A Scenario-Based Study of Root Tip Mitosis

Janice M. Bonner
College of Notre Dame of Maryland
4701 North Charles St.
Baltimore, MD 21210

This workshop presents a scenario-based method of introducing students to the cell cycle. In the scenario, students are presented with a crop that might be affected by a soil fungus that secretes a lectin-like substance into the soil. In a blind study of exposed and unexposed root tips, students stain the tips, count the cells, compare the percentage of cells in mitosis in the two sets of tips, and use chi-square analysis to determine whether the difference is significant. This activity can be carried out in a 3-hour laboratory period if adequate pre-lab preparation is done by students.

Introduction

Participants will be given the opportunity to experience this laboratory exercise as a student in an introductory biology course. They will be led through a discussion of the problem found in the scenario. They will be presented with the question: How can you determine whether mitosis is proceeding quickly or slowly if you are only observing dead root tip cells? They will be led through an analogy that helps them to make the connection between the time needed to a stage of the cell cycle and the number of cells observed in that stage. Time will be allotted for groups of participants to stain and squash the two sets of root tips and obtain sufficient class data for analysis. Participants will be led through steps of data analysis to convert total number of cells in each cycle stage to percentage of cells. They will be led through setting up a contingency table and carrying out a chi-square analysis.

Student Outline

Prior to beginning the laboratory session, students are presented with five pieces of background information. Students must prepare answers to all the bulleted questions as their pre-lab assignment.

1. The first part of the background is a letter addressed to the president of the college that asks for the involvement of students in the introductory biology course.

CROPS WITHOUT BOUNDARIES

Mary Pat Seurkamp, President
Dear Dr. Seurkamp,

Let me introduce myself and explain the package that I hope will arrive soon at the Notre Dame campus in Baltimore. I am Anna Fazio, a graduate (’79) of the Biology department of College of Notre Dame of Maryland. Currently, I am working as a mycologist with Crops without Boundaries (CWB), a consortium of private foundations and universities. The goal of CWB is to develop and promote sustainable agriculture in developing areas of the world. Agricultural specialists in Thailand recently have suspected the involvement of a soil fungus in the death of many soybean plants (*Glycine max*) and they requested a mycologist to help with its discovery and identification. When I arrived, I soon found a fungus in many samples of the local fields, generally planted with soybeans. This fungus appears to thrive in the near-anaerobic conditions resulting from heavy rains. I noticed that the roots of many soybean plants looked unusual when I dug around them to obtain my soil samples. Our current hypothesis is that the soil fungus interferes somehow with the growth of the roots, because the roots are in greatest contact with the soil.

We are planning to conduct our research on two fronts. First, we are studying the fungus to identify it and to determine if it produces a toxin. This work is being carried out by mycologists and chemists at CWB. On the second front, we would like to know more about the effect of the fungus on *G. max.* It struck me that this aspect of the research could be addressed by some NDM biology students—can they determine whether the fungus has an effect on the growth of the roots of *G. max?* I have taken

the liberty of assuming that some biology students, always ready for a challenge, will be able to help us, so I have supplied you with two sets of root tips of *G. max*, shipped in 70% ethanol. (International law prohibits the transport of the actual fungus out of the country.) One set of tips is from *G. max* plants exposed to the fungus, and the other is from the same plants without this exposure. I hope your students will be able to apply their understanding of biological concepts to this very real problem.

Sincerely,
Anna Fazio, NDM '79, Ph.D.
Supervising Mycologist, Crops without Boundaries

2. The second part of the background is an analogy that prepares students to think about the situation with which they will be confronted when they develop their hypothesis.

Developing an Analogy

For the sake of this analogy, let's say that everyone who eats lunch in the cafeteria takes 30 minutes to do so. Estimate how much of that time would be spend on each of these aspects of getting lunch. Be sure the total is 30 minutes.

Checking in	____
Getting a tray	____
Standing in line to get food	____
Selecting your food	____
Finding a seat	____
Eating	____
Getting ice cream	____
Cleaning up	____

Now, let's say a photographer goes into the cafeteria at lunchtime with a wide-angle lens camera. She stands in the center of the room and takes pictures around the circumference of the entire room.

- What would most of the people in the pictures be doing?
- What would the fewest people in the pictures be doing?
- What is the connection between the number of people doing an activity in the pictures and the amount of time spent doing that activity?

Now, let's say the management of the cafeteria decided to change the system to make it more "eater-friendly." They streamline the process so that you only have to spend 2 minutes standing in line.

You still have 30 minutes for lunch. So now, redistribute the time. Again, make sure the total number of minutes is 30.

Checking in	____
Getting a tray	____
Standing in line to get food	____
Selecting your food	____
Finding a seat	____
Eating	____
Getting ice cream	____
Cleaning up	____

To get a photo history of the effects of this system change, the photographer comes back to take a second set of pictures. She again stands in the middle of the room and takes pictures of the entire circumference of the room.

- What will most people in the pictures be doing now?
- What will the fewest number of people be doing?
- What is the connection between the number of people doing an activity in the pictures and the amount of time spent doing that activity?
- What effect did speeding up the time spent standing in line have on the number of people in the two sets of pictures?

3. The third part of the background is an explanation of the staining protocol the students will be using.

The Feulgen -Schiff DNA Reaction

The Feulgen-Schiff's Reaction is a simple staining procedure that is specific for DNA. It works in the following manner:

Step 1: The root tip is treated in strong (6 M) hydrochloric acid (HCl), which breaks the hydrogen bonds that hold the complementary nucleotides of the DNA ladder together at the rungs, splitting the double helix of DNA apart into single strands. The acid treatment then removes the purine bases, leaving an exposed aldehyde group where each purine had been located on the deoxyribose.
Step 2: The root tip is placed in Schiff's Reagent, which contains carbol fuschin, a dye. The dye molecules bind at the exposed aldehyde sites on the depurinated DNA and, in doing so, become a reddish-purple color. Whole tissue samples can be treated by this protocol and then the nuclei can be viewed under the compound microscope.

Constance G. MacIntosh and Bailey L. Porter. (1996). *Basic Histological Methods*. New York: Wiley-Liss.

- What would be the overall appearance of the nucleus of a non-dividing cell after this staining? (HINT...How is the DNA distributed in the nucleus: as chromosomes or as chromatin?)
- What structures in the nucleus would be evident in dividing cells following this staining? Why? (See the HINT above.)

4. The fourth part of the background is practice in identifying stages of the cell cycle.

Identifying cells in the cell cycle
Students download the following webpage - (http://porpax.bio.miami.edu/~cmallery/150/mitosis/c8.12.concept.onion.jpg), print it out, and identify at least two cells each in interphase, prophase, metaphase, anaphase, and telophase.

5. The fifth part of the background is an explanation of how to set up and use a contingency table for chi-square analysis.

Setting up and using a contingency table for chi-square analysis

You know that chi-square is used to statistically analyze data that is categorical in nature. A chi-square test determines whether the difference between observed and expected results is significant. Before you can statistically analyze the data from this experiment, however, you have to organize it in a contingency table to obtain your expected values. Here is a sample problem:
Professor Marge Genovera has hypothesized that leaders are recognized because of their height: tall people are leaders, short people are followers. She conducted a survey of 95 students in which she measured their height and studied their leadership skills. She entered her observed data into a contingency table. To determine each expected value, multiply the *row combined total* times the *column combined total* and divide this product by the *overall total*. The first two expected values have been determined for you:
(36 x 43) / 95 = 16.3 and (36 x 52) / 95 = 19.7.

- Calculate the other expected values and enter them in the contingency table.

Variable	Group		Combined
	Short	Tall	
Followers	22 16.3	14 19.7	36
Unclassifiable	9	6	15
Leaders	12	32	44
Total	43	52	95

- Enter all the observed and expected values in a chi-square entry table in two columns: observed and expected. Calculate chi-square. The only difference is this: the *df* = (r – 1) (c – 1), not the value for *df* given in the chi-square results.

- Is Marge Genovera's hypothesis supported by her survey? Explain.

This is the information that is provided to students prior to conducting the experiment.

STAINING AND OBSERVING CELLS IN THE CELL CYCLE

Preparing the chromosome squash slides

You will carry out the procedure below for two sets of root tips—one set of tips from plants exposed to the fungus and one set of tips from plants not exposed to the fungus. You will conduct the experiment blind; that is, you will not know which tips are which until you have completed your analysis. The tips will be referred to as "blue" and "red" tips to distinguish between them.

Use this procedure to stain and squash one tip at a time. Do not set up an "assembly line" or stain/squash more than one tip at a time because the cells will dry up before you can observe them.

Label a small beaker for HCl and another for Carnoy fixative. Use forceps to remove a tip from either jar of the class supply of root tips and transfer it to the beaker of 6 M HCl. Allow the tip to remain in the acid for 4 minutes. Then transfer the tip to the beaker of Carnoy fixative. Allow the tip to remain in the fixative for 4 minutes. Meanwhile, remove several slides and coverslips from the 70% alcohol; dry and label them. Place the tip on a microscope slide. With a razor blade, cut off the distal 2 mm portion of the root tip. Discard the remainder of the tip. Cover the short piece of root tip with 2 drops of carbol fuschin stain. After 2 minutes, blot away the excess stain with a Kimwipe,

trying not to touch the root tip. Cover the stained tip with 1 or 2 drops of dH_2O. Gently lower a coverslip over the root tip. Cover the coverslip with a Kimwipe and, with your thumb, firmly press down. The pressure will spread the cells into a single layer. Be careful not to twist the coverslip. If the tip is difficult to squash using this method, press down on the coverslip with the eraser end of a pencil. Use this method to stain and squash three tips from each set.

Collecting the data

It is best to study your squash preparations in areas where the cells are one cell thick so that you do not see overlapping and confusing images. Therefore, observe the preparation under the low magnification scanning objective (4X) to locate areas that have a pattern of distinct nuclei arranged in rows. Place one of these areas in the center of the field of view and focus on it. Increase the magnification to low power (100X) and find a section in which you can distinguish the cells, the nuclei and the chromosomes within some of the nuclei. Now, increase the magnification to high power (430X). Fill the field of view with an area of the squash with clearly distinct, well stained, cells and nuclei. If you can determine the state of each of the cells that you can see in the field, the slide is a good candidate for the next phase of the protocol. Move the slide to the microscope in the lab to which the camera is attached. Use the directions at the camera to download the picture. **D**etermine the stage of the cell cycle for each of the cells in the image. Record your observations.

Table 1. Individual group identification of cells in "blue" *G. max* root tips

.

Tip	Number of cells				
	Interphase	Prophase	Metaphase	Anaphase and Telophase	Total
1					
2					
3					
Total					

Table 2. Individual group identification of cells in "red" *G. max* root tips

.

Tip	Number of cells				
	Interphase	Prophase	Metaphase	Anaphase and Telophase	Total
1					
2					
3					
Total					

Table 3. Class totals of cells in two sets of *G. max* root tips.

Exposure to fungus	Number of cells				
	Interphase	Prophase	Metaphase	Anaphase and Telophase	Total
Exposed					
Unexposed					

Analyzing the Data

Because the data are categorical, you will use chi-square to conduct your statistical analysis. Use the contingency table below to determine the expected values. Then compare the observed and expected values in *BioStats*.

Table 4. Contingency table to compare interphase and mitotic cells in class data.

Variable	Group		Combined
	Unexposed tips	Exposed tips	
Interphase cells			
Mitotic cells			
Total			

Assignment

Go to http://labwrite.ncsu.edu/www/. Select postLAB, Guide to Writing Partial Lab Reports. Write the parts in the order suggested in LabWrite, but assemble them in the correct order before you hand in your report.

You are responsible for a partial lab report that includes:

- Title—separate page
- Introduction— entire section; follow the specific directions in LabWrite; must include two citations (but not the lab directions) each in a different CSE format
- Methods— entire section; must include a citation; follow the specific directions in LabWrite
- Results—entire section; follow the specific directions in LabWrite; refer specifically to the figure(s) at least once
- Figures—must include at least one bar graph with error bars
- Discussion—bulleted items only
- References in Name-Year format

Fazio, A.W. (2008). Preliminary report of the effect of the fungus *Rhizoctonia anaerobis* on the growth of *Glycine max. Journal of Mycological Interactions*; 56, 47.

Mycologists working in Thailand report that a recently identified fungus, *Rhizoctonia anaerobis*, may be implicated in the death of several seasons of growth of *Glycine max.* Following periods of heavy rain that occurred during the growing seasons of 2003-2006 (total rainfall > 50 cm), agricultural observers noted that the normal growth rate of *G. max* decreased significantly ($p < .005$). Preliminary examination of these plants suggests that the root systems are not developing adequately. Examination of the soil in which the plants are grown reveals the presence of the fungus *R. anaerobis*. Initial chemical analysis suggests the presence in the soil of a lectin-like substance, presumably a secretion of the fungus. Lectins are known to accelerate mitosis.

Materials

Per lab group:

Bench paper
Coplin jar of 70% ethanol with 5-6 slides
Syracuse dish of 70% ethanol with 5-6 cover slips
Microscope
Sharpie
Label tape
Razor blade
Fine forceps
Scissors
Dropper bottle of dH_2O
Dropper bottle of carbol fuschin (Ziehl-Neelsen)
60mL beaker of ~ 20 ml 6M HCl
60mL beaker of ~ 20 mL Carnoy's fixative
2- 60mL jars with lids of ~ 20 mL 70% ethanol each
Kimwipes
100mL beaker of ~ 80 mL dH_2O (to dip forceps into after acid to prevent damage to forceps)

Per lab section:
~ 100 unexposed onion root tips, in ~ 50mL 70% ethanol in 100 mL beaker: red tape
~ 100 exposed onion root tips, in ~ 50mL 70% ethanol in 100 mL beaker: blue tape
Extra slides and coverslips
Equipment for capturing images, for example, Moticam

Preparing onion root tips
Can use bulblets from several sources: Carolina Biological P/N 17-1143 or onion sets from seed store. Bulblets can be stored for 4-5 months at 4°C and still be used. Each bulblet will yield 15-25 root tips.

- Obtain two jars with ~10-cm diameter opening, straight sides, and loose-fitting lids. Fill both jars with ~ 1.5-cm of fine sand. Label one jar "Unexposed" and one "Exposed."
- Wet down the "Unexposed" sand thoroughly with dH_2O.
- Wet down the "Exposed" sand with lectin solution. This will probably take 50-75 mL.
- Refrigerate the remaining lectin solution.

To prepare bulblets:
- Prepare ~6 bulblets for each jar
- Peel off the dried outer skin of each bulblet.
- Make a long incision into the top pulp layer and pull it entirely off.
- Use a razor blade to cut off the dried roots back to the bulb base.
- Insert the bulblets into the moistened sand until they touch bottom.
- Place the lids on the jars and store in dark for 1½ -2 days. Tips should be ~ 2 cm long.

To harvest the tips:
- Put on disposable gloves.
- Pull bulblets out one at a time from jar and rinse the sand off in a 600 mL beaker of 500 mL dH_2O.
- Use fine dissecting scissors to cut off the roots of each bulblet, cutting back to the base of the bulblet.
- Have 2 100-mL beakers of Carnoy's fixative, each with ~50 mL of fixative, one for each set of root tips.
- Place cut tips directly into Carnoy's fixative for 4 hours. (They can remain in the fixative for up to 18 hours.)
- Decant off Carnoy's fixative, rinse tips with ~ 25 mL 705 ethanol. Place tips in beakers of 70% ethanol for storage. Parafilm and store at 4°C.
- Carolina Biological claims that these root tips can be used for up to 1 year. We have found that as the tips age, they become harder to squash.

Solutions:
70% ethanol (2 L)
1400 mL 95% ethanol (reagent grade ethanol is also OK to use)
600 ml dH_2O

Lectin (200 mL)
10 mg lectin (Sigma-L-2646) dissolved in 200 mL dH_2O.
Store in 250 mL amber bottle.

6M HCl (100 mL)
50 mL concentrated HCl added to 125 mL Nalgene bottle of 50 ml dH_2O.

Carnoy's fixative (500 mL)
125 ml glacial acetic acid added to 500 mL Nalgene bottle of 375 mL 95% ethanol.

Carbol-fuschin stain (Ziehl-Neelson) ~ 100 mL
Dissolve 0.30g basic fuschin in 9.5 mL 95% ethanol
Dissolve 5.0g phenolin in 85 mL dH_2O
Combine two solutions
Add 0.4 mL methyl isobutyl ketone (MIBK)
Add 0.1 mL of hexane

Notes for the Instructor

In our experience, this laboratory exercise only works if students can collaborate on their identification of cell stages. If you do not have a way to make group viewing of each squash possible, this experiment will not work because it will rely on the identification skills of individual students working alone.

Preparing the solutions:
Reagent grade ethanol can be substituted for 95% ethanol with no compromise of integrity of the experiment.

Prelab comments:

We have completed our discussion of the cell cycle prior to beginning this laboratory exercise.

It is essential to the understanding of the laboratory exercise that students understand how "pictures" of dead cells can reveal information about the rate at which the cells were dividing. That is the reason for the cafeteria analogy. After we discuss the cafeteria situation, I ask the students to identify each part of the analogy in the exercise they will be conducting: who /what is equivalent to the cafeteria, students in the cafeteria, activities carried out by students in the cafeteria, photographer, and pictures taken by the photographer. The interpretation, of course, is that cafeteria = the root tip, students in the cafeteria = cells in the root tip, activities carried out by students in the cafeteria = stages of the cell cycle, the photographer = people carrying out the experiment, and pictures taken by the photographer = microscope views of each root tip. The main conclusion for students to draw from this analogy is that the number of cells in a particular stage of the cell cycle is dependent on the amount of time that the average cell spends in that stage. The longer the stage of the cycle, the greater number of cells that can be expected.

Once this is established, I ask students to develop their hypothesis. I refer them to the diagram of the cell cycle in their textbook and ask them to interpret it in the light of the time relationships we have just discussed. Then I ask them to fill in the blanks in the following statements:
If mitosis is speeded up in the exposed root tips, there will be (more/fewer) cells in _____ than in _____.

If mitosis is slowed down in the exposed root tips, there will be (more/fewer) cells in _____ than in _____.

I ask the students to work together in their lab groups to complete the two statements. Then we compare notes. The most usual result of their hypothesis development is:
If mitosis is speeded up in the exposed root tips, there will be (more/fewer) cells in mitosis than in interphase
If mitosis is slowed down in the exposed root tips, there will be (more/fewer) cells in mitosis than in interphase.

This requires an extended discussion through which they realize why their hypotheses are incorrect. Admittedly, I have set them up to form these incorrect statements. But I have learned that if the class doesn't labor over the understanding of the hypotheses, they will never understand the data. Eventually, we arrive at the correct hypotheses:
If mitosis is speeded up in the exposed root tips, there will be (more/fewer) cells mitosis than in the unexposed root tips.
If mitosis is slowed down in the exposed root tips, there will be (more/fewer) cells in mitosis than in the unexposed root tips.

It is also helpful to provide students with an opportunity to identify cells at various stages of the cell cycle before they view their squashes. This webpage is a good sample of cells. http://porpax.bio.miami.edu/~cmallery/150/mitosis/c8.12.concept.onion.jpg. Alternately you can provide them with a page from a textbook. *Molecular Biology of the Cell* 3rd edition (1994). Alberts, B et al. has such a picture on p. 862.

Conducting the experiment:
The staining procedure that we use generates good stains in about 90% of the tips. Some tips just don't stain well and should not be used.

The lectin-exposed tips are harder to squash than the unexposed tips. Sometimes, we have had to use pencils or flattened glass stirring rods to obtain adequate squashes.

Make sure students squash the tips hard enough to produce a single layer of cells. The cells should look like an aerial view of a parking lot if the squash is good. A squash with layers of cells that overlay each other is very difficult to analyze. To illustrate this, I make a transparency of the page of cells they used for their pre-lab assignment and fold it over so that there are two layers of cells superimposed.

This exercise only works if students can collaborate on their identification of cell stages. If a single student is making the identification, the laboratory does not work.

We generally have each lab group obtain results from at least two unexposed and two exposed root tips. If the lab section is made up of 6 groups, this provides sufficient data for further analysis. Ideally, each group should stain/squash three of each type of tip.

As the experiment progresses, I have student groups record their results for exposed and unexposed tips on the board. This enables me to see easily whether a group is mis-identifying cells; for example, if a group records that they observed 95 out of 112 cells in anaphase, most likely they didn't correctly identify a cell in anaphase.

Data analysis:
Students must understand why absolute numbers of cells in each stage cannot be compared to each other, but must instead be converted to %. Students also must remember that, when using a contingency table to determine expected results, df will not be (number of observed/expected pairs) – 1 but will instead be the product of (rows -1) x (columns -1).

I wait until after the students have completed the laboratory exercise and the data analysis to reveal which set of root tips is which. What is nice about this exercise is that, whether the data support the hypothesis or refute it, students can still complete their report. I distribute the "journal article" from Dr. Fazio to students by e-mail after the lab is completed.

About the Author

Janice Bonner has been a member of the biology department at College of Notre Dame of Maryland for over 20 years. Her primary teaching responsibilities are general education courses and the introductory course for biology majors