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# Genetic Complementation Testing in *C. elegans*

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Complementation testing of alleles facilitates genetic identification of phenotypes. The nematode *Caenorhabditis elegans* rich history of genetic discovery due to its relatively short life cycle, ease of culturing, and a variety of available phenotypes. Many important genetic pathways were first elucidated in C. elegans, and later found to play critical roles in more complex metazoans. This laboratory will apply the technique of genetic complementation of alleles to up to four easy to score phenotypes. After completing the experiment, students analyze their data and present a poster of their results and interpretation of the data to their peers.

**Keywords**: *Caenorhabditis elegans,* complementation, genetics, presentation, nematode

## Introduction

Genetic inheritance is an essential component of understanding biology. In the classical forward genetic screen, organisms are mutagenized and then screened for particular phenotypes. On caveat of the mutagenesis screen protocol is that the genetic identity of the DNA lesion resulting in the phenotype is not known. Genetic mapping by recombination of chromosome during meiosis allows for the linkage to a chromosome and to a region of a chromosome to be obtained; however, it does not directly identify the gene involved in mutant phenotype. One technique to confirm a genes identity is complementation testing. Complementation testing of alleles facilitates genetic identification of phenotypes. The nematode *Caenorhabditis elegans* rich history of genetic discovery due to its relatively short life cycle, ease of culturing, and a variety of available phenotypes. Many important genetic pathways were first elucidated in *C. elegans*, and later found to play critical roles in more complex metazoans. This laboratory will apply the technique of genetic complementation of alleles to up to four easy to score phenotypes.

## Background Information

Logistically, *C. elegans* is small and easy to maintain; adults are 1 mm long, it is a harmless, free living, nematode, and it grows well at temperatures of 15-25˚C. Cultures of worms are easily maintained on small agar plates inoculated with a non-pathogenic, laboratory strain of *E. coli*. *C. elegans* can be viewed easily using a 40X stereo-zoom microscope, and the learning curve to effectively transfer individual worms between plates using platinum wire worm picks has been rapid with students.

Students will take the role of a *C. elegans* geneticist who has isolated a mutant worm line with a desired phenotype. Students will observe both wild type hermaphrodite and wild type male nematode plates, and then will be given a plate with a mutant phenotype to characterize. After carefully observing, sketching, and describing the worms, the student will perform a cross to determine if his or her worm line has mutant alleles in the same gene as three tester strains. This will be performed as a series of two crosses that address the low frequency of males in most *C. elegans* populations and the inability of many males with mutant phenotypes to mate effectively with hermaphrodites.

*C. elegans* are diploid nematodes with six pairs of chromosomes; XX animals are hermaphroditic and self-fertile while XO animals are male (Brenner, 1974). Normally *C. elegans* is a hermaphroditic strain, although males appear in the population by non-disjunction of sperm spontaneously at a rate of about 0.02%. Male sperm are larger than hermaphrodite sperm and always out-compete hermaphrodite sperm; therefore, a mating between a hermaphrodite and male results in all cross-progeny. A hermaphrodite mated with a male will produce 50% male offspring (Brenner, 1974). However, males are not maintained well in populations and generally a culture plate of worms will have few to no males. Males can be enriched in a population by heat-shocking L4 hermaphrodite larvae that are producing

sperm. Each hermaphrodite produces approximately 300 sperm during late larval development before switching to oogenesis for the remainder of her life.

The mutant phenotypes the students will be testing all interfere with the male's ability to mate, either by affecting the musculature or nervous system, or by affecting the mating structures such as the fan, hook, and sensory rays of the tail necessary for attachment to the hermaphrodite (Sulston et al., 1980). To remedy overcome this obstacle, students will mate a pure mutant line with another line that has a higher than normal frequency of males; mutations resulting in an increase in spontaneous male progeny are referred to as Him (High Incidence of *M*ales) (Hodgkin et al., 1979) The strain used in the this laboratory is CB4951, whose genotype is *spe-12(hc76) I; him-8(e1489) IV* (Table 1) (Wormbase.org). This is male/female strain which in which the *spe-12(hc76)* prevents self-fertility in hermaphrodites but does not impair spermatogenesis in males. Presence of *him-8(e1489)* increases male frequency by promoting non-disjunction of the X chromosome during spermatogenesis. The CB4951 strain is otherwise wild type for all other genes and behaves as a wild type worm.

Students will be given one of the mutant phenotype lines to test for complementation with three known strains. The phenotypes are Bli (blistered cuticle), Unc (uncoordinated movement), Coil (a type of Unc that coils like a snake), and Dpy (dumpy or short, fat worms). The strains, genotypes, and phenotypes are shown in Table 1. The phenotypes are described in the Student Outline and more information for each strain and genotype can be found by typing the URL with the strain name appending the address as shown for CB769: http://www.wormbase.org/species/c_elegans/strain/CB769. During week one, students will "mate" hermaphrodites of their unidentified mutant line with the males of the CB4951 strain to generate F1 generation worms that are heterozygous for the mutant phenotype. These worms will be carriers of the mutation and show the wild type phenotype, and should be half male and half hermaphrodite (Figure 1). The second week, students will mate their known carrier males with three different known strains that contain alleles resulting in the same phenotype. At the third week, students will score the progeny from these sets of three crosses and identify the strains that show complementation and non-complementation (Figure 2). The students prepare their data and present it as a poster to their peers in a future lab session.

## Time and Equipment Requirements

This laboratory requires two sets of genetic crosses of *C. elegans* and a session to record results; therefore, it requires three weeks to complete in a standard once a week laboratory setup. It would be possible to complete the lab in as little as two weeks if students met twice a week. In addition, this laboratory is much easier for the students if under-lit, minimum 30X magnification zoom stereomicroscopes of good quality are used. Moving microscopic worms with a platinum wire while observing through a microscope requires some practice; however, most people get the hang of it relatively quickly.

**Table 1.** Mutant phenotype strains used in this laboratory.

| Strain | Genotype | Phenotype |
|---|---|---|
| | **Bli** | **Blistered Cuticle** |
| CB769 | *bli-1(e769) II* | Blistered cuticle. Male tale abnormal |
| CB768 | *bli-2(e768) II* | Blistered cuticle |
| CB518 | *bli-5(e518) III* | Blistered cuticle. Small. Bursae abnormal |
| | **Coil** | **Coiler phenotype** |
| CB933 | *unc-17(e245) IV* | Coiler Unc. Severe; Small and thin |
| CB189 | *unc-32(e189) III* | Coiler Unc. Severe |
| CB262 | *unc-37(e262) I* | Coiler Unc. Severe |
| | **Dpy** | **Dumpy, Short Fat** |
| CB61 | *dpy-5(e61) I* | Strong dumpy; Early larvae are non-Dpy |
| CB364 | *dpy-18(e364) III* | Strong dumpy; Mapping marker standard. |
| CB27 | *dpy-3(e27) X* | Strong dumpy |
| | **Unc** | **Uncoordinated movement** |
| CB57 | *unc-14(e57) I* | Uncoordinated |
| CB4845 | *unc-119(e2498) III* | Small, Severely uncoordinated. |
| CB444 | *unc-52(e444) II* | Larvae move. Paralyzed adult. Dystrophic body muscle. |

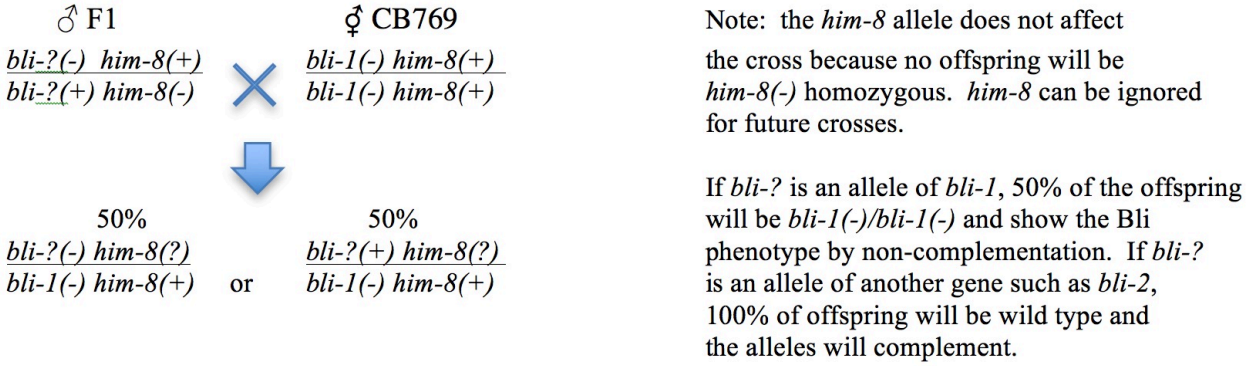


**Figure 1.** Sample cross I: Unknown Bli phenotype worm crossed with CB4951 male.

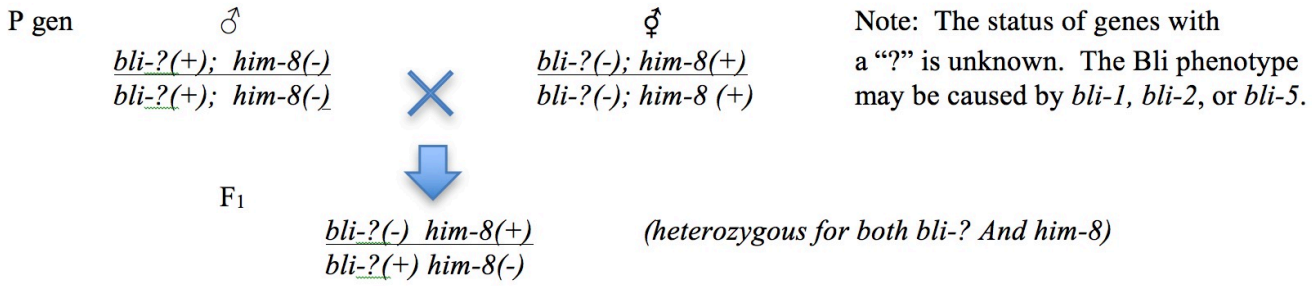


**Figure 2.** Sample cross II: Bli x CB4951 F1 progeny crossed with CB769.

# Student Outline

## Objectives

- Students will learn the life stages and sexing of *C. elegans*.
- Students will diagram a series of two genetic crosses with the goal of identifying if two strains with similar phenotypes are homozygous for the same allele for that trait.
- Students will collect data, analyze the data, and provide realistic conclusions from their experiment.
- Students will describe their results to an audience of their peers.

## Introduction

This laboratory investigation tests for complementation of one of four phenotypes in the free-living soil nematode, *Caenorhabditis elegans*. You will work with either worms that have blistered cuticles (Bli), coil back on themselves (Coil), are much shorter and fatter than normal (Dpy), or are slow and uncoordinated (Unc). No matter which type of worm you examine, you will follow the same procedures.

*Caenorhabditis elegan's* size, short life cycle, and ease of culture have made it one of biology's major model organisms. *C. elegans* is a hermaphroditic strain, meaning that the majority of worms produce sperm, followed by producing eggs (Figure 3). Most worms self-fertilize and produce about 300 offspring during their short lifespan. Occasionally, a hermaphrodite, which is XX, will generate a sperm without an X chromosome by non-disjunction during meiosis. This sperm will fertilize an oocyte to produce an XO zygote that will develop into a male (Figure 4). The presence of males allows for cross progeny. Males are extremely rare in normal populations of worms; however, alleles of genes involved in meiosis exist that can increase the number of XO sperm and therefore the percentage of male progeny in unmated hermaphrodites.

A fertilized *C. elegans* egg will develop in 2 - 3 days into a feeding larva. *C. elegans* eats bacteria and grows rapidly. The larva molt four times before assuming the final reproductive adult form. These larval stages are named L1, L2, L3, and L4 (Figure 5). Under times of stress such as population crowding, high temperatures, and low availability of food, developing L1 larvae may bypass the L2 and L3 stages by an alternative molt into a dauer. A dauer can survive much harsher environmental conditions. Adults live for approximately two weeks under normal conditions.

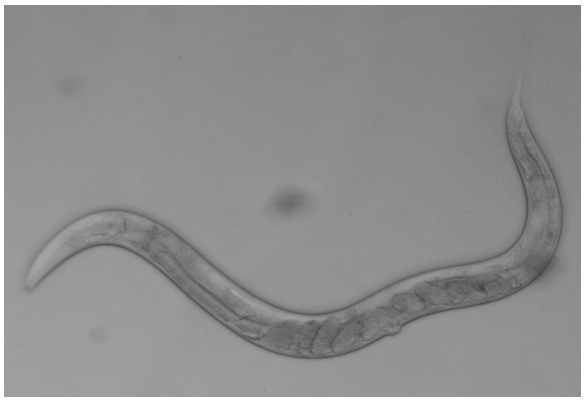

**Figure 3.** *C. elegans* Hermaphrodite

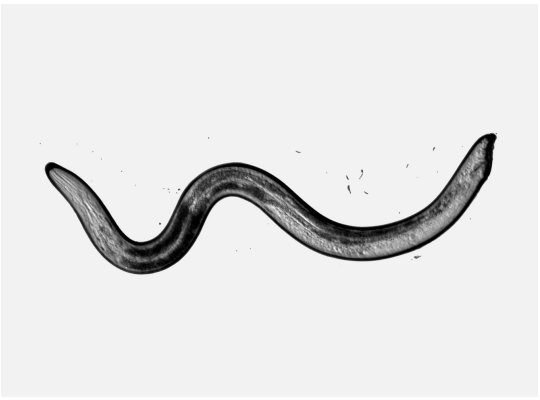

**Figure 4.** *C. elegans* Male

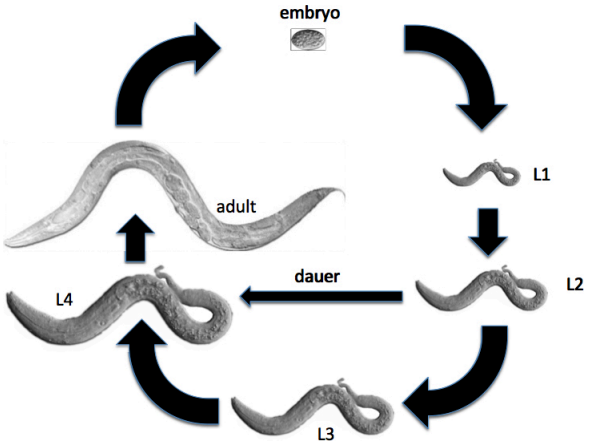


**Figure 5.** *C. elegans* Life Cycle

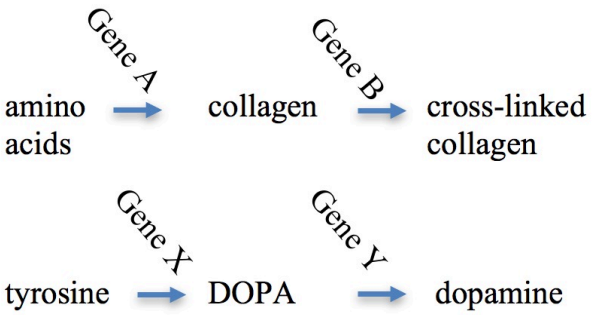


**Figure 6.** Genetic pathways.

The activity of more than one gene is often responsible for phenotypes, such as the formation of stable collagen in the skin, or synthesis of a neurotransmitter (Figure 6). For example, if either Gene A or Gene B are homozygous for a loss-of-function allele, crossed linked collagen will not be present in the skin. However, mutations in Gene X and gene Y will not affect collagen. Complementation occurs when a worm homozygous for a loss of function allele of Gene A is mated with a worm homozygous for a loss of function allele for Gene B. While neither parents could complete the synthesis pathway, all of the F1 progeny will be heterozygous for both Gene A and Gene B, and therefore will appear wild-type. Most structure and functions are the culmination of many different proteins, chemical reactions, and signaling steps, each which must function correctly for the end phenotype to be normal. In this laboratory, you will test for complementation of a mutant phenotype of an unknown genetic source with three phenotypically similar strains of known identity. In this laboratory you will receive one *C. elegans* culture with either a Bli, Coil, Dpy, or Unc phenotype to test for complementation with three *C. elegans* cultures of the same phenotype over a three-week period. The phenotypes are described in Table 2.

**Table 2.** Description of phenotypes used in this laboratory.

| **Phenotype** | **Description of Phenotype** |
|---|---|
| *Blistered phenotypes* | The skin, or cuticle of *C. elegans* is composed of numerous proteins, including collagens just like out skin. When the cuticle of *C. elegans* is abnormal, it forms fluid filled sacs that look like blisters, which we call a blistered (Bli) phenotype. We will be examining multiple *bli* gene sand testing alleles of it and other cuticle formation genes. |
| *Coiler phenotypes* | The normal movement of the worm requires neurons to signal to muscles in the worm's body. If neurons do not connect to the correct muscles, specific neurotransmitters are absent, or receptors do not function, animal movement may be disabled. The coiler phenotype prevents the forward locomotion of the animal, and instead, the worm coils back onto itself. |
| *Dumpy phenotypes* | The worm's exterior structure is a result of body muscles, collagens, and epithelial cells. If any of these does not function correctly, a worm may not elongate to its normal long, thin form. Dumpy worms are generally short and fatter than normal worms; however, their movement is rarely affected by the rounder shape. |
| *Uncoordinated phenotypes* | Uncoordinated worms generally have defects in their nervous or muscular system and are slow or paralyzed. The Unc phenotype is very common; a result of the vast number of genes necessary for proper muscular-nervous system integration. |

## Methods and Data Collection

*Materials*

- Platinum wire worm pick
- Alcohol lamps
- Worm strains
- N2 (wild type)
- CB4951 (Him strain, male enriched)
- Unknown strain A, B, C, or D
- Unknown strain A, B, C, or D plus CB4951
- Fresh NGM agar plates (60x15mm) inoculated with *E. coli*
- Zoom stereo microscopes with at least 30X magnification
- Plastic boxes for plate storage

*Week 1:*

Skills of the Worm Picker

1. Locate a plate of N2 strain worms. These are the wild type strain used by laboratories all over the world.
2. Mark one of your unlabeled plates with your initials and "N2 test." It is best to use small letters along the edge of the bottom of the plate when labeling. Mark the other plate "Sticky Food."
3. Sterilize the platinum wire worm pick using the flame of an alcohol lamp. The tip of the wire should briefly glow orange/red. Remove the pick from the flame; it generally cools completely in a matter of seconds. Be careful with the worm pick; it is very fragile.
4. Very briefly flame the worm pick in a lit alcohol lamp. Only the tip of the wire needs to glow.
5. Carefully scrape some of the bacteria from the "Sticky Food" plate onto the tip of the worm pick, being careful not to break the surface of the agar. This bacteria will act as an adhesive for the next step; therefore, it is often referred to as "sticky" bacteria.
6. Place the plate of N2 worms under the microscope, remove the lid and locate and gently touch the tip of the worm pick (covered in sticky food) to the worm. The worm should adhere to the worm pick.
7. Quickly replace the lid of the N2 plate, and move the "N2 Test" plate under the microscope, taking off the lid.
8. Using the stereomicroscope, gently place the tip of the pick on the agar surface of the destination plate. Do not try to force the worm off, rather, hold the pick still, enabling the worm to crawl off by creating a continuous surface between the sticky food on the pick and the agar surface.
9. Briefly flame your worm pick and repeat multiple times until you feel comfortably locating and moving worms.
10. Have your instructor verify that your worms are successfully transferred.

*C. elegans* Cross, Preparing the First Cross

1. You will be provided with three plates of worms.
    a. One plate will be of CB4951 Him worms, which are highly enriched with males
    b. Another plate will have a *C. elegans* mutant phenotype A, B, C, or D. Write that letter here _____ and in the remaining blanks of this procedure
    c. The last plate will be a combination of Him worms and the same mutant phenotype worms as above (A, B, C, or D) _____ that have been allowed to mate overnight.
2. View the plate of Him worms to identify the phenotype of males. Sketch and describe the male anatomy and movement in your notes.
3. View the plate of _____ mutants under the microscope. Sketch and describe the anatomy and movement of the mutant strain.
4. Label four of your unlabeled worm plates with your initials, the date, and the phrase "_____ x CB4951 Him Cross" on the bottom surface of the plate, not the lid.
5. Place the premixed plate of Him worms and your mutant phenotype ("CB4951 Him x ____) under the microscope and search for a healthy adult hermaphrodite mutant phenotype worm.
6. Use your worm pick to transfer the worm to one of your labeled plates.

7. Repeat this three more times so that all four plates have one healthy adult mutant phenotype worm. (If a worm appears damaged, add a second worm to the plate)
8. Place worms in a plastic box to be incubated at 13˚C until next week.

*Week 2*

1. Check your "CB4951 Him x ____" mutant worm crosses.
    a. For each cross that was successful, you see many young male worms on the plate.
    b. All worms should appear wild type.
2. Set up six plates labeled on the bottom with the date and your initials.
3. Divide these six plates into three sets of two and label each set as follows: "Comp Test 1", "Comp Test 2", and "Comp Test 3" as referenced in Table 3.
4. Use Table 3 to determine the strains you will need for your phenotype. Set up crosses by placing worms on the plates as follows:
    a. Place two healthy hermaphrodite worms from Strain 1 of your phenotype listed in Table S2 on each of the two plates labeled Comp Test plate 1
    b. Place two healthy hermaphrodite worms from Strain 2 of your phenotype listed in Table S2 on each of the two plates labeled Comp Test plate 2
    c. Place two healthy hermaphrodite worms from Strain 3 of your phenotype listed in Table S2 on each of the two plates labeled Comp Test plate 3
5. Place as many as five males from your successful "CB4951 Him x ____" cross plates on each of the six plates from step 4.
6. Place worms in your plastic box to be incubated at 13˚C until next week.

**Table 3.** Strains available with the Bli, Coil, Dpy, and Unc phenotypes.

| Strain | A - Bli | B - Coil | C - Dpy | D - Unc |
|---|---|---|---|---|
| **1** | CB518 / *bli-5* | CB189 / *unc-32* | CB27 / *dpy-3* | CB57 / *unc-14* |
| **2** | CB768 / *bli-2* | CB262 / *unc-37* | CB61 / *dpy-5* | CB444 / *unc-52* |
| **3** | CB769 / *bli-1* | CB933 / *unc-17* | CB364 / *dpy-18* | CB4845 / *unc-119* |

*Week 3*

1. Observe Each test plate from last week
    a. Each plate should have ~ 50% male worms if the mating was successful.
    b. Determine if any worms on the plate have the phenotype
2. For each plate successfully mated plate, score the number and types of progeny.
3. Determine the percentage of wild type to mutant progeny for plates where male offspring are present.
4. A strain has an allele that shows complementation when all offspring appear wild type. This means that the genes involved in your unknown strain and the tester strain are different.
5. A strain has an allele that does not complement when a proportion of the offspring show the original mutant phenotype. This should be at least 25%, but up to 50% of the offspring. This means that the two phenotypes are the result of alleles of the same gene. This identifies your unknown as a mutant of this gene.

## Poster of Results

You will create a research poster describing your experiment and the genetics that result in phenotype all three of your strains exhibit. You should use sources such as WormBase: http://wormbase.org, WormBook: http://wormbook.org, and Wormatlas: http://wormatlas.org.

*General Guidelines for Poster Format*

1. *Title and Headings* -- Include a large title that identifies your topic and suggests the theme that you are exploring for the poster. Include headings for each of the major sections of information that you intend to discuss on your poster.

2. *Content* -- Provide background information about *C. elegans*, your phenotype, and the genes you tested for complementation in an appropriate context.
3. *Color* – Use color effectively. Avoid distracting backgrounds and clashing color schemes. Coordinating different sections of your poster with color is okay.
4. *Text* -- Use large readable fonts for your poster (at least 16 point). Keep text to a minimum. Use bullet points or short sentences to convey concepts on your topic. Only include the key points on your poster, your will fill in the details when you talk about the poster. Explain terms that might be unfamiliar to your audience. Remember that your poster is also a visual tool for you to explain your topic to the listener.
5. *Organization* -- Organize your poster in a manner that is easy to understand and follow.
6. *Figures* -- Use figures to enhance the features of your topic and the physiological principles. Prepare figure legends to describe how the figure supports the information about your topic. If you use a figure from an outside source, be sure to cite the source.
7. *References* – Include a reference section that lists all of the sources that you used for the development of your poster. In the text of your poster, make sure to include the references cited for your material.
8. *Space* - Use your poster space widely. You should not leave large amounts of "white" space; however, it is important for the poster to be pleasing and not overcrowded.
9. *Grammar* – Avoid grammar mistakes and misspelled words.

### *Sections of Poster Content*

Information should be subdivided into sections:

1. Introduction – Description of *C. elegans*, your phenotype, and the genes you tested.
2. Terminology Used - List any special words or concepts used in your topic.
3. Your experimental design. Show figures describing and diagraming your crosses with appropriate genetic nomenclature. Describe the expected results for the experiment for genes that complement the unknown strain and for genes that do not complement.
4. The results of your crosses in table format showing numbers and percentages of phenotypes.
5. Conclusions and Discussion – How is studying these genes relevant to other fields of biology?
6. References

## ***C. elegans*** **Notes for Students:**

### *Genetic Nomenclature*

A *C. elegans* strain name consists of one to three CAPITAL letters followed by a number. This identifies the laboratory that created the strain (a genetically distinct and defined line of worms). For example, PAS77 denotes the 77th strain that Pliny Smith's lab created.

A *C. elegans* gene is denoted by three letters followed by a dash and a number. Generally, the three letters are an abbreviation or an acronym for a phenotype or protein. Because a gene may have more than one allele, there is often included an allele designation. This code consists of one to three lowercase letters followed by a number that identifies the alleles source. For example, *dpy-17(e164)* represents the 164 allele isolated by Jonathan Hodgkin's laboratory (*e*).

When listed the genotype of a worm strain, the alleles are listed, starting from the left arm to the right arm of each chromosome, which is denoted by a Roman numeral following the alleles as in the following example: *unc-4(e120) bli-1(e769) II.* Heterozygous strains would add a slash mark describing the alternate alleles on the homologous chromosome. Capitalize the first letter of a phenotype description and do not use italics. For example, Uncoordinated animals such as those with a homozygous *unc-32* genotype would display an Unc phenotype.

### *Types of Mutations*

Below is a list of some common classes of mutations of *C. elegans.*

- ***dpy*** Produces a "dumpy" (short and fat) phenotype. Different *dpy* mutants have characteristic shapes from "football" to slightly fat and may be dominant or recessive.
- ***unc*** Uncoordinated movement. Unc mutants do not crawl the same as wild type, but this may vary from total paralysis, constant coiling of their tails, a loss of touch response, or the inability to back up. We will use the term Coil for Unc mutants that coil their tails.

- ***bli*** Blister phenotype. Adult animals have defects in their skin (cuticle) resulting in a bubbled or blistered appearance.
- ***him*** High incidence of males. Chromosomal non-disjunction of the X chromosome occurs more frequently than in wild type, resulting in an increased number of spontaneous males.

### *Contamination*

Care should be taken to prevent foreign material from contacting the worm plates or bacterial or fungal contamination can occur. Bacteria other than the normally used OP50 strain will make it more difficult to see the worms. Fungus will decrease the fertility, survival of the worms and obstruct microscopic observation as well. Sterile technique should always be observed when working with *C. elegans*.

# Materials

## Materials and Equipment for Students

- 12 Zoom stereo microscopes with at least 30X magnification
- 15˚C Incubator
- 12 Plastic boxes for student experiment storage (one box per pair)
- 12 Worm platinum-wire worm picks
- 12 Alcohol lamps
- Worm strain culture plates
- NGM agar plates (60 mm) inoculated with 250 uL of OP50 bacterial solution (see instructor materials for numbers)

## Materials and Equipment for Instructor Set Up

- 1 Zoom stereo microscopes with at least 30X magnification
- 13˚C Incubator
- 20˚C Incubator (useful for staging worms, not necessary)
- 25˚C Incubator (useful for staging worms, not necessary)
- Multiple plastic boxes for plate and strain storage
- Parafilm
- 2 feet Platinum/iridium wire (worm picks may be used by multiple lab sections; therefore, 2 feet may accommodate multiple lab sections)
- 1 Box standard glass Pasteur pipettes
- 1 Alcohol lamp
- 1 Bunsen burner
- Sterile transfer pipettes (1 mL with 0.1 ml gradations) or P-200 and P-1000 pipetters with sterile tips
- 14 Worm strains
- OP50 *E. coli* strain
- NGM agar plates (60 mm) inoculated with 250 uL of OP50 bacterial solution (see instructor notes for number)

# Notes for the Instructor

The set-up of this experiment assumes at least 2-hour lab sessions spaced once per week. Therefore, the experiment requires three lab periods.

### *Worm Cultures*

*C. elegans* reproduce and grow rapidly; always have plenty of worm plates prepared and ready for use. Table I3 is a guideline of the minimum number of plates needed for the lab. Extra plates may be required if cultures show fungal contamination or if mistakes occur. We use a Tritech Research PourBoy 4 automatic plate-pouring machine to ensure our plates have consistent volumes of media and to decrease the incidence of contamination. Plates should be allowed to sit 24 hours before being seeded with OP50 bacteria, and then should be left at room temperature for another few days to allow the bacteria solution to dry.

Always use sterile techniques to prevent the introduction of non-OP50 bacteria or fungal species to the worm cultures. Worm cultures may be cleaned by a bleaching protocol; however, this takes time. Two guides for removing contamination are provided in Appendix A.

### *Equipment*

Good-quality stereomicroscopes with a bright light source and under stage lighting is extremely helpful in observing the worms. We use Leica S6E, MZ6, and GZ6 series microscopes with an under stage rotating mirrors and fiber-optic light source to direct the light for the best contrast of the worms. The procedure will work with less-capable microscopes; however, it will be more difficult for students to observe the worms.

### *Picking Worms*

Students generally learn to pick worms with a platinum wire pick within an hour. In this protocol, students will only need to pick worms and transfer them to a new plate as an exercise; it is not essential to the working of the experiment. It is important to understand worms will stick to the semi-desiccated bacterial glob on the end of the pick, rather than the platinum wire itself.

### *Safety*

1. The *C. elegans* strains are non-pathogenic. They will not survive on skin or lab bench.
2. The OP50 *E. coli* strain is a non-pathogenic lab strain auxotrophic for uracil.
3. Care should be taken when students are using alcohol lamps to prevent unintentional burns or fire.
4. Used plates should be sterilized by autoclaving before disposal.

## Other Resources

**WormBase:**

http://wormbase.org/

WormBase is a publically funded, online database of nematode genomics, literature, reagents, and

anything else worm related. WormBase can be used to research the phenotypes of alleles that students are investigating.

**WormBook:**
http://wormbook.org/
Contains chapters concerning *C. elegans* development, genetics, cell biology, evolution, as well as worm methods and protocols (http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html)

**Wormatlas:**
http://wormatlas.org/
A free source of diagrams, descriptions, and photographs of worm anatomy.

***C. elegans* Genetic Center (CGC):**
http://www.cbs.umn.edu/CGC/
Source of worm and bacterial strains used in *C. elegans* labs.

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## About the Author


Pliny A. Smith obtained his B.A. in Biology from Grinnell College in 1992 and his Ph.D. from the University of Missouri in 2001. He is an assistant professor in the Biology Department of Dominican University in River Forest, IL. Pliny teaches Developmental Biology, Genetics, Organism Biology,

and the Biology of Aging. He uses *C. elegans* both in the classroom as well as to train undergraduates to carry out original research.

# Appendix A
# Lab Set-Up Protocols

## Creating a Worm Pick

1. Cut a 1.0 cm piece of platinum/iridium wire (Ladd Research, part #30575).
2. Insert 1/3 of the wire into a shortened Pasteur pipette, holding the wire in place with a pair of forceps.
3. Place the end of the pipette containing the wire into a gas flame and twist the wire with the forceps to melt the glass and close the end.
4. Let the pick cool and flatten and bend the end of the wire to form a scoop.

## Obtaining Worms for the Experiment

*C. elegans* stocks may be obtained from the *C. elegans* Genetics Center (CGC) at the University of Minnesota (see Appendix B). For this lab, the strains listed in Table 4 were used.

**Table 4.** Strains used in this laboratory.

| Strain | Genotype |
|---|---|
| N2 | *C. elegans* wild isolate. (Brenner, 1974) |
| CB4951 | *spe-12(hc76) I; him-8(e1489) IV (Nance et al., 1999) (Phillips et al., 2005)* |
| CB769 | *bli-1(e769) II (Johnstone, 2000)* |
| CB768 | *bli-2(e768) II (Johnstone, 2000)* |
| CB518 | *bli-5(e518) III (Hodgkin, 1983)* |
| CB933 | *unc-17(e245) IV (Alfonso et al., 1993)* |
| CB189 | *unc-32(e189) III (Pujol et al., 2001)* |
| CB262 | *unc-37(e262) I (Flowers et al., 2010)* |
| CB61 | *dpy-5(e61) I (Thacker et al., 2006)* |
| CB364 | *dpy-18(e364) III (Hill et al., 2000)* |
| CB27 | *dpy-3(e27) X* |
| CB57 | *unc-14(e57) I (Ogura et al., 1997)* |
| CB4845 | *unc-119(e2498) III (Maduro and Pilgrim, 1995)* |
| CB444 | *unc-52(e444) II (Rogalski et al., 1995)* |
| OP50 | *E. coli* bacterial strain used for food |

To view information about the strain on the online database Wormbase, you may create a URL as follows:
In this example, CB4951 is the strain name to be recalled.

## Growing Worms

*6 Weeks Pre-Lab:*

1. Order worm strains from CGC if necessary (see appendix).
2. Prepare NGM agar plates for the experiment using the chart (Table 5) as a guide to the number of plates needed per set of eight students:

**Table 5.** Number of plates need per set of eight students.

| | Plates Required | | | |
|---|---|---|---|---|
| | **8 students** | **16 students** | **24 students** | **32 students** |
| 3.5 weeks pre-lab | 28 | 28 | 28 | 28 |
| 2 weeks pre-lab | 28 | 28 | 28 | 28 |
| 2 week cultures | 16 | 32 | 48 | 64 |
| 2 day cultures (week 1) | 16 | 32 | 48 | 64 |
| Student's plates for week 1 | 16 | 32 | 48 | 64 |
| 2 day cultures (week 2) | 12 | 12 | 12 | 12 |
| Student's plates for week 2 | 16 | 32 | 48 | 64 |
| Total plates: | 132 | 196 | 260 | 324 |

3. Refrigerate the NGM agar plates in plastic shoe boxes until the OP50 *E. coli* strain arrives.

*4 Weeks Pre-Lab:*

1. Grow cultures of OP50 *E. coli* in Luria Broth (LB) without antibiotics for overnight or longer. You will need 30 mL of OP50 culture per set of eight students.
2. Seed the NGM agar plates with 250 µl OP50 culture and incubate at room temperature for 2 days to allow the liquid to be absorbed or evaporate from the plate.
3. Store the seeded plates agar-side up in plastic shoe boxes. Leave at least 16 plates per group of eight students at room temperature. You may refrigerate the remaining plates until needed.

*3.5 Weeks Pre-Lab:*

1. Preparing stock cultures of all 14 strains. You will need 28 plates for this procedure as each strain will be duplicated on two plates.
2. If worm strains are stored on premises from previous years:
    a. Thaw frozen worm strains from the -80˚C or Liquid- N2 storage rapidly at room temperature.
    b. Pour the contents of the freezer vial on labeled NGM plates and store agar-side down overnight to allow liquid to be absorbed.
    c. 72-hours post thaw, move at least five thawed worms to two new OP50 seeded NGM plate by liquid transfer.
        i. For liquid transfer, add 0.5 mL of sterile M9 or sterile water to the plate.
        ii. Pipette ~200 mL of suspended worm to each of the three NGM plates.
3. If worms arrived from the CGC
    a. Check that none of the plates has contamination (fungus). Set aside any that do.
    b. Label to two new OP50 seeded NGM plates for each strain and use liquid transfer to move worms to the plates.
    c. If CGC stock plates have fungus or a thick-growing layer of bacteria, see Appendix A for strain decontamination.
4. Store worm plates at 20˚C or room temperature.

*2 Weeks Pre-Lab:*

1. You will need 28 plates for this procedure in order to passage the worms to fresh media.

2. Check all plates for fungus. If plates show fungal growth, decontaminate by transferring worms to fresh NGM plates using a worm pick over three days.
3. Transfer a small number of worms from each plate to fresh OP50 seeded NGM plates, either by using a worm pick or by diluting worms.
4. Store at 20˚C or room temperature.

*2-3 Days Pre-Lab:*

1. You will need 16 plates per set of eight students for this procedure.
2. For each multiple of eight students in lab, label four sets of four plates as follows:

**Table 6.** Instructions for labeling plates for sets of eight students.

| **Set 1 - Bli** | **Set 2 - Coil** | **Set 3 - Dpy** | **Set 4 - Unc** |
|---|---|---|---|
| A | B | C | D |
| Male ♂ | Male ♂ | Male ♂ | Male ♂ |
| WT | WT | WT | WT |
| A + ♂ | B + ♂ | C + ♂ | D + ♂ |

3. Examine the *C. elegans* culture plates from 2 weeks pre-lab and select plates that uncontaminated and rich in L1 larvae for each of the following strains: 1 x CB769, 1 x CB933, 1 x CB61, 1 x CB57, 2 x N2, and 2 x CB4951.
4. Using a pipette with sterile tips, transfer 1.0 mL of sterile M9 or sterile water to one of the plates, gently swirl it to suspend the worms, and transfer ~50 µl of the solution to the appropriate OP50 seeded NGM agar plate(s) as shown in Table 7.

**Table 7.** Plating worms 2-3 days before first week lab.

| | **Set 1 - Bli** | | **Set 2 - Coil** | | **Set 3 - Dpy** | | **Set 4 - Unc** |
|---|---|---|---|---|---|---|---|
| A | 50 µl CB769 | B | 50 µl CB933 | C | 50 µl CB61 | D | 50 µl CB57 |
| ♂ | 50 µl CB4891 | ♂ | 50 µl CB4891 | ♂ | 50 µl CB4891 | ♂ | 50 µl CB4891 |
| WT | 50 µl N2 | WT | 50 µl N2 | WT | 50 µl N2 | WT | 50 µl N2 |
| A+♂ | 50 µl CB769 + 50 µl CB4891 | B+♂ | 50 µl CB933 + 50 µl CB4891 | C+♂ | 50 µl CB61 + 50 µl CB4891 | D+♂ | 50 µl CB57 + 50 µl CB4891 |

5. Incubate at room temperature or 20˚C until lab day.

*2-3 Days before Second Lab (4-5 days post lab day 1)*

1. You will need 12 plates per set of eight students for this procedure.
2. For each multiple of eight students in lab, label four sets of three plates as is noted in Table 8.

**Table 8.** Plating worms 2-3 days before second week lab.

| **Set 1 - Bli** | **Set 2 - Coil** | **Set 3 - Dpy** | **Set 4 - Unc** |
|---|---|---|---|
| CB769 | CB933 | CB61 | CB57 |
| CB768 | CB189 | CB364 | CB4845 |
| CB518 | CB262 | CB27 | CB444 |

3. Examine the *C. elegans* culture plates from 2 weeks pre-lab and select plates that uncontaminated and rich in L1 larvae for each of the following strains listed in the table.

4. Using a pipette with sterile tips, transfer 1 mL of sterile M9 or sterile water to one of the plates, gently swirl it to suspend the worms, and transfer ~50 µl of the solution to the appropriate OP50 seeded NGM agar plates.
5. Incubate at 20˚C or room temperature until lab day.

## Decontaminating Worm Strains

*Method 1: Fungus Only (does not clean up bacterial contamination)*

1. Mark a small circle near one side of a 60 mm NGM agar plate seeded with OP50 with a marker.
2. Carefully use a worm pick to transfer a ten worms to a fresh NGM plate, ensuring that you place each worm on the agar above the marked spot near an edge of the plate.
3. The next day, transfer all worms that are not near the marked circle to another fresh NGM plate, marked the same way as the day before.
4. After another day, transfer the worms one final time, three to each of two plates. This should isolate the worms from the fungus.

*Method 2: Fungus or Bacteria*

1. The following steps should be performed quickly. Make sure your worm pick, plates, and 20% Alkaline Hypochlorite Solution ready before proceeding.
2. Obtain a fresh OP50 seeded NGM plate and place a drop of 20% Alkaline Hypochlorite Solution (AHS) in the middle.
3. Carefully use a worm pick to transfer up to ten gravid adult worms into the drop of AHS. The worms should quit moving and begin to break apart after a few minutes.
4. Incubate the plate until the next day and check for hatched L1 larvae. If no worms are present, repeat the procedure.
5. After another day, transfer the worms to a new NGM plate. This should decontaminate the worms.

## Solution

*Making Normal NGM Agar Plates (recipe makes ~ 100 60mM plates)*

**1.** Autoclave the following:
   2.5 g Bacto Peptone
   3.0 g NaCl
   20 g Bacto Agarose
   975 ml distilled $H_2O$

**2.** After autoclaving, add:
   25.0 ml 1M Sodium Phosphate, pH 6.0
   1.0 ml 1M $CaCl^2$
   1.0 ml 1M $MgSO_4$
   1.0 ml 10 mg/ml Cholesterol in Ethanol

**3.** Pour ~10 ml of media into each 60 x 15mm plastic Petri dish, an automatic plate pouring machine is very useful for making consistent plates

**4.** Inoculate NGM plates with 50 – 100 µl of fresh OP50 culture and allow to grow overnight.

*M9 Solution (1 Liter)*

**1.** Mix the following
   5.8 g $Na_2HPO_4$ $7H_2O$

3.0 g $KH_2PO_4$
5.0 g NaCl
0.25 g $MgSO_4$ $7H_2O$
$ddH_2O$ to 1 L

**2.** Sterile filter (0.22 **µ**m) and bottle.

*20% Alkaline Hypochlorite Solution (10 mL)*

**1.** Mix the following in a 15 conical tube:
5.5 mL $ddH_2O$
2.5 mL 1 M NaOH
2.0 mL Bleach (ordinary laundry bleach, regular strength)

**2.** This solution should be made fresh for each worm decontamination and preparation protocol.

# Appendix B
# Suppliers

## Ladd Research

http://www.laddresearch.com
83 Holly Court
Williston, VT 05495
Phone (800) 451-3406 (USA)
Fax: (802) 660-8859
Part # 30575 - Platinum Iridium Wire 80:20, 8 mil, 2 feet

## *C. elegans* Genetic Center (CGC)

http://www.cbs.umn.edu/CGC/
University of Minnesota, Dept. of GCD
6-160 Jackson Hall
321 Church Street S.E.
Minneapolis, MN 55455
Phone: 612-625-2265

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