

The Creature from Beneath: An Inquiry Genetics Exercise for Introductory Non-Science Majors

Peggy Brickman and Cara Gormally

Plant Biology Department
University of Georgia
Athens, Georgia 30602
(706) 542-1690
brickman@uga.edu
cgormally@plantbio.uga.edu
FAX: (706)-542-1695

Abstract: This lab provides non-science majors taking an introductory biology laboratory course with an opportunity to enjoy genetics in a way that they are rarely afforded –through self-guided discovery using live organisms. The challenge that engages students in this lab is to discover the genotype of “mystery mutants,” prepared *C. elegans* worms that have a normal phenotype. Possible hidden mutations include alleles that are incompletely dominant, autosomal recessive, X-linked recessive, or dihybrids. Students choose one of four different mystery mutants and then plan and execute genetic crosses to uncover the hidden mutation.

Introduction

The possibility to both self and cross-fertilize worms is just one of the benefits of using *C. elegans*. There is also an abundance of easily distinguishable mutant phenotypes (long, thin, slimy ones; short fat juicy ones, just as the saying goes), and the worms are low maintenance: a complete generation of *C. elegans* can be raised in as little as 2 days to as many as 7 days on simple agar plates seeded with *E. coli*. In the first week of this 3-week lab, students observe several different mutant strains and compare them to normal males and hermaphrodites. They then choose one of four different mystery mutants and plan the genetic crosses they will perform to uncover the hidden mutation. The mutations include an incompletely dominant allele, a simple autosomal recessive allele, an X-linked recessive allele, and a strain carrying two different mutations. In their experimental designs, students must explain the rationale for performing the crosses and make predictions for the expected results in both the F₁ and F₂ generations. The F₂ generation can be observed in week 3 of the lab. To insure student preparation prior to lab, students complete two genetics problem sets. Further progress is monitored via weekly experimental designs and a final written report. Extensive rubrics are provided for both.

Although originally developed for non-majors taking an introductory biology laboratory course, this exercise could successfully be used in an upper-division genetics course as well. The primary

difficulties students have in this lab is identifying the different mutations when compared with normal (wild-type), distinguishing the two genders (male from hermaphrodite), and manipulating the worms. From an instructor's point of view, the only requirements for adopting this lab are access to dissecting microscopes with underneath illumination and a small amount of space in an incubator if you want to see the next generation of offspring after 7 days instead of 2.

The objectives of this investigation are to:

1. Develop a thorough understanding of inheritance, and test that understanding by solving a genetic mystery.
2. Conduct all aspects of experimental design, including using observations to help choose appropriate tests, explain how these tests will provide an answer to a question, and predict the outcome of genetic crosses if a mutation were recessive, dominant, or sex-linked.
3. Collect data from an experiment and analyze that data to refute a prediction.
4. Communicate the results of an experiment and explain how the results solved the mystery.

Student Time Table

Week 1:

- Students practice distinguishing *C. elegans* of known genotypes from wild type males and hermaphrodites.
- Students compose experimental designs to discover the genotype of an unknown *C. elegans* "mystery mutant."
- Students set up their crosses on Petri dishes (2 dishes for each group).

Week 2:

- Students observe outcomes of their matings from Week 1 and plan subsequent crosses to further elucidate the genotype of "mystery mutant."
- Students set up their crosses on Petri dishes (2 dishes for each group).

Week 3:

- Students observe final outcomes of their experiments and start lab reports.

Student Outline

Introduction:

Imagine that you took a scoop of soil from your backyard compost heap and placed it onto a Petri dish. You'd be amazed at what could crawl out, like the 1-mm little guys shown in Figure 1. They are harmless roundworms, specifically, nematodes, of the species *Caenorhabditis elegans*. Grown in the lab on Petri plates, Figure 1 shows them swimming through a lawn of their favorite food, *E. coli* bacteria. Developed as a genetic model organism by Sydney Brenner in the 1970s, *C. elegans* has been instrumental in uncovering the secrets of some of the most basic cellular processes in animals. Embryonic development, sex determination, and even aging and alcoholism has benefited from the studies of *C. elegans* mutants.

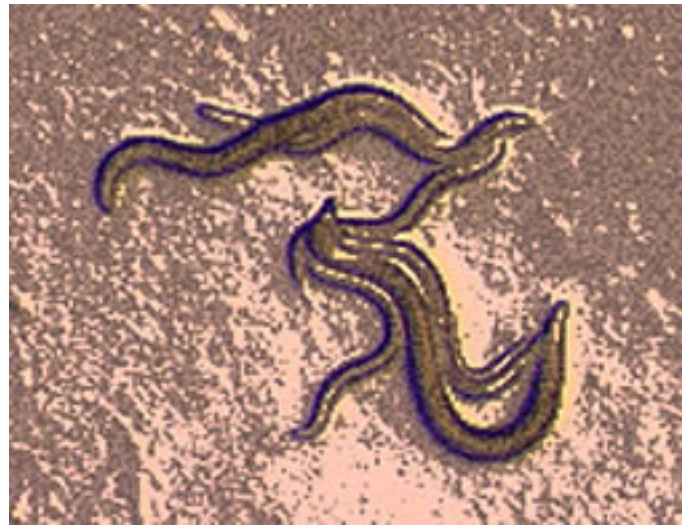


Figure 1. Mix of adult wild-type *C. elegans* adult males and hermaphrodites.

Assignment:

During the next few labs, you will test your understanding of inheritance by developing genetic crosses that should help you uncover the genotype of a *C. elegans* "Mystery Mutant." The materials available to you include: petri dishes upon which to rear your Mystery Mutant's F₁ and F₂ offspring, a two-week time limit, and a selection of *C. elegans* genotypes to breed with your mutant. In order to prepare you to set up and run your own experiments, please complete the following genetics problem set. Your answers are due at the beginning of lab this week.

Pre-Class Genetics Problem Set I:

Adult *C. elegans* are usually hermaphrodites –meaning they make both eggs and sperm and can reproduce ONLY by self-fertilization; they cannot breed with another hermaphrodite. However, 5 out of every 10,000 eggs actually develop as males. Hermaphrodites have 2 X-chromosomes; males occur due to non-disjunction of the X-chromosome during meiosis, thereby producing XO males.

1. For the following *C. elegans*, predict what percent offspring would be male or hermaphrodite.

Table 1: Predictions of gender of matings between hermaphrodites and males.

		Parents:		
		1 hermaphrodite	1 male	1 male plus 1 hermaphrodite
Predicted Offspring:	% hermaphrodite			
	% male			

2. Mutant nematodes of *C. elegans*, have been isolated with a mutation (*sma-2*) that causes them to have a small body size. You place a hermaphrodite that is homozygous for *sma-2* and a hermaphrodite that is homozygous for the normal allele (**wild-type**) on a single plate.
 - a. How would you determine if the offspring were the result of self-fertilization rather than from cross-fertilization from mating?
 - b. If you look at the F1 offspring of these two hermaphrodites a week later, what would be the predicted ratio of the phenotypes and genotypes?

3. The Petri dish on the left hand side of the diagram below shows the parents of a genetic cross between a *sma-2* homozygous hermaphrodite and a true-breeding, **wild-type** male (normally sized).
 - a. Give the expected phenotype(s)/genotype(s) of the F1 progeny if the mutation were dominant or recessive (use the symbol + for wild-type and (*sma-2*) for small body).

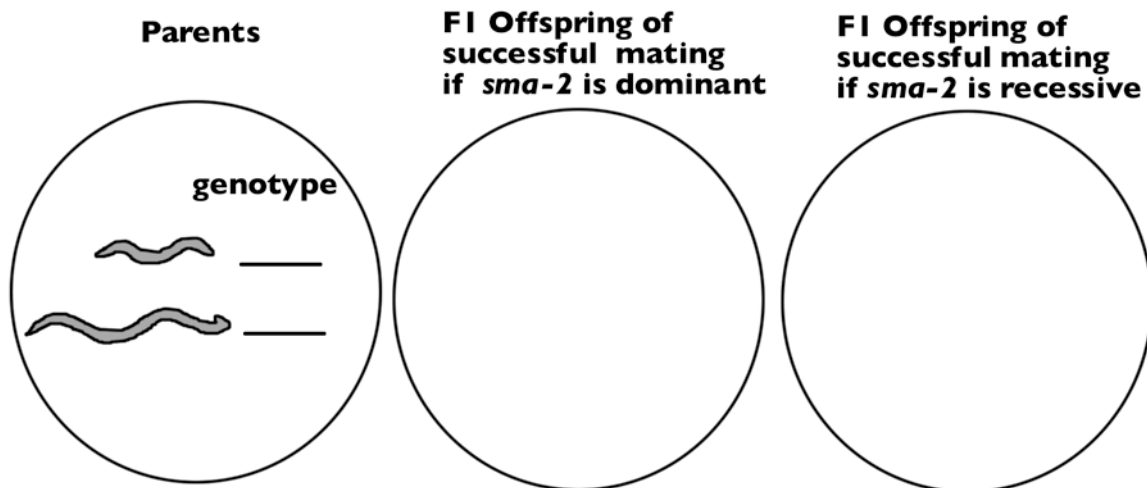
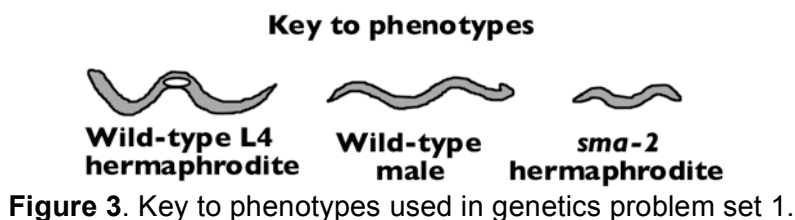


Figure 2: Possible progeny of a *sma-2* worm mated to a wild-type normal male.



- b. Give the expected phenotype(s) /genotype(s) of the F1 progeny from the hermaphrodite alone (use the symbol + for wild-type normal body and (*sma-2*) for small body).

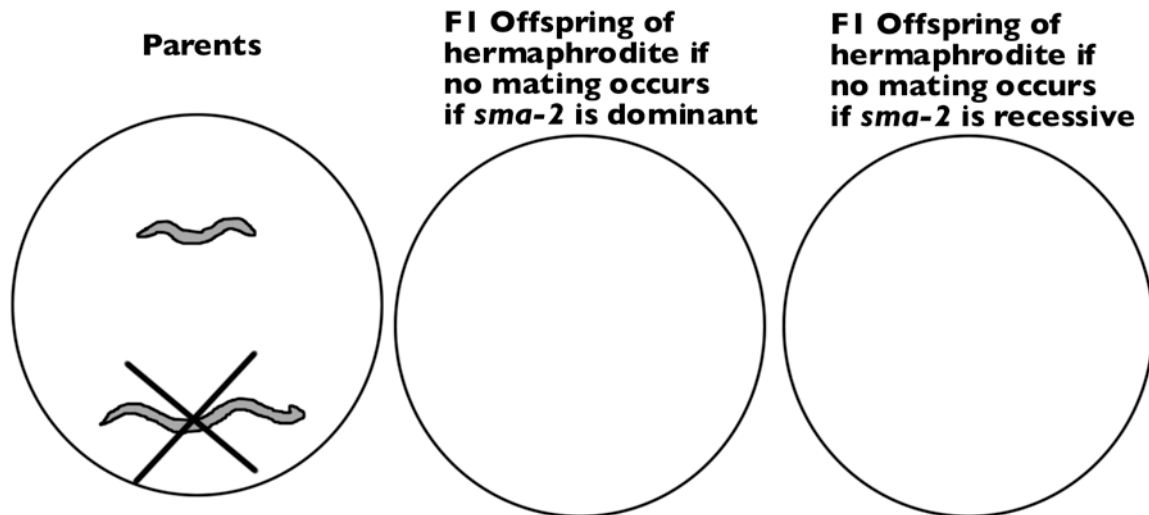


Figure 4. Possible progeny of a *sma-2* worm self-fertilized.

4. You isolate a heterozygous F₁ hermaphrodite offspring from a successful mating between a *sma-2* hermaphrodite and a wild-type male parent and place it on the plate below. Draw out the phenotype(s) of the offspring in the F₂ generation you would expect to see the following week and fill in the genotypes in a Punnett Square for the F₂ offspring.

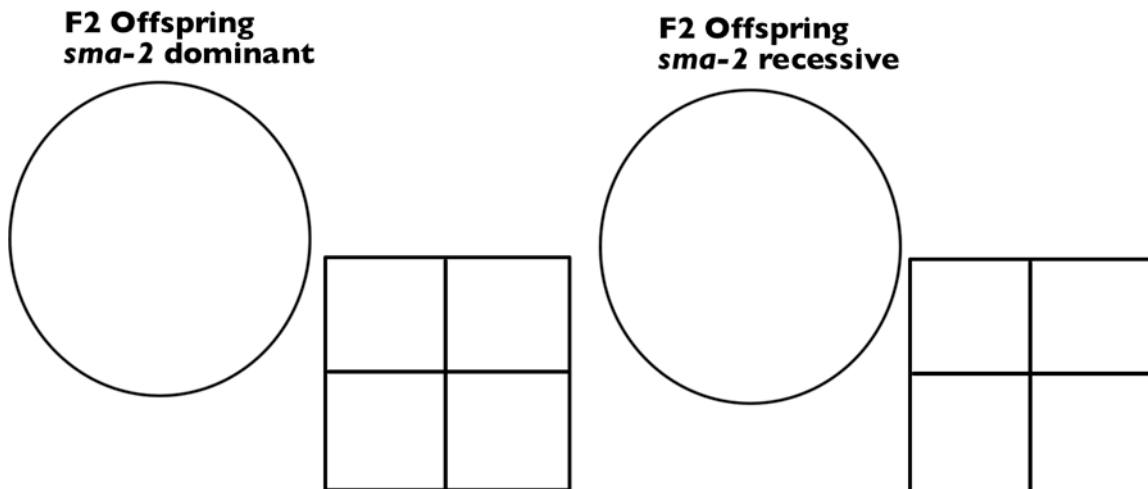


Figure 5. Possible F₂ progeny of a *sma-2* heterozygote.

Observations and Experimental Design for Week 1

Background: “Like a Virgin”

Adult *C. elegans* are usually hermaphrodites, meaning they make both eggs and sperm and can reproduce by self-fertilization. This would limit their usefulness for genetic experiments, except for the rare 5 out of every 10,000 eggs laid that develop as males.

Males have to mate to reproduce. Every mating between a male and a hermaphrodite results in about 50% male offspring, IF your male gets to mate before the adult hermaphrodite goes it alone. This means you need to be able to distinguish adults from juveniles (larval stages L1-L4) to pick out non-adults (i.e. virgins, namely L4 larvae) to mate with a male. L4 hermaphrodites have a smoothly tapered tail and a distinguishing white oval with a small black dot in the middle of the animal that will develop into the vulva (structure used to lay eggs).

There are four different mystery mutant *C. elegans* in addition to wild-type (normal) *C. elegans*. How could you determine the genotype of one mystery mutant?

Assignment:

Working with your group, use your homework notes and the following guidelines to discuss and agree on an approach that will allow you to determine the genotype of ONE of the 4 “mystery mutant” *C. elegans* using genetic crosses.

Available tools and equipment for genetics sleuthing:

1. Petri dishes upon which to rear your mystery mutant’s F1 and F2 offspring
2. A two-week time limit
3. A selection of *C. elegans* of known genotypes to choose from to cross to your mystery mutant.

Safety Note:

In the laboratory, *C. elegans* are grown on agar plates spread with a lawn of *E. coli* bacteria and then incubated at temperatures between 15-25°C. To keep molds and wild bacteria from taking over the plates, aseptic (sterile) technique should always be used when transferring worms. Keep your plates covered as much as possible, and make sure not to touch the surface of the plate with anything that is not sterile. Although the strain of *E. coli* used in this experiment is not normally harmful, be sure to disinfect your hands after touching the plates. When you are finished with them, bacterial plates should NOT be thrown into the trash, please place all *C. elegans* plates back in the plastic storage boxes provided.

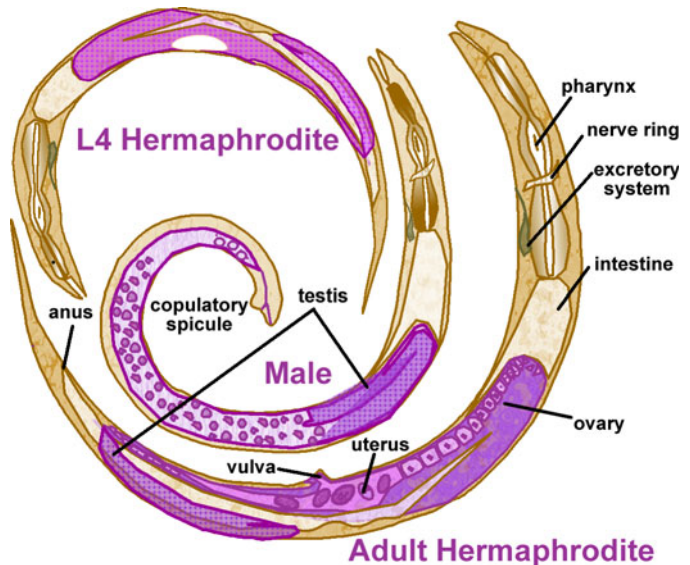


Figure 6. Differences between adult males and hermaphrodites and L4 juveniles

Suggested Steps:

1. Start by looking at the N2 plates (containing normal, wild-type strain of *C. elegans*) that are a mixture of hermaphrodites and males. Using a dissecting microscope illuminated from above with the light hitting the frosted side of the mirror underneath, place a plate of *C. elegans* onto the stage. The worms are swimming on the upper surface of the agar, so make sure that the cover is facing up. On low magnification, try turning the wheel that aligns the mirror until you can see the shadowy outline of the worms through the eyepieces. Now focus and increase the magnification. You should be able to identify larvae, adult hermaphrodites, and males. Make a list of distinguishing characteristics in the table below.

Table 2. Distinguishing characteristics of males and hermaphrodites.

	Sex Chromosomes	Appearance:
Hermaphrodites	XX	
Males	X	

2. All the mystery mutants carry mutations that you can observe in the provided known mutants. Examine each of these known mutants, one at a time, and describe them below including movement and how to distinguish each from wild-type.

Table 3. Distinguishing characteristics of mutant strains of *C. elegans*.

Mutant Name	Description, including movement and how to distinguish from wild-type.

Care and Feeding of Your Mutant & Moving Your Worms Around:

You will be using little worm “spatulas” to move the worms around under the microscope. These are slender pieces of platinum wire embedded in a glass pipette that are fragile and can bend easily, so always store picks upright in the holder supplied. You will be using alcohol lamps to flame-sterilize your picks. Keep your hair tied back and keep other flammable objects away from the alcohol lamps.

3. Practice moving the worms around:

- Sterilize your worm “spatula” by passing it briefly through the flame of an alcohol lamp and then cooling it completely by CAREFULLY pressing it against the surface of the agar plate. You do not need to push the spatula through the agar. Repeat this before and after using a plate.
- Using the dissecting microscope, pick up worms by gobbing some bacteria onto the back of your spatula, and then placing it on the surface of a worm. Gently lift the worm up.
- Looking through the dissecting microscope, you can transfer this worm to another location on the plate by gently touching the spatula to the agar surface of the plate and waiting for the worm to crawl off.
- Some tips:
Be careful not to poke holes in the plates; the worms can burrow down and escape from the surface, making them impossible to observe or manipulate.
Be careful that you don’t inadvertently transfer eggs or larvae over to your mating plates. Look for the little S-shaped slalom tracks worms leave in the lawn.
When using the pick steady your hand by resting its edge on a flat surface.

Questions to Guide You:

Discuss the following points of consideration with your group: Keep in mind that it in the interest of time it may be more productive to discuss them as you are planning your experiment; your ideas can be used in your in-class experimental design write-up.

- Each group will have 2 plates available on which to set up matings. You can do whatever cross you want in order to discover more about your unknown mutant. Since you have two plates, you can set up more than one cross. You may want to consider replication or some sort of control for comparison.
- If you placed a single virgin hermaphrodite (L4 or earlier) on a plate of *E. coli*, what would be the expected phenotype and sex of the offspring, be and how could you determine the genotype of the parents?
- If you placed a single L4 hermaphrodite with a male on a mating plate, what would be the expected phenotype and sex of the offspring?
- Why would you want to cross your mystery mutant with a male instead of just allowing the hermaphrodite to self-fertilize? What would you predict for the result of each cross?
- How do you determine how many worms (# hermaphrodites and # males) should be placed on each plate? Would you expect successful mating 100% of the time? Would it be better to have many males and one hermaphrodite or many hermaphrodites and one male on a plate?

Take some notes for the experimental design for your proposed tests:

Remember, although you may discuss and write up notes as a group, you must write up an individual experimental design to turn in for assessment. Your writing should be in your own words. Your description of your methods should be clear and complete enough so that someone else (as well as yourself, at a later date!) can follow your logic and repeat your tests if necessary. Doing a complete job now will make writing your report later that much easier. Use the rest of this page for notes, but you should also use the checklist on the next page to guide your writing. You will be turning in your experimental designs to your GLA at the end of class today, and they will be graded!

In your notes, include observations about your parents, and one expected result from your crosses. If you are performing a mating, state how you would tell if the mating worked and what the results would look like if the gene for the trait were on an autosome, or on the X-chromosome, or if it were an incompletely dominant allele.

Observations of the mystery mutant plate:

- Phenotypes of Mystery Mutant parent(s):

- What do you think the hidden mutation is?

Plate 1: Description of parent(s):

- Why did you choose this cross?

- Predicted phenotypes of F1 offspring:

- Predicted phenotypes of F2 offspring:

Plate 2: Description of parents:

- Why did you choose this cross?

- Predicted phenotypes of F1 offspring:

- Predicted phenotypes of F2 offspring:

Experimental Design Checklist:

- Context:** Provide the observations/knowledge that helped you select your tests.
 - Observations:* Did you describe how you viewed the known mutants and what observations you made about the differences between them?
 - Name:* Did you write down the name of the mystery mutant you have chosen to investigate and the mutation you believe your mystery mutant carries?
 - Gender:* Did you describe the physical and/or behavioral differences you used to distinguish males from hermaphrodites?
 - Lifestage:* Did you describe how you distinguished the different lifestages?
 - Phenotype:* Did you describe the appearance/behavior of your mystery mutant?
- Question:** State what you hope to learn or conclude from your tests (either those assigned to you or those you came up with on your own.)
- Justification:** Include relevant background information about why these tests are interesting or important.
- Tests:** Explain how your tests will provide answers to your question; include what these tests are designed to find.
 - Procedures:* Describe how you moved the worms from plate to plate including the sterilization procedure you used.
 - Descriptions:* Explain why you set up the plates with the worms that you chose, indicate the gender and lifestage or age of the worms you chose for your parents, and if you use more than one parent describe why.
 - Mating:* Explain if you hope to see a cross or self-fertilization from the worms on your plate.
- Detail:** Provide sufficient description of your tests so that another classmate could repeat them.
- Prediction:** What is one result you expect?
 - Observations:* What differences might you observe next week if your mutation is dominant? Recessive? Sex-linked? Autosomal? Some other words you might include are heterozygous, homozygous, and incompletely dominant.
 - Phenotypes:* Did you describe the physical characteristics you will look for?
- Claims:** Describe what you may be able to conclude as a result of your tests.
- Evidence:** Describe how you will evaluate the information from your tests.
 - Data:* Do you describe how you will collect the data on your observations?
- Explanation:** Explain how you will be able to use the data to conclude if your question has been answered?
 - Analysis:* Did you describe expected ratios or Punnett squares? Do you describe how you will use quantitative data to conclude if your hypothesis is correct?

Pre-Class Genetics Problem Set II:

- You find a mutant *C. elegans* that is very long and thin called the *lon-2* mutation. Crossing a *lon-2* hermaphrodite with a normal male yields F₁ hermaphrodite offspring shown in the left hand column (A) of the figure below. (Males are indicated with a bump on their tails, hermaphrodites by their larger size and white oval at their center.) Explain why this occurred:
- The resulting F₁ offspring from a cross between a *lon-2* hermaphrodite and a normal male are shown in (A). In the empty Petri dishes shown below, draw in the expected phenotypic ratios of the F₁ offspring for the self-fertilization of a *lon-2* hermaphrodite (B) and a *lon-2* male crossed to a normal hermaphrodite (C).

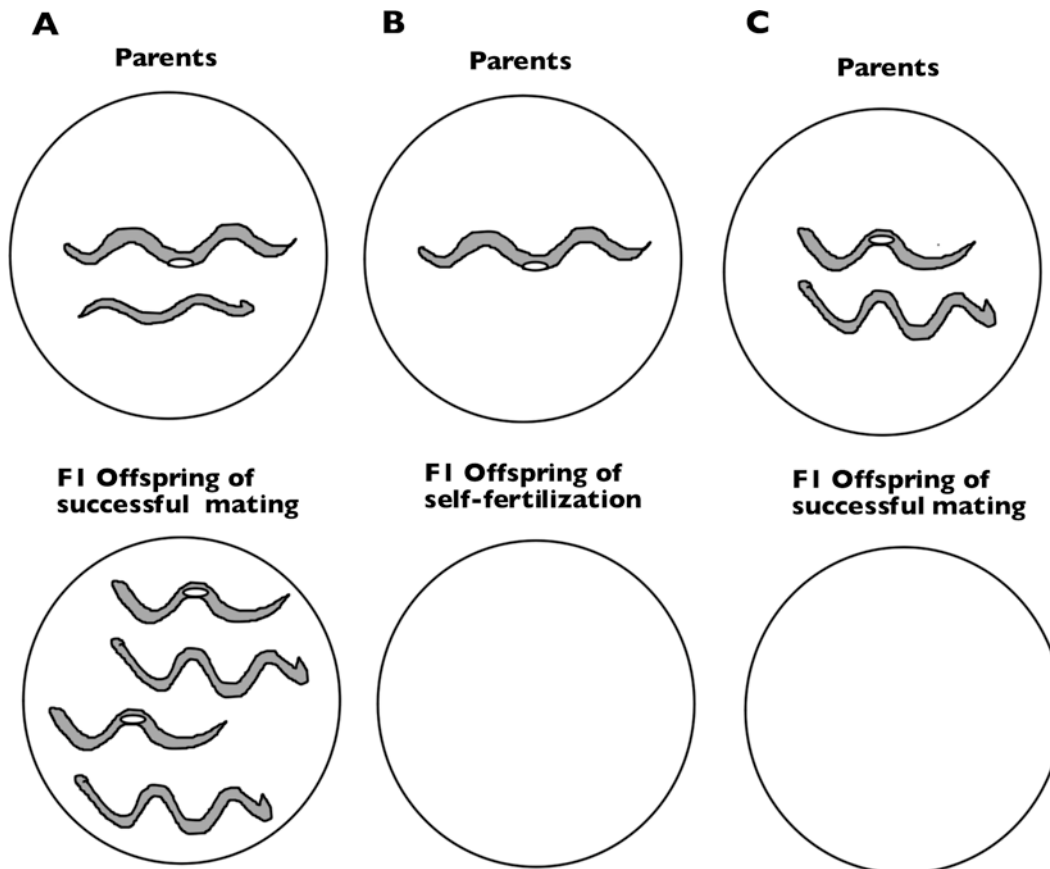


Figure 7. F₁ Progeny of a *lon-2* hermaphrodites or males.

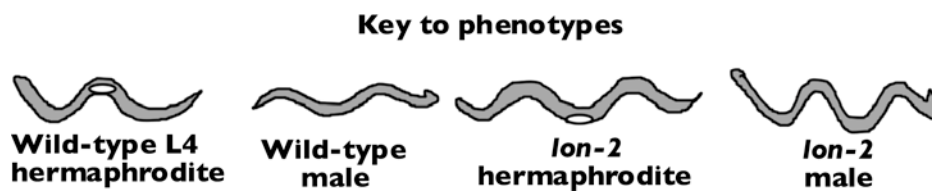


Figure 8. Key to phenotypes of *lon-2* questions

3. If you were to cross one of the F1 male offspring from column A with one of its F1 hermaphrodite siblings what would be the genotypic and phenotypic ratios of the offspring produced in the F2 generation?

4. You cross two different mutant *C. elegans* to create offspring with a combination of their traits. One of the mutants, a roller, rolls around on the plate (*rol-2*, use *r*) and is caused by a mutation that is recessive to wild-type (*R*). The other mutant is dumpy (*dpy-5*, use *d*) which is also caused by a mutation that is recessive to normal wild-type (*D*). The problem is that male rollers and dumpies can't mate; they can't catch up with the hermaphrodites, so it is impossible to cross a roller to a dumpy. But, you can cross a roller to a normal male and a dumpy to a normal male to make heterozygous offspring that you can cross together.

What are the genotypes and phenotypes of the F₁ progeny for these crosses?

Parents: ***ddRR*** hermaphrodite X ***DDRR*** male ***DDrr*** hermaphrodite X ***DDRR*** male

F₁ = genotype F₁ = genotype
 _____ phenotype _____ phenotype

5. If you crossed these two different F1 progeny from Question #4 together, what would be the ratio of expected phenotypes and genotypes of the F₂ offspring?

6. What percent of the F₂ offspring from Question #5, if allowed to self-fertilize, would be able to produce at least a few offspring that were dumpy rollers?
7. Dogs of the boxer breed that have brown fur with black stripes are referred to as brindle. The number and darkness of the stripes in brindle boxers is highly variable; some have so many stripes they look almost black, some have a medium number of stripes, while others have just a few stripes. You breed your medium-striped boxer with one having very prominent dark stripes. Half of the resulting puppies have medium stripes and half have dark stripes. If this brindle gene shows incomplete dominance, what would be the expected phenotypic ratio of puppies produced by crossing two dogs with medium stripes?

Observations and Experimental Design for Week 2

Recording Your Experimental Results:

Use the experience of solving the previous genetics problems and working out your Punnett Squares to tabulate the numbers of offspring that you observe on your plates this week.

Plate 1: Re-Write a Description of the parent(s):

- Description of the observed F_1 offspring:

- How does this compare with your original prediction?

- Do you wish to revise your original expectations about the genotype of the mystery mutant?

Plate 2: Re-Write a Description of the parent(s):

- Description of the observed F_1 offspring:

- How does this compare with your original prediction?

- Do you wish to revise your original expectations about the genotype of the mystery mutant?

Planning Experiments for Next Week:

This week you will have the opportunity to continue your experiments in hopes of uncovering the hidden genotype of your mystery mutant. Each group will have 2 plates available on which to set up matings. You can do whatever cross you want in order to discover more about your mystery mutant. You may want to consider replication or some sort of control for comparison.

Plate 3: Description of parent(s):

- Why did you choose this cross?

- Predicted phenotypes of F_1 offspring:

Plate 4: Description of parent(s):

- Why did you choose this cross?

- Predicted phenotypes of F_1 offspring:

Experimental Design Checklist:

- Context:** Provide the observations/knowledge that helped you select your tests.
Observations: Did you describe how you viewed the F₁ offspring on your plates and what observations you made about the differences between them?
- Question:** Did you state what you hope to learn or conclude from your tests (either those assigned to you or those you came up with on your own.)
- Justification:** Did you describe the success of your matings from last week and how you might improve on them for this week?
- Tests:** Explain how your tests will provide answers to your question; include what these tests are designed to find.
Description: Did you explain why you set up the plates with the worms that you chose, indicating the gender and lifestage or age of the worms you chose for your parents? If you use more than one parent, did you describe why?
Mating: Explain if you hope to see a cross or self-fertilization from the worms on your plate.
- Detail:** Provide sufficient description of your tests so that another classmate could repeat them.
- Prediction:** What is one result you expect?
Observations: What differences could you observe next week if your mutation is dominant? Recessive? Sex-linked? Autosomal? Some other words you might include are heterozygous, homozygous, and incompletely dominant.
Phenotypes: Did you describe the physical characteristics you will look for?
- Claims:** Describe what you may be able to conclude as a result of your tests.
- Evidence:** Describe how you will evaluate the information from your tests.
Data: Did you describe how you will collect the data on your observations?
- Explanation:** Explain how you will be able to use the data to conclude if your question has been answered?
Analysis: Do you describe expected ratios or Punnett squares? Did you describe how you will use quantitative data to conclude if your hypothesis is correct?

Observations for Week 3*Recording Your Experimental Results:*

Tabulate the numbers of offspring that you observe on your plates this week.

Plate 3: Re-Write a Description of the parent(s):

- Description of the observed F₁ or (F₂) offspring:

- How does this compare with your original prediction?

- Do you wish to revise your original expectations about the genotype of the mystery mutant?

Plate 4: Re-Write a Description of the parent(s):

- Description of the observed F₁ offspring:

- How does this compare with your original prediction?

- Do you wish to revise your original expectations about the genotype of the mystery mutant?

Lab Report Guidelines

Assignment: You have just designed and completed an experiment to determine the genotype of an unknown mystery mutant. Now, we would like you write a report that describes your tests. Your report should have the following elements:

Content Criteria:

- _____ *Context:* Provide the observations/knowledge that helped you choose your tests.
- _____ *Question:* State what you hoped to learn or conclude from your tests (either those assigned to you or those you came up with on your own.)
- _____ *Tests:* Describe *exactly* what you did to try to answer the question and what the tests were designed to find.
- _____ *Detail:* Provide sufficient detail so that someone else could repeat your tests.
- _____ *Prediction:* What was one result you expected?
- _____ *Justification:* Include relevant information about why your testes were appropriate and would provide answers to your questions.
- _____ *Claims:* Describe what you were able to conclude as a result of your tests.
- _____ *Evidence:* Describe how you collected and evaluated the information from your tests.
- _____ *Graphs/tables:* Produce graphs and tables to display your data effectively.
- _____ *Explanation:* Explain what you think the mystery mutant's genotype is or explain what you could have done better or differently if you extended your project.

Grammatical and style criteria*:

- _____ *Grammar/spelling:* Follow correct conventions for grammar and spelling.
- _____ *Format:* The ideas are organized in a logical format.
- _____ *Clarity:* The paper is clear and concise.

2 points will be awarded if the criteria is fully addressed and discussed; 1 point will be awarded if the criteria is partially discussed, and 0 points will be given if the criteria is ignored.

Format: Your lab report should be approximately 4 to 6 pages, double-spaced.

Photo Atlas of *C. elegans* Mutants**Figure 9.** Adult Hermaphrodite**Figure 10.** Adult Male**Figure 11.** Mating pair.**Figure 12.** *lon-2* Adult Hermaphrodite**Figure 13.** *unc-76* Adult Hermaphrodite**Figure 14.** *dpy-5* adult hermaphrodite**Figure 15.** *dpy-10* adult hermaphrodite

Notes for the Instructor

C elegans:

Strains can be ordered from the Caenorhabditis Genetics Center, an NIH supported stock service: <http://biosci.umn.edu/CGC/Strains/strains.htm>. They pay the shipping charges but state: “You must send a request in writing, either letter, fax or email, stating the strains or genotypes that you would like to receive, and a brief statement of what you will do with the strains (one sentence is fine). Include your complete mailing address and your phone number. Strains are sent growing (or starved) on agar plates. They are sent via first class or airmail. It generally takes about 7-10 days, plus shipping time, for a request to be filled. There is no charge for strains that we send to educational or non-profit organizations.”

Life Cycle:

- Life cycle at 24°C takes about 2 days.
- Life cycle at 20°C takes about 3 days
- Life cycle at 14-15°C takes about a week

Teaching Notes:

Background Knowledge Required:

The success of this lab hinges on students’ abilities to understand enough genetics to be able to 1) plan their own genetics crosses using predictions about the potential offspring and 2) use those predictions to draw conclusions about the parental genotype. We have found that students need to have studied genetics recently in class, especially basic Mendelian patterns of inheritance for dominant and recessive alleles, as well as extensions of genetics to explain incompletely dominant and sex-linked alleles.

Teaching Challenges:

Adult hermaphrodites are the largest worms visible on the plates. Males are smaller and more slender, approximately the size of juvenile L4 hermaphrodites, which can be distinguished by a small white oval at their middle. Males are usually more active, swimming quickly around the plate, often backwards, so it might be fruitful to make sure students can identify the tails of males. Males will also stroke objects (including other worms and themselves) with their tails, attempting to mate. You can most often distinguish them by this behavior. Students have a difficult time distinguishing males, so it might be good to have a plate with just a few isolated males on to it. Alternatively, if you have a video camera attached to a dissecting microscope, you could show students several males, adults, and L4 hermaphrodites. If you have access to a computer, there are excellent movies at the following sites:

- <http://elegans.swmed.edu/>
- <http://www.bio.unc.edu/faculty/goldstein/lab/movies.html>

Suggested Further Experiments:

After gaining proficiency manipulating the worms, instructors may wish to investigate further experiments. There are very interesting protocols for testing alcohol effects on worms and using a chemo-attractant butanone at: <http://www.biologicalprocedures.com/bpo/arts/1/79/m79.htm>.

Making Males:

Hermaphrodites have 2 X-chromosomes. Males occur due to rare non-disjunction of the X-chromosome during meiosis producing XO males. Non-disjunction can be promoted by placing young hermaphrodite worms under heat stress (30°C for 6 hours) before shifting them back to 20°C. In practice, I was able to generate 1 male out of almost 300 offspring. An alternate strategy would be to order a *him-5* strain that increases the incidence of males in an otherwise wild-type background.

Preparing culturing plates:

Worms are fed using a strain of *E. coli* that will not overgrow the plates, called OP50, a uracil auxotroph. You will need to streak OP50 out on a 5 cm NGM agar plate and incubate the plate at 37°C overnight. You can then parafilm the plate and keep it at 4°C for months. When you want to grow a lawn for worm consumption, pick a single colony using a flamed wire hoop and transfer to a 100 ml bottle of B broth. Set the bottle (shaking) at 37° overnight, or at room temperature for 1-2 days with occasional agitation. It should be cloudy the next day. This bottle can be stored at 4°C and used for ~3 months. OP50 can also be frozen in 20% glycerol. Mix 1 ml of cultured OP50 in B broth with 1 ml autoclaved 40% glycerol. Mix well and freeze at -20°C or -70°C. To inoculate from frozen stocks, scrape the surface of the frozen mix with a sterile toothpick and drop it into the B broth.

Seeding plates:

NGM plates should be allowed to sit at room temperature in the bags the plates came in or a box for a couple of days after pouring to allow them to dry so that plates with bacterial or fungal contaminants become obvious. Using a sterile pipette, drop 1-2 drops OP50 suspension on the middle of each 5 cm NGM plate. It is best if the bacterial lawn does not touch the sidewall as worms then tend to crawl up the wall and die. (Don't move the plates soon after seeding, or the puddle of bacteria will definitely slosh against the wall). Take care not to damage the surface of the agar with the pipette tip; worms will then burrow under the surface. Let the plates sit overnight at 37°C or 2 days at room temperature to form a bacterial lawn before using. Plates can be used for about 7 days after seeding, and are best 2-4 days after seeding.

“Chunking out” worms from plates:

Strains will arrive on Petri dishes and will need to be transferred in a chunk to a new plate using a sterile spatula. Use a small, capped bottle of ethanol next to your dissecting microscope. Sterilize a small spatula by dipping it into the ethanol and then flaming the spatula in a Bunsen burner for about 15 seconds until the ethanol burns away and the spatula glows slightly. After a second or two, dip the hot spatula back in the ethanol until the spattering and fizzling stops, then quickly pass the spatula through the flame to ignite the ethanol and sterilize the spatula. Use the now sterile spatula to cut a ~1 cm square chunk of the old plate and place it upside down on the new plate. Worms will crawl out onto the fresh bacterial lawn

Culturing plates:

Worms are usually cultured at 20° C, with the plates upside down. The boxes you store them in can be baked at 65° for >1 hour before use to sterilize, we use stackable towers from a big box store. Worms can be cultured between 15°C and 25°C and will grow slower or faster, respectively, at these temperatures. To keep a stock available for a long time without maintenance, parafilm the plate and set it at 15°C. You can then recover the worms for up to 5 months afterwards. The limiting factor is that the

worms will eventually die if the plate dries out. If you're leaving town for a while a good way to keep your worms is to put the plates in a 12.5° C incubator. The worms will be almost in stasis and will show only barely perceptible development after 1 week. Apparently prolonged incubation at 12.5°C kills worm stocks by sterilizing them. One week is certainly safe, and after 2-3 weeks you'll probably still have some fertile worms.

Making a worm "spatula:"

Take a hammer and bang on the very tip of ~32 gauge platinum wire to flatten it. You only need a few millimeters of flat wire attached to about an inch of round wire. Bend the flat section so that it is at a shallow angle to the rest of the wire (resembling a miniature spatula). Holding the wire with a pair of forceps in one hand, place the non-flattened end of the wire into the tip of a pulled out glass Pasteur pipette. About 2 cm of wire should protrude from the glass. Now, seal the wire in place by holding both in the flame of a Bunsen burner. Once the wire sticks inside the glass, use the forceps to squeeze the glass down onto the wire, thus cementing it into place. Check your "spatula" under the dissecting microscope and trim off the sharp corners of the flat end with the razor blade. When the tip of the pick eventually wears out, the end can be cut off and a new end flattened and bent.

Freezing worm stocks:

1. Wash worms off of 1, 9 cm plate or 3, 5 cm plates. Worms should be harvested off of plates when they are slightly starved, about 1 day after the bacteria have been exhausted. To wash the worms off plates, add ~2 ml S Basal buffer to each small plate (w/sterile 5-ml glass pipette and a Pasteur pipette bulb), swirl briefly to dislodge worms, and suck off the suspension with the sterile glass pipette and place into a 15 ml sterile plastic centrifuge tube.
2. Spin down in a clinical centrifuge for ~30 sec. Remove all but 1.5 ml of the supernatant.
3. Add 1.5 ml freezing solution, mix well, and aliquot 1 ml each into 3 sterile freezing vials (Nunc CryoTubes #363401).
4. Freeze slowly to -80°C. This is accomplished by placing the vials in a Styrofoam rack (the kind that 15 ml disposable sterile centrifuge tubes come in), placing another inverted such rack on top of the first, fastening the two racks together with rubber bands, and placing in a -80 °C freezer.
5. The next day, move two vials to a permanent location. Record the strain number, genotype, and comments in a computer database.
6. The third vial should be used for a test thaw. Take the vial out of the freezer, thaw quickly by holding in your hand or in a 37°C water bath (leave in the bath only until the ice is gone so as not to heat it up). Can dump the whole vial out in a seeded large plate, or use a sterile 200ul pipette tip to withdraw the bottom 200ul (containing the settled worms) and place it on a small plate. The next day, pick live worms to a new plate.

Answer key for Genetics Problems I:

1.

		Parents:		
		1 hermaphrodite	1 male	1 male plus 1 hermaphrodite
Predicted Offspring:	% hermaphrodite	100%	0%	50%
	% male	0%	0%	50%

2. Monohybrid Cross

- They can't mate with each other without a male.
- Each hermaphrodite would mate with itself giving homozygous *sma-2* genotype and all small phenotype. The other parent would give homozygous wild-type offspring. There would be no offspring of the two hermaphrodites as a cross-fertilization

3. There are different outcomes in the F1 of a cross if a mutation is recessive or dominant:

- If *sma-2* is dominant, 50% male small, 50% hermaphrodite small phenotype, all genotype *sma-2/+*. If *sma-2* is recessive, 50% male and 50% hermaphrodite all with a wild-type phenotype, genotype *sma-2/+*.
- If *sma-2* is dominant, 100% hermaphrodite small phenotype, all genotype *sma-2/sma-2*. If *sma-2* is recessive, 100% hermaphrodite all with a small phenotype, genotype *sma-2/sma-2*.

4. There are different outcomes in the F2 of a cross if a mutation is recessive or dominant:

- If *sma-2* is dominant, 100% hermaphrodite, 3/4 small phenotype (2/3 *sma-2/+*, and 1/3 *sma-2/sma-2*, 1/4 wild-type, all genotype *+/+*.
- If *sma-2* is recessive, 100% hermaphrodite 3/4 with a wild-type phenotype (1/3 genotype *+/+*, 2/3 genotype *sma-2/+*, and 1/4 with *sma-2* phenotype and *sma-2/sma-2* genotype.

Answer key for Genetics Problems II:

1. Explanations for the outcome of the *lon-2* genetics crosses: Crossing a *lon-2* hermaphrodite with a normal male yields F₁ hermaphrodite offspring that are all normal sized but male offspring that are all long. This indicates this trait is recessive and sex linked, i.e. on the X chromosome X^{*lon-2*}. Males inherit only one X chromosome, the one from their mother. If the mother has two of the *lon-2* alleles, then the males will also be long in phenotypes X^{*lon-2*}/0. Hermaphrodites inherited an X from both parents and will be heterozygous X^{*lon-2*}/X⁺, and have a wild-type phenotype.

2. Predicted outcomes for B and C:

- lon-2* hermaphrodites yield all long phenotype offspring with homozygous genotype X^{*lon-2*}/X^{*lon-2*}.
- lon-2* males crossed to wild-type hermaphrodite should yield all wild-type male offspring. Half will be males with only one dominant + allele for the *lon-2* gene X⁺/0. All hermaphrodite offspring will be heterozygous X^{*lon-2*}/X⁺ and thus have a wild-type phenotype. F₁ male offspring would be X^{*lon-2*}/0, hermaphrodite siblings will be X^{*lon-2*}/X.

3. Predicted outcomes in the F1 between lon-2 males and heterozygous hermaphrodites:

	X^{lon-2}	X^+
X^{lon-2}	X^{lon-2}/X^{lon-2} Hermaphrodite long	X^{lon-2}/X^+ Hermaphrodite wild-type
	X^{lon-2} Male wild-type	X^+ Male wild-type

4. Parents: *ddRR* hermaphrodite X *DDRR* male *DDrr* hermaphrodite X *DDRR* male

F₁ = *dDRR* genotype

F₁ = *DDRr* genotype

Wild-type phenotype

Wild-type phenotype

5. Punnett Square:

	<i>dR</i>	<i>DR</i>
<i>DR</i>	<i>DdRR</i>	<i>DDRR</i>
<i>Dr</i>	<i>DdRr</i>	<i>DDRr</i>

6. 1/4 of the F₂ offspring, if allowed to self-fertilize, would be able to produce at least a few offspring that were dumpy rollers.

7. Stripes are inherited as an incompletely dominant allele:

Let gene **B** = stripes

BB = dark prominent stripes

Bb = medium stripes

bb = few stripes

Parents = medium stripe (**Bb**) X dark stripes (**BB**)

Offspring: 50% dark stripes and 50% medium

	B	b
B	BB (dark)	Bb (medium)
B	BB (dark)	Bb (medium)

Materials

	Item	Vendors	Catalogue #
<i>Chemicals:</i>	S Basal buffer	IPM scientific http://www.ipmscientific.com	11006-503
	NaCl	Sigma	S9888
	MgSO ₄ -7H ₂ O	Sigma	M9397
	CaCl ₂	VWR	
	KPO4 buffer	VWR	
	Peptone	Difco	
	Bacto-agar	Difco	
	Bactotryptone	Difco	
	Cholesterol	Baker, 100g for \$34.11	STF676-5
	Glycerol	VWR	
<i>Materials:</i>			
	Dissecting microscopes (1 per pair of students)	It is imperative that you have underneath illumination of the worms (frosted mirror with illumination from the top of the scope works best.)	
	32-gauge platinum wire (makes about 20 worm spatulas, which can be re-used)	SPI 1-800-2424SPI, 1 ft for \$48.14	01707-AC
	6-cm culture dishes (1 per student, plus 20 for preparing worm stocks)	Can be purchased pre-made with antibiotics at http://www.ipmscientific.com/nematode.htm	11006-500
	Sterile boxes to store worm plates (stores 60 plates total)	Plastic box towers with drawers from Walmart, 3-drawer containers	
	24 worm "spatulas"	Made with glass pipettes and 32 gauge platinum wire before lab.	
	12 alcohol lamps and matches		
	1.2-ml Nunc CryoTubes for freezing stocks	VWR, 100 for \$43.52	#16001-100

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About the Authors

Peggy Brickman received her bachelor's degree in biology from Columbia College in 1987, and her Ph.D. in genetics from U.C. Berkeley in 1993. She is an assistant professor of plant biology at the University of Georgia where she teaches non-majors introductory biology to approximately 650 students each semester. She is currently researching the effectiveness of technology in biology education, particularly to aid large-group instruction. She has been actively involved in developing a number of CD-ROM and web-based activities for her course and its lab component. She wrote this lab as part of a NSF-CCLI research project to convert all the non-majors labs to an inquiry-based approach aimed to increase science literacy skills rather than just acquisition of knowledge.

Cara Gormally received her bachelor's degree in philosophy from St. John's College in 2002, and is currently a graduate student in plant biology at the University of Georgia, studying evolutionary ecophysiology. She is involved in a collaborative effort to convert non-majors labs to inquiry-based labs and she has taught the pilot labs for the past two semesters. She is currently researching how students' writing may reflect changes in their science literacy skills and understanding of the scientific process. She became interested in whether guided questions which are incorporated into lab material might decrease student reliance on instructor guidance after teaching new inquiry lab material and finding that student groups may rely heavily on prompting from the instructor. In her other life, her dissertation research focuses on understanding the ecological and evolutionary responses of plant populations to selective pressures in the extremely dynamic coastal dune habitat.

Appendix A
Recipes for Culturing Solutions

NGM agar:

NaCl	3.0	grams
Peptone (Difco)	2.5	grams
Bacto-agar (Difco)	17.0	grams
Dissolve in dH ₂ O	975	ml

After autoclaving add sterile:

(5 mg/ml) cholesterol in ethanol	1	ml
1 M CaCl ₂	1	ml
1 M MgSO ₄	1	ml
1 M KPO ₄ (pH 6.0)	25	ml

This typically can be used to pour 120 5-cm plates. Store at room temperature for a day or two before use to allow the plates to dry and check for contamination. They can then be stored in the refrigerator for weeks in the same bags the plates came in.

B broth:

NaCl	5.0	grams
bactotryptone	10.0	grams
dH ₂ O	Add to 1	liter

S Basal Buffer:

NaCl	5.8	grams
1 M KPO ₄	50.0	ml
dH ₂ O	950	ml
(5 mg/ml) cholesterol in 95% ethanol	1	ml

Swirl to disperse and then autoclave.

Freezing Solution:

1 M NaCl	200	ml
1 M KPO ₄ (pH 6.0)	100	ml
glycerol	600	ml
dH ₂ O	1100	ml

Distribute to 200 ml bottles: autoclave

Add 0.06 ml sterile 1M MgSO₄ per 200 ml bottle

Appendix B

Timetable For Lab Preparation

- Several weeks ahead:*
1. Obtain OP50 stock
 2. Prepare about 150 NGM agar plates, after leaving at room temperature for a few days, they can be stored at 4°C in their original bags for weeks.
 3. Steak out OP50 on an NGM agar plate, grow overnight at 37°C. Prepare a liquid culture in LB broth by growing overnight and then store the plate with parafilm and the culture bottle at 4°C.
 4. Obtain *C. elegans* stocks including N2 wild-type with males
 5. Seed a few NCM agar plates with OP50 cultured overnight at 37°C or at room temperature for a day or two.
 6. Chunk-out the stocks when they arrive from in the mail.
- Two week before lab week 1:* Check to make sure mutant stock plates are healthy. Set up fresh plates with several healthy L4 hermaphrodites onto 4 plates and place at 15°C. Make sure you set up a couple of mating plates with 4 L4 hermaphrodites for every 16 wildt-type N2 males. Set up mating plates of *lon-2/lon-2* X *+/+* animals and place on fresh plates at 15°C. Select *lon-2* males
- One week before lab week 1:* Check to make sure mutant stock plates are still healthy. Set up fresh plates with several healthy L4 hermaphrodites for each mutant strain onto 4 plates and place at 15°C. Make sure you set up a 6 mating plates of wild-type N2 males. Set up 3 mating plates for each of the mystery mutants.
- Prepare P generations: ***dpy-5/dpy-5*** X *+/+* wildtype males
 - Prepare P generations: ***lon-1/unc-76*** X *+/+* wildtype males
 - Prepare P generations: ***lon-2*** males X *+/+* wildtype hermaphrodites
 - Prepare P generation: ***dpy-10/dpy-10*** X *+/+* wildtype males
- 2 days before lab week 1:* Seed 20 NGM agar plates with OP50 cultured overnight at 37°C and then placed at 4°C. For each lab section of 20 students.
- One week before lab week 2:* Set up fresh plates with several healthy L4 hermaphrodites for each mutant strain onto 4 plates and place at 15°C. Make sure you set up a 6 mating plates of wild-type N2 males. Set up 3 mating plates for each of the mystery mutants.
- Prepare P generations: ***dpy-5/dpy-5*** X *+/+* wildtype males
 - Prepare P generations: ***lon-1/unc-76*** X *+/+* wildtype males
 - Prepare P generations: ***lon-2/lon-2*** X *+/+* wildtype hermaphrodites
 - Prepare P generation: ***dpy-10/dpy-10*** X *+/+* wildtype males
- 2 days before lab week 2:* Seed 20 NGM agar plates with OP50 cultured overnight at 37°C and then placed at 4°C. For each lab section of 20 students.

Appendix C

Genetic Stocks for Generating Mystery Mutants

<i>dpy-5 (e61)</i>	Linkage group (I) <i>dpy-5</i> encodes a cuticle collagen. <i>e61/e61</i> strong dumpy, <i>e61/+</i> medium dumpy. Recessive allele http://www.wormbase.org/db/gene/gene?name=dpy-5
<i>dpy-10 (e128)</i>	Linkage group (II) <i>dpy-10</i> encodes a cuticle collagen, <i>e128/e128</i> small dumpy non-roller. Incompletely dominant allele. http://www.wormbase.org/db/gene/gene?name=dpy-10
<i>lon-1 (e185)</i>	Linkage group (III) <i>lon-1</i> encodes a protein belonging to the PR-protein superfamily that is a target of DBL-1 (TGF-beta) signaling, and that regulates polyploidization and body length, <i>lon-1/lon-1</i> is 50% longer than wt, eggs are even elongated. Recessive allele. http://www.wormbase.org/db/gene/gene?name=lon-1
<i>unc-76 (e246)</i>	Linkage group (V) <i>unc-76</i> codes for a kinesin-associated fasciculation and elongation protein involved in axonal transport. <i>unc-76/unc-76</i> sluggish will tend to curl or coil. Recessive allele http://www.wormbase.org/db/gene/gene?name=unc-76
<i>lon-2 (e678)</i>	Linkage group (X) <i>lon-2/lon-2</i> is 50% longer than wt at all stages. X-linked recessive allele http://www.wormbase.org/db/gene/gene?name=lon-2
