

# Environmental Effects on Mitosis

## AP\* Biology Big Idea 3, Investigation 7

### An Advanced Inquiry Lab

#### Introduction

All new cells come from previously existing cells. New cells are formed by the process of cell division, which involves the replication of the cell's internal structures and the division of the cytoplasm (*cytokinesis*).

#### Concepts

- Biotic and abiotic factors
- Chi-square analysis
- Mitosis
- Cell cycle
- Interphase
- Plant hormones

#### Background

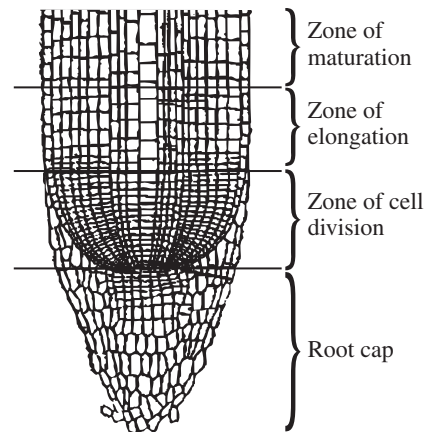
The health of a plant or animal depends upon both biotic and abiotic factors. Imagine the parking lot of your school. A few plants may be growing in cracks and crevices of the pavement. In these cracks there is at least a subsistence level of nutrients and water for a plant to survive. A few meters away an unpaved area with soil and little foot traffic may have more plants. The plants compete for space, water, nutrients, and light but, more than one plant is in the soil area. If you were to compare plants from the paved and soil areas you would likely see differences in the height of the stems, the number of leaves, and the number and length of the roots. This is a simple example of abiotic factors in the environment affecting plant growth.

Many biotic factors also affect plant growth. A classic example of a beneficial biotic effect is the mutualistic relationship between legumes (beans, peas, clover, and alfalfa) and the nitrogen-fixing bacterium, rhizobia. *Rhizobia* (singular=rhizobium), live in nodules on the roots of beans and other plants. Chemicals released by the plant cause the bacterium to migrate toward the plant roots. Entry of the bacterium into the root causes a cascade of cell signals. That area of the root enters a phase of rapid cell division producing a nodule where the bacteria flourish.

Not all biotic interactions benefit a plant. Parasitic interactions may harm a plant by increasing mitosis. For example, the plant pathogen *Agrobacterium tumefaciens* (now called *Rhizobium radiobactor*) causes plant cancer or galls. By triggering a plant to undergo rapid cell division, the pathogen forces the plant to expend more energy in that location and not in the other roots, stems, and leaves. This weakens the plant and may cause death.

*A. tumefaciens* enters the plant through a wound and infects cells by inserting a plasmid into the cell. The plasmid inserts into the DNA and causes several important genes to be transcribed. One gene codes for the plant hormone indole-3-acetic acid, IAA. IAA is a plant hormone that triggers cell division—it has been used commercially as a rooting compound for many years.

The plant of choice when studying mitosis is the common onion. Onions germinate easily without soil so the chemicals provided to the plant can be easily controlled. Onion root tips also grow quickly and are only a few cells thick. A stain is used to dye condensed chromosomes like those undergoing mitosis a very dark color. By viewing the onion root tip using a light microscope it is easy to determine if a particular cell is in interphase or mitosis. See Figure 1 for a graphical representation of the anatomy of an onion root tip. Note that cell division occurs only in the meristem region, not in the other regions of the root tip. Recall also that 90% of the time a cell in this region will be in interphase, since mitosis typically makes up only 10% of a full cell cycle. Onions



**Figure 1.** Apical meristem

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are alive and therefore the onion slide preparation will have more than one layer of cells present in each preparation. In order to reduce the total depth of the slide preparation the onion root tip needs to be treated and then squashed between the cover slip and the microscope slide.

## Experimental Overview

Onion root tips germinated in a solution containing IAA will be compared to onion root tips germinated in water only. A minimum number of cells will be tallied to determine the percentage of cells in interphase and mitosis for each treatment. The data from this baseline activity will be analyzed using a Chi-square statistical analysis test to determine if any observed variation in percent mitosis is statistically significant. The results of this baseline activity will provide a procedure and model for open inquiry and student-designed experiments—see the *Opportunities for Inquiry* section on page 4 for further information. Explore biotic and abiotic factors that may affect the rate of mitosis in plants.

## Materials

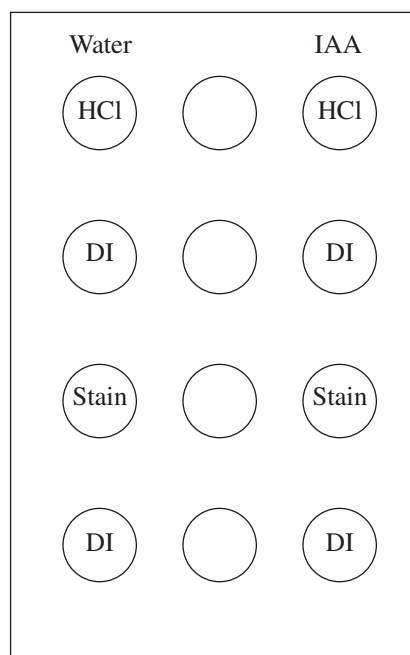
Hydrochloric acid, HCl, 1 M, 2 mL	Glass slides, 6
Onion root tips, control treatment, 3	Compound microscope with 40X objective
Onion root tips, IAA treatment, 3	Paper towels
Carbol fuchsin solution, 2 mL	Pencil with eraser
Deionized water (DI)	Permanent marker or wax pencil
Cover slips, 6	Pipets, disposable, 2
Dissection scissors	Spot plate
Forceps	

## Safety Precautions

*Hydrochloric acid solution is toxic by ingestion or inhalation and corrosive to skin and eyes. Carbol fuchsin solution contains phenol and is moderately toxic by ingestion and inhalation. Avoid breathing the vapors. It is a skin irritant and a permanent stain that will stain skin, clothes, and other objects. Wear chemical splash goggles, chemical-resistant neoprene gloves, and a chemical-resistant apron. Avoid contact of all chemicals with eyes and skin and wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.*

## Baseline Activity

- Fill the spot plate as follows: top row with 1 M hydrochloric acid, second and fourth rows with deionized water, and the third row with stain.
- Cut three roots from actively growing onions using dissection scissors. Remove the entire root. Only harvest roots that are less than 1.5-cm long.
- Trim the tip of each root to 0.5 cm. Only the tapered end of the root tip will be used. The remainder may be discarded in the trash.
- Soak each tip in the hydrochloric acid treatment for 5 minutes.
- Rinse in the deionized water for 1 minute.
- Stain for 2–3 minutes.
- Rinse in deionized water for at least 1 minute. The water may need to be changed. The final color of the rinse water should be a clear raspberry.
- Prepare squash mounts of the onion root tips.
- Locate the meristem area of the root tip and identify mitosis or interphase for a minimum of 300 cells or completely count two fields of view, whichever is greater.
- Pool data with that of the other lab groups.



## Disposal

Consult your instructor for appropriate disposal procedures.

## Statistical Analysis

The observed distribution of onion root tip cells in mitosis versus interphase for the treated samples will likely not coincide exactly with the percentages observed for the control group. The question, however, is whether the difference is statistically significant, that is, whether the observed difference in the percent mitosis for the two sets of samples may be due to chance. A chi-square ( $\chi^2$ ) “goodness of fit” test is commonly used to determine whether a frequency distribution of results in various categories (in this case percent mitosis versus interphase) fits a predicted or expected distribution. Applying this statistical test to experimental results is done by formulating the so-called *null hypothesis* in which the observed distribution for the treated group can be described by the expected or control distribution.

The chi-square statistic is calculated using the following formula:

$$\chi^2 = \sum \frac{(o_i - e_i)^2}{e_i} + \frac{(o_m - e_m)^2}{e_m} \quad \text{Equation 1}$$

where  $o_i$  is the number of cells observed in interphase  
 $e_i$  is the number of cells expected to be in interphase  
 $o_m$  is the number of cells observed in mitosis  
 $e_m$  is the number of cells expected to be in mitosis

The calculated chi-square value is then compared with a critical value ( $\chi^2_c$ ) that depends on two factors, the degrees of freedom (DF) for the distribution and the selected probability ( $p$ ) for statistical significance (see Table 1). The degrees of freedom is equal to the number of categories for the results ( $k$ ) minus one ( $DF = k - 1$ ). The results in this experiment fall into two categories (percent mitosis and percent interphase), so  $DF = 1$ . The probability is usually selected at a 95% confidence level,  $p = 0.05$ , corresponding to a 5% probability that the observed difference is due to chance.

Probability	Degrees of Freedom (DF)				
	1	2	3	4	5
0.1	2.71	4.61	6.25	7.78	9.24
0.05	3.84	5.99	7.82	9.49	11.1
0.01	6.64	9.21	11.3	13.2	15.1
0.001	10.8	13.8	16.3	18.5	20.5

**Table 1. Critical Values**

**If the calculated chi-square value is greater than the critical value** obtained from the table for the degrees of freedom and the selected probability value, then the **null hypothesis is rejected** and the observed difference in percent mitosis or rate of mitosis between the treated sample and the control sample is considered statistically significant.

1. The number of cells in interphase and mitosis for the IAA-treated root tips will be the observed ( $o$ ) values.
2. To calculate the expected values, complete the following steps.
  - a. Determine the percentage of cells in interphase and mitosis in the control group (root tips germinated in water).
  - b. Multiply the percentages by the total number of cells in the IAA treatment. This will give the expected numbers ( $e$ ).
3. Calculate the chi-square ( $\chi^2$ ) value for the test (see Equation 1).
4. Compare the calculated chi-square value to the critical value found in the table above, using the information from steps 5 and 6.
5. The degree of freedom (DF) equals the number of groups minus one. In this case, there are two groups, interphase and mitosis. Therefore,  $DF = 2 - 1$ , or 1.
6. The  $p$  value is 0.05, and the critical value is 3.84. If the calculated chi-square value is greater than or equal to the table value, then the null hypothesis is rejected. If the calculated chi-square value is less than the table value, the null hypothesis is not rejected.

7. In terms of this part of the investigation, what does it mean if your null hypothesis is rejected?

## Opportunities for Inquiry

1. Consider the following questions while reflecting upon your knowledge of biotic and abiotic factors that may influence root growth and mitosis in plants.
  - a. In areas where there are very few plants growing, what biotic and abiotic factors may be affecting the rate of mitosis and the ability of plants to thrive?
  - b. What chemicals may be expected to increase or decrease the rate of mitosis in plants?
  - c. Of the factors identified in the above questions, which can be replicated as an experiment in the laboratory?
2. Plan, discuss, execute, evaluate, and justify an experiment to test a question regarding the rate of mitosis in plants.
  - a. Decide upon one question that your group would like to explore.
  - b. Develop a testable hypothesis.
  - c. Discuss and design a controlled experiment to test the hypothesis.
  - d. List any safety concerns and the precautions that will be implemented to keep yourself, your classmates, and your instructor safe during the experimental phase of this laboratory.
  - e. Determine what and how you will collect and record the raw data.
  - f. How will you analyze the raw data to test your hypothesis?
  - g. Review your hypothesis, safety precautions, procedure, data tables, and proposed analysis with your instructor prior to beginning the experiment.
  - h. Once the experiment and analysis are complete, evaluate your hypothesis and justify why or why not the hypothesis was supported by your data.
  - i. Present and defend your findings to the class.
  - j. Make suggestions for a new or revised experiment to modify or retest your hypothesis.

# Teacher's Notes

## Environmental Effects on Mitosis

### Materials Included in Kit (for 8 groups of students)

Carbol fuchsin solution, 100 mL	Cover slips, 100
Carnoy's plant fixative, 50 mL	Cups, plastic, 5 oz, 15
Ethyl alcohol, 95%, 100 mL	Glass slides, box of 72, 2
Hydrochloric acid, HCl, 1 M, 100 mL	Pipets, disposable, 32
Indole-3-acetic acid, IAA, 1 mg	Spot plates, 8
Sand, 2 kg	

### Additional Materials Needed (for each lab group)

Deionized water	Paper towels
Dissection scissors	Pencil with eraser
Forceps	Permanent marker or wax pencil
Compound microscope with 40X objective	

### Additional Materials Needed (for *Pre-Lab Preparation*)

Onion bulbs, 10+	Permanent marker
Water, tap	Plastic wrap
Graduated cylinder, 10-mL	Volumetric flasks, 1-L, 2

### Pre-Lab Preparation

1. Prepare the indole-3-acetic acid solution (1 mg/L)
  - a. In a 1-L volumetric flask, dissolve 1 mg of indole-3-acetic acid in 10 mL of ethyl alcohol.
  - b. Fill the volumetric flask to the 1-L mark with tap water and mix well.
  - c. The solution is stable at room temperature for several weeks.
2. Prepare the (control) water solution.
  - a. Add 10 mL of ethyl alcohol to a 1-L volumetric flask.
  - b. Fill the volumetric flask to the 1-L mark with tap water and mix well.
  - c. The solution is stable indefinitely if stored at room temperature.
3. Germinate root tips.
  - a. Fill 8 plastic cups about  $\frac{1}{3}$  full with sand.
  - b. Label 4 of the cups "control" and the remainder "IAA treatment."
  - c. Remove any long roots or dried roots from the bottom of each onion.
  - d. Insert onions into the cups. Place either one large onion or several small onions per cup.
  - e. Add enough control solution to the "control" cups to completely wet the sand.
  - f. Add enough of the IAA solution to the "IAA treatment" cups to completely wet the sand.
  - g. Loosely place plastic wrap on top of each cup to prevent excess evaporation.
  - h. The onions do not need to be placed in direct light but the room should be 65–75 °F. Check for root growth every day. Roots are typically ready after 36 hours.

## Teacher's Notes *continued*

3. Preserve the onion root tips as they reach 1.5–2 cm using the procedure below. (optional)
  - a. Cut roots that are longer than 2-cm and place into 10–20 mL of Carnoy's plant fixative in a labeled polypropylene jar. *Caution:* Carnoy's fixative solution is acidic and corrosive to skin, eyes, and mucous membranes.
  - b. Allow the roots to remain in the fixative for 4–24 hours.
  - c. Use forceps to transfer the roots into 10–20 mL of ethyl alcohol in a second labeled polypropylene jar. The roots may be stored indefinitely in the ethyl alcohol.

### **Safety Precautions**

*Carnoy's plant fixative contains glacial acetic acid and ethyl alcohol. Acetic acid is corrosive to skin and tissue and poses a moderate fire risk. It is toxic by ingestion or inhalation. Ethyl alcohol is a flammable liquid and dangerous fire risk—keep away from flames and other sources of ignition. It is irritating to the eyes and skin and toxic by ingestion. Hydrochloric acid solution is a corrosive liquid and is toxic by ingestion and inhalation. Carbol fuchsin solution contains phenol and is moderately toxic by ingestion and inhalation. Wear neoprene gloves and use in a chemical fume hood. It will also stain skin, clothes, and other items. Wear chemical splash goggles, chemical-resistant neoprene gloves, and a chemical-resistant apron. Avoid contact of all chemicals with eyes and skin and remind students to wash their hands thoroughly with soap and water before leaving the laboratory. Some of the chemicals used in the inquiry portion of this laboratory may be toxic or poisonous in very low concentrations. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information before using these or any other chemicals.*

### **Disposal**

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Excess hydrochloric acid may be neutralized with base and then flushed down the drain with plenty of excess water according to Flinn Suggested Disposal Method #24b. The left-over Carnoy's plant fixative and carbol fuchsin solution may be disposed of by neutralizing with base and then disposing of down the drain with plenty of excess water according to Flinn Suggested Disposal Method #24a. Ethyl alcohol may be flushed down the drain with an excess of water according to Flinn Suggested Disposal Method #26b.

### **Alignment with AP Biology Concepts and Curriculum Framework**

**Big Idea 2: Biological systems utilize free energy and molecular building blocks to grow, to reproduce and to maintain dynamic homeostasis.**

#### **Enduring Understandings**

- 2A3: Organisms must exchange matter with the environment to grow, reproduce and maintain organization.
- 2B1: Cell membranes are selectively permeable due to their structure.
- 2B2: Growth and dynamic homeostasis are maintained by the constant movement of molecules across membranes.
- 2D1: All biological systems from cells and organisms to populations, communities, and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.

**Big Idea 3: Living systems store, retrieve, transmit and respond to information essential to life processes.**

#### **Enduring Understandings**

- 3A1: DNA, and in some cases RNA, is the primary source of heritable information.
- 3A2: In eukaryotes, heritable information is passed to the next generation via processes that include the cell cycle and mitosis or meiosis plus fertilization.
- 3A3: The chromosomal basis of inheritance provides an understanding of the pattern of passage (transmission) of genes from parent to offspring.
- 3C2: Biological systems have multiple processes that increase genetic variation.

## Teacher's Notes *continued*

**Big Idea 4: Biological systems interact, and these systems and their interactions possess complex properties.**

### Enduring Understandings

4A4: Organisms exhibit complex properties due to interactions between their constituent parts.

4A6: Interactions among living systems and with their environment result in the movement of matter and energy.

### Learning Objectives

- The student can make predictions about natural phenomena occurring during the cell cycle (3A2 & SP 6.4).
- The student can describe the events that occur in the cell cycle (3A2 & SP 1.2).
- The student is able to construct an explanation, using visual representations or narratives, as to how DNA in chromosomes is transmitted to the next generation via mitosis, or meiosis followed by fertilization (3A2 & SP 6.2).

### Science Practices

- 1.2 The student can describe representations and models of natural or man-made phenomena and systems in the domain.
- 5.3 The student can evaluate the evidence provided by data sets in relation to a particular scientific question.
- 6.2 The student can construct explanations of phenomena based on evidence produced through scientific practices.
- 6.4 The student can make claims and predictions about natural phenomena based on scientific theories and models.
- 7.2 The student can connect concepts in and across domains to generalize or extrapolate in and/or across enduring understandings and/or big ideas.

### Lab Hints

- Enough materials are provided in this kit for 8 groups of students. The baseline activity can reasonably be completed in one 50-minute class period provided pre-lab discussion occurred in the previous class. The data compilation and calculations may be completed the day after the lab.
- Red kidney bean lectin (*Phytohemagglutinin*) is used in the College Board lab. Lectin is a lyophilized powder that must be kept refrigerated. It is available from Flinn Scientific as Catalog No. L0114. The recommended concentration is 10 mg in 200 mL of deionized water. Once diluted, it must be stored in the refrigerator. It is only active for a few days. Lectin increases mitosis in the roots however, it is also hemolytic and must be used with appropriate personal protective equipment to limit exposure.
- Student groups should count a minimum of 2,000 cells when analyzing their inquiry experiment. This is to compensate for the pooling of class data in the *Baseline Activity*.

### Teaching Tips

- Use prepared onion root tip slides to review what interphase and mitosis in root tips look like and to demonstrate that mitosis only occurs in the meristem. Contact Flinn Scientific and request BioFax 10875 for an activity to determine the time spent in each phase of the cell cycle using prepared slides.
- For a more complete procedure on how to prepare onion root tip squash slides contact Flinn Scientific and request BioFax 10945.
- IAA is an auxin. It is thought that lectins may enhance the function of auxins.
- *A. tumefaciens* is a very successful plant pathogen and as such is regulated by the USDA. Its success as a pathogen has led to its use as a carrier in biological modification experiments. *A. tumefaciens* genes have been successfully inserted into HeLa cells and other cells as a way to insert genes into a host organism.



## Teacher's Notes *continued*

### Sample Results

#### Calculated Chi-Square Sample Data

Control (water) Treatment		IAA Treatment	
No. of Cells in Interphase	No. of Cells in Mitosis	No. of Cells in Interphase	No. of Cells in Mitosis
2909	67	2171	117
Total number of IAA treated cells counted=2288			

2. To calculate the expected values, complete the following steps:

a. Determine the percentage of cells in interphase and mitosis in the control group (root tips germinated in water).

$$2909/(2909 + 67) = 0.977$$

$$67/(2909 + 67) = 0.023$$

b. Multiply the percentages by the total number of cells in the IAA treatment. This will give the expected numbers (*e*).

$$0.977\% \times 2288 = 2236$$

$$0.023 \times 2288 = 53$$

3. Calculate the chi-square ( $\chi^2$ ) value for the test.

$$\chi^2 = \frac{(2171 - 2237)^2}{2237} + \frac{(117 - 52)^2}{52}$$

$$\chi^2 = \frac{4356}{2237} + \frac{4225}{52}$$

$$\chi^2 = 1.95 + 81.25$$

$$\chi^2 = 83.2$$

4. Compare the calculated chi-square value to the critical value found in the table above, using the information from steps 5 and 6.

$$83.2 > 3.84$$

5. The *p* value is 0.05, and the critical value is 3.84. If the calculated chi-square value is greater than or equal to the table value, then the null hypothesis is rejected. If the calculated chi-square value is less than the table value, the null hypothesis is not rejected.

*The null hypothesis is rejected.*

6. In terms of this part of the investigation, what does it mean if your null hypothesis is rejected?

*The chemical indole-3-acetic acid does increase the rate of mitosis in onion root tips at a concentration of 1 mg/L.*

#### Additional Sample Data

- The baseline activity uses a 1 mg/L indole-3-acetic acid solution. Higher concentrations of IAA inhibit mitosis.
- Caffeine inhibits mitosis. Onions treated with 1% aqueous caffeine failed to produce any roots after several weeks. A 0.1% aqueous caffeine solution did produce short roots. However, when stained the cell cycle phase is difficult to determine and may not produce quantifiable results. Caffeine is very toxic with an oral (rat) LD<sub>50</sub> of 192 mg/kg. As little as 65 mg can be life-threatening. Protect against skin contact when working with caffeine. Use strict hygiene and store in a locked poison cabinet. Caffeine is available from Flinn Scientific, Catalog No. C0344.
- Colchicine interferes with spindle fiber formation and halts mitosis in metaphase. As little as a 0.03% colchicine solution will stop spindle fiber formation. Colchicine is acutely toxic with an oral (mouse) LD<sub>50</sub> of 6 mg/kg. Use strict hygiene and store in a locked poison cabinet. Colchicine is available from Flinn Scientific, Catalog No. C0407.



## Teacher's Notes *continued*

- The auxin 2,4-dichlorophenoxyacetic acid was also tested at a concentration of 1 mg/L using the above procedure. Numerous root tips germinated but they remained very short. 2,4-D is available from Flinn Scientific, Catalog No. D0056.
- Other factors that may affect the mitotic rate are salinity, temperature, mineral limiting factors, pH, roundworms, soil bacteria or fungus, other plant hormones, amount of light, acetaminophen, aspirin, ibuprofen, vitamins and minerals, heavy metals, antibiotics, and certain plant competitors that excrete inhibiting chemicals.

## References

*AP Biology Investigative Labs: An Inquiry-Based Approach*. College Entrance Examination Board: New York, 2012.

*Biology: Lab Manual*; College Entrance Examination Board: New York, 2001.

**The *Environmental Effects on Mitosis* and supporting supplies are available from Flinn Scientific, Inc.**

Catalog No.	Description
FB2031	Environmental Effects on Mitosis
FB1468	Onion Sets, 100 bulblets
L0114	Lectin-Phytohaemagglutinin, 10 mg
C0344	Caffeine, 25 g
C0407	Colchicine, 100 mg
D0056	2,4-Dichlorophenoxyacetic acid, 100 g

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.