  (4 pairs of autosomes and an X chromosome). There is no equivalent to the Y chromosome in this species. In this exercise we will use the mutants *dpy-11(e224)V* and *unc-32(e189)III* as \_\_\_\_\_ genetic markers in an exercise designed to illustrate Mendel's principle of independent segregation of gene pairs. These mutant alleles serve as genetic "tags" for chromosomes III and V.

### *C. elegans* Nomenclature:

In *C. elegans* nomenclature, gene names usually reflect the phenotype of mutations in the gene. For example, a gene defined by a mutation that results in a paralyzed or uncoordinated phenotype would be designated as an *unc* gene. The phenotype would be referred to as an Unc phenotype. Likewise, if mutations in a gene result in a short, fat or "dumpy" phenotype, the gene is called *dpy* and the phenotype is a Dpy phenotype. A strain containing both such mutations would have an Unc Dpy phenotype. The formal genetic nomenclature for such a strain that has a mutation in the *unc-32* gene and in the *dpy-11* gene would be:

*unc-32(e189) III; dpy-11(e224) V*

*e189* and *e224* are alleles of the *unc-32* and *dpy-11* genes, respectively. The *unc-32* gene is located on chromosome III and the *dpy-11* gene on chromosome V. The semi-colon is used when the two genes are on different chromosomes.

## Experimental Protocol:

Strains needed: N2 (wild-type)  
*unc-32(e189) III; dpy-11(e224) V*

### Generation:

**P** Cross N2 X *unc-32; dpy-11*  
Put cross at 16°C.

**F1 SELF** Pick wild-type (non-Unc; non-Dpy) cross-progeny L4  
Pick 4/plate X 2 plates  
(Do not transfer eggs and other larvae along with the L4 larvae. Why?)  
**Put plates at ~20 °C.**

*After ~48 hrs, remove the (now adult) parental hermaphrodites. (This step reduces the number of progeny on the plate and synchronizes the cultures somewhat.)*

***Place the plates in the 16°C. incubator***

*Note that these F1 heterozygotes that you have generated are equivalent to Mendel's F1 dihybrids.*

**F2** Score the phenotypes of the self progeny of the F1. You should observe 4 phenotypic categories. What are these categories and what genotypes do they correspond to?

- Score the phenotype of at least 200 adult progeny and record the number in each phenotypic class (*DATA SET I-personal*).
- Also record the combined class data (*DATA SET I-class*).
- Pick wild-type (non-Unc; non-Dpy) adult F2 progeny. (Do not transfer eggs and larvae along with the adults).
  - 1/plate X 6 plates. Place the plates at 16°C.

**F3** Score the progeny of these F2 hermaphrodites for the presence or absence of each phenotypic class (*DATA SET II-personal*). You do not need to count the number of animals in each class. (Why not?)

- Based on these observations, deduce the genotype of each F2 hermaphrodite.
- Collate your results and indicate the number of F2 hermaphrodites in each genotypic class. Also record the combined class data (*DATA SET II-class*).

## FORMAL LAB WRITE UP (due: 4/25/2012)

This lab requires a formal report that must include:

1. **An introductory statement** (of a sentence or two only) stating the purpose of this exercise. Don't just copy the introduction. State it in your own words.
2. The **complete genotype(s)** of the animals at each step and the **expected frequency of each genotypic and phenotypic class**. Be sure to define your allele symbols.
3. **Results:** Data Sets I and II (personal and class for both) should be clearly presented.
4. **Data Analysis:** State the expected results--phenotypic or genotypic ratios-- (assuming independent assortment) of these experiments. State the results you obtained. Do a chi-square test data sets I (class and personal) and data set II (class). Do not do a chi-square test on your personal data set II because this statistical test is not valid for this sample size. [No expected category should be less than 1 and no more than 20% of expected categories should be less than 5] Show your calculations. If your data deviate from the expected results, the chi-square test can tell you if your experimental data are statistically significant.

Your  $\chi^2$  calculations should be carried out to the four decimal places (including your expected values). The  $\chi^2$  value itself should be expressed to 3 decimal places -- reflecting the  $\chi^2$  values in the table.

### 5. Discussion/Conclusions:

- (iv) Briefly summarize your data and the results of the  $\chi^2$  analysis.
- (v) What do your data show?
- (vi) Be sure to **state in words what the p value means**.
- (vii) Can this statistical test **prove** that your hypothesis is true or false? Explain.
- (viii) How does sample size affect the analysis?
- (ix) Do your data deviate significantly from the class data or the expected results? If so, how can you explain this deviation?