Introduction

GOALS OF THE LABORATORY INVESTIGATIONS

Knowing a collection of facts about biology is beneficial only if you can use that information to understand and investigate a particular aspect of the natural world. AP® Biology lab investigations allow you to explore the natural world, and provide opportunities for you to choose to study what interests you most about each concept. Science is about the process of investigating, and should be a central part of your experience in AP Biology. Performing labs also gives you insight into the nature of science, and helps you appreciate the investigations and processes that result in the collection of facts that your textbook and your teacher often present to you.

This suite of AP Biology laboratory investigations helps you gain enduring understandings of biological concepts and the scientific evidence that supports them. The investigations allow you to develop and apply practices and skills used by scientists. You make observations, ask questions, and then design plans for experiments, data collection, application of mathematical routines, and refinement of testable explanations and predictions. As you work through your experiments, your teacher will ask follow-up questions to assess how well you understand key concepts. Finally, you will communicate your findings and your interpretation of them to your classmates and instructor(s).

For each investigation in this manual, you will find the following:

- Background information and clear learning objectives for each investigation
- Prelab questions, activities, software simulations, and other supplemental resources
- "Checklists" of prior skills and skills that will be developed
- Tips for designing and conducting investigations
- Safety concerns
- Lists of materials and supplies
- Methods of analyzing and evaluating results
- Means of communicating results and conclusions
- Postlab questions and activities
- Suggestions for extending the investigation(s)

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CHAPTER 1:

What Is Inquiry?

How do we know what we know? Inquiry begins with observations you make about the natural world — a bare spot under a tree, a bird chirping repeatedly, or an unusual spot on your skin. If you follow such observations by a question, such as *What is causing that?*, you have begun an inquiry. Inquiry-based laboratory investigations allow you to discover information for yourself, and model the behavior of scientists as you observe and explore. Through inquiry, you use a variety of methods to answer questions you raise. These methods include laboratory and field investigations; manipulation of software simulations, models, and data sets; and meaningful online research. By designing experiments to test hypotheses, analyze data, and communicate results and conclusions, you appreciate that a scientific method of investigation is cyclic, not linear; each observation or experimental result raises new questions about how the world works, thus leading to open-ended investigations.

There are four levels of inquiry that lead to the student question. It is not reasonable to think that every part of a particular lab in AP Biology will be completely student directed. However, as written, the labs lead to a student-directed, inquiry-based investigation(s). The four levels of inquiry are as follows:

- **Confirmation**. At this level, you confirm a principle through an activity in which the results are known in advance.
- **Structured Inquiry**. At this level, you investigate a teacher-presented question through a prescribed procedure.
- **Guided Inquiry**. At this level, you investigate a teacher-presented question using procedures that you design/select.
- **Open Inquiry**. At this level, you investigate topic-related questions that are formulated through procedures that you design/select.

As you work on your investigations, your teacher may walk around the room and ask probing questions to provoke your thinking (e.g., *How are you changing the temperature? How are you recording the temperature?*). Your teacher may also ask about data and evidence (e.g., *Is there an alternative way to organize the data? Is there some reason the data may not be accurate? What data are important to collect? What are you hoping to find out? How will you communicate your results?*). This strategy will allow your teacher to diagnose and address any misconceptions immediately.

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CHAPTER 2:

Written, Verbal, and Graphic Communication

Experimental results must be communicated to peers to have value. To understand the relationship among your hypothesis, procedures, and results, you should first take part in an informal small-group or class discussion of the experiment, including possible errors, changes in procedures, and alternative explanations for your data. Since many of the laboratory experiences described in this manual contain suggestions for further investigation, discussion of a given experiment can be a launching pad for independent work, culminating in a formal written report, poster, or oral presentation. Some possibilities for more permanent presentations are described below.

Mini-Posters and Presentations

At scientific conferences, many experiments are presented orally or via posters. Posters provide the advantage of clarity and brevity that articulate the essential elements of the research. In a class, an alternative to the standard oral presentation or a full-sized poster is a mini-poster session, which requires fewer materials and less time than a formal presentation. You can include the most important elements of a full-sized poster, present your work, and get feedback from your classmates in an informal setting. The essential elements of a mini-poster are as follows:

- Title
- Abstract
- Introduction with primary question, background context, and hypothesis
- Methodology
- Results, including graphs, tables, charts, and statistical analyses
- Conclusions, or your interpretation of your results based on your hypothesis
- Literature cited

An example of a mini-poster session can be found at ***http://www.nabt.org/blog/2010/05/04/mini-posters-authentic-peer-review-in-the-classroom***. Such a session allows you to evaluate information on your own, and then discuss it with other students, mimicking authentic presentations and peer review.

Lab Notebooks/Portfolios

A lab notebook allows you to organize your work so that you have the information for a more formal report. Your lab notebook should contain the information necessary for making a formal report, which may include a prelab experimental outline with the following information:

- Members of work group
- Primary question for investigation
- Background observations and contextual information
- Hypothesis and rationale for the investigation
- Experimental design — strategies for testing hypothesis, using appropriate controls and variables
- Materials required
- Safety issues
- Procedure in sufficient detail so that someone could replicate your results

In addition, your lab notebook should contain the following:

- Results, including graphs, tables, drawings or diagrams, and statistical analysis
- Conclusion and discussion — Was the hypothesis supported? What additional questions remain for further investigation?
- References

A lab portfolio might contain finished lab reports, notes on individual projects, library research, reflections on particular lab experiences, and connections with other parts of the course, or a combination of these elements as requested by your teacher.

Lab Reports/Papers

A formal report or paper provides an effective method for you to organize your work, and mimics papers in scientific journals. Your teacher might provide a rubric for what information should be included. This type of report gives you writing experience and opportunities to reflect on your work. (Refer to page 10 for tips on constructing informative graphs to include in your report.) The writing center at the University of North Carolina has published an excellent guide for writing lab reports. Find it at ***http://www.unc.edu/depts/wcweb/handouts/lab_report_complete.html***.

You also can see a good example of a descriptive lab report, "Examination of Protozoan Cultures to Determine Cellular Structure and Motion Pattern," at ***http://www.ncsu.edu/labwrite/res/labreport/sampledescriptlab.html***.

Technology

There are numerous websites for posting class data, which can then provide a larger sample for analysis, comparison of different conditions in the experiment, or collaboration between students in different class sections and different schools. Your school's technology or media center personnel may recommend appropriate Web-based options.

Graphs

A graph is a visual representation of your data, and you want your graph to be as clear as possible to the reader for interpretation. First, you have to decide whether to use a scatter plot in order to draw a "best fit" line through data points, a bar graph, or some other representation with appropriate units. Use a line graph if your data are continuous (e.g., the appearance of product over time in an enzyme reaction). If your data are discontinuous (e.g., the amount of water consumption in different high schools), use a bar graph. Your teacher might have other suggestions.

A graph must have a title that informs the reader about the experiment. Labeling a graph as simply "Graph Number Four" doesn't tell the reader anything about the experiment, or the results. In comparison, the title "The Effect of Different Concentrations of Auxin on Root Growth" tells the reader exactly what was being measured. Make sure each line or bar on your graph is easily identifiable by the reader.

Axes must be clearly labeled with units.

- The x-axis shows the independent variable. Time is an example of an independent variable. Other possibilities for an independent variable might be light intensity, or the concentration of a hormone or nutrient.
- The y-axis denotes the dependent variable, or what is being affected by the condition (independent variable) shown on the x-axis.
- Intervals must be uniform. For example, if one square on the x-axis equals five minutes, each interval must be the same and not change to ten minutes or one minute. If there is a break in the graph, such as a time course over which little happens for an extended period, note this with a break in the axis and a corresponding break in the data line.
- For clarity, you do not have to label each interval. You can label every five or ten intervals, or whatever is appropriate.
- Label the x-axis and y-axis so that a reader can easily see the information.

More than one condition of an experiment may be shown on a graph using different lines. For example, you can compare the appearance of a product in an enzyme reaction at different temperatures on the same graph. In this case, each line must be clearly differentiated from the others — by a label, a different style, or color indicated by a key. These techniques provide an easy way to compare the results of your experiments.

Be clear as to whether your data start at the origin (0,0) or not. Do not extend your line to the origin if your data do not start there. In addition, do not extend your line beyond your last data point (extrapolation) unless you clearly indicate by a dashed line (or some other demarcation) that this is your prediction about what may happen.

For more detailed information about graphs, see Appendix B: Constructing Line Graphs.

CHAPTER 3:

Quantitative Reasoning in AP® Biology

Which would you choose? A brain biopsy or a CAT/MRI scan? A vaccine for 90%+ of the population with a risk of 0.001% suffering from side effects, or no vaccine at all? Fresh vegetables sprayed with competing bacteria, or vegetables sprayed with sterilants that are hazardous to ecosystems? To risk conviction of a crime based on a detective's hunch, or to be acquitted based on evidence provided by DNA markers? These are routine questions affected by the use of mathematics in science, including biology, medicine, public health, and agriculture.

To have a rich foundation in biology, you need to include and apply quantitative methods to the study of biology. This is particularly true for a laboratory experience. Quantitative reasoning is an essential part of inquiry in biology. Many mathematical tools (e.g., statistical tests) were developed originally to work out biological problems.

Mathematics can help biologists (and biology students) grasp and work out problems that are otherwise:

- Too big (such as the biosphere)
- Too slow (macroevolution)
- Too remote in time (early extinctions)
- Too complex (human brain)
- Too small (molecular structures and interactions)
- Too fast (photosynthesis)
- Too remote in space (life in extreme environments)
- Too dangerous or unethical (how infectious agents interact with human populations)

The laboratory investigations in this manual were chosen to provide you with an opportunity to do biology — to explore your own questions and try to find answers to those questions. Many of the investigations provide a preliminary, guided exploration to introduce you to a way of looking at a biology problem, or method for studying it, providing just enough familiarity with the topics so that you can begin asking your own questions and investigating them. An essential part of that exploration includes an introduction to various quantitative skills — mathematical routines, concepts, methods, or operations used to interpret information, solve problems, and make decisions — that you will need in order to explore the investigative topic adequately.

The quantitative skills you'll apply as you carry out the investigations in this lab manual are for the most part the same skills you have been acquiring in your mathematics courses. For many of the skills required in these labs, you already understand how to do the math, and these investigations simply extend the application of those math skills. Your teacher can help to guide you as you supplement and review the quantitative skills required for the various laboratory investigations in this manual.

To conceptually organize the scope and nature of the skills involved, refer to Figure 1:

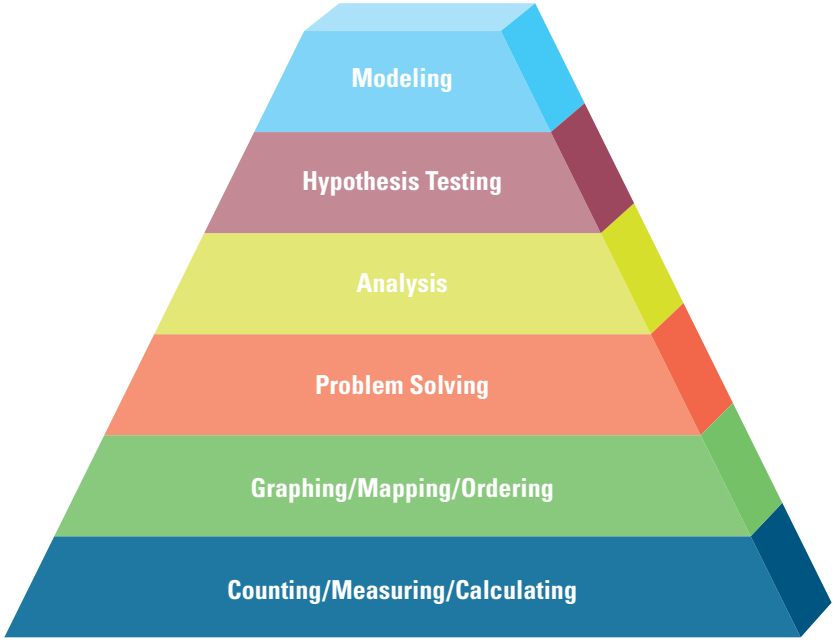


Figure 1. Pyramid of Quantitative Reasoning

The figure graphically organizes the quantitative skills featured in this lab manual. The skills labeled on the bottom of the pyramid are generally less complex, and require the application of standard procedures. As you move up the pyramid, the applications become more complex as you try to make sense out of data and biological phenomena. One of the important lessons about quantitative reasoning is that real data are "messy." The increasing complexity as you move up this pyramid does not necessarily indicate that the mathematical operations themselves are more complex. Good, first approximations of mathematical models often require only simple arithmetic. This chapter describes how the quantitative skills listed in the pyramid are applied when answering questions generated by various lab topics in this manual.

Counting, Measuring, and Calculating

At this point in your education, you may not feel that counting, measuring, and calculating represent much in the way of a "skill." And you'd be right in a theoretical world. The problem is that your investigation will explore the real world of biology, and that is messy.

For example, Investigation 1: Artificial Selection presents the problem of selection of quantitative variation in a population of plants. You identify a trait that can be quantified (counted), and then measure the variation in the population of plants by

counting. This is not always as easy as it sounds. You will notice that some of the plants in your population are more hairy than others, so this is the trait you select. What do you count? All the hairs? Some of the hairs on specific parts of the plant? On how many plants? After observing one of your plants more closely, you see that it has very few (if any) hairs, but another plant has hundreds. These hairs are small. You have a limited amount of time to make your counts. How do you sample the population? After discussion with your lab partner(s), you and your class decide to count just the hairs on the first true leaf's petiole (stalk attaching the blade to the stem) — a much smaller and more reasonable amount to count, but you'll still need to work out whether or not it is a representative sample.

Measuring phenomena in the real world presents similar challenges. Investigation 10: Energy Dynamics introduces you to energy dynamics by measuring the biomass of growing organisms. How do you measure the mass of a small caterpillar? What about the water in the organism? Is water included in "biomass"? It is your challenge to come up with solutions to these problems, and to define all measurements carefully so that someone could measure in the same way you did and replicate the experiment. Perhaps you could measure a quantity of caterpillars and sacrifice a few caterpillars to estimate how much the "wet mass" of a caterpillar is biomass, and how much is water. You will have to perform relatively simple calculations, including percentages, ratios, averages, and means.

Nearly every lab investigation requires these kinds of operations and decisions. What is different about this manual is that the decisions are up to you. The manual doesn't make the decisions for you. There are almost always a number of reasonable, productive solutions to such problems. Make sure that your decisions are reasonable and provide a good solution to the problem you are studying.

Precision needed in the experiment is also a consideration and a decision you have to make. Increasing precision requires more time and resources. How precise do your data need to be for you to support or reject your hypothesis?

Graphing, Mapping, and Ordering: Histograms of Variation and/or Energy Flow Diagrams

To build on the previous two examples, consider how the data counted and measured should be represented — not numerically, but with graphs or diagrams. For example, consider the examination of the variation of a quantitative trait in a population of plants. How do you best represent these data? If you count the hairs (trichomes) in a population of 150 plants, do you present each data point on a graph, or do you compile the data into an overall picture? If all data points are the same, then there would be no need to present data graphically, but the messy reality is that the counts likely could vary from 0 to more than 50 hairs per plant. For this reason, a histogram (see Figure 2) is often used to represent the variability and distribution of population data.

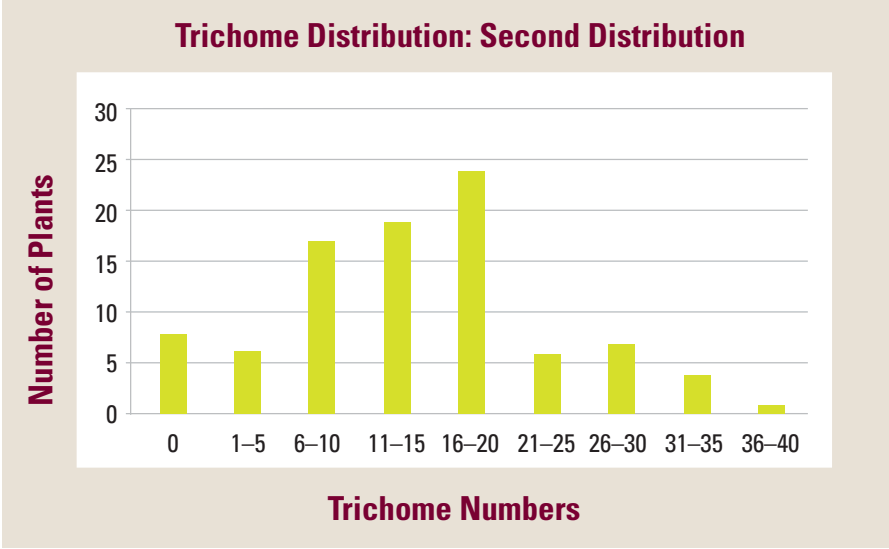


Figure 2. Trichome Distribution: Second Distribution

In a histogram, the data are organized into bins with a defined range of values. For example, for the hairy plants the bin size might be 10 hairs, and bins defined in this manner might include 1–10 hairs, 11–20 hairs, 21–30 hairs, and so on. You simply count the number of plants that fall into each bin, and then graph the distribution as a bar graph — or in this case, a histogram. There are several challenges and decisions you'll have to make where your quantitative skills will be tested. For instance, what should you do about plants with 0 plant hairs? Do you include a separate bin for this one plant? How do you know what the "correct" bin size is? It is usually best to try several bin sizes, but you'll have to make the decision which bin size best captures the nature of the variation you are working with — messy.

Creating Diagrams, Charts, and Maps

Biology is the study of systems at several levels of organization, from molecules and cells to populations and ecosystems. When exploring a topic, such as energy dynamics in Investigation 10, creating a chart or map can help you to logically define the system components and the flows between those components, while simplifying a very complicated process. Creating such a chart is an exercise in logic and graphic design. Such a graphic representation of your work helps to communicate your thinking, and organizes your analysis and modeling structure. Figure 3 is one model of how a disease might infect a population.

Figure 3. A Disease's Infection of a Population

Your teacher may have suggestions on investigations of graphic representation methods you may want to employ to summarize your data and thoughts.

Problem Solving

All sorts of questions and problems are raised and solved during biological investigations. Such questions include the following:

- What is the inheritance pattern for a particular trait?
- What is the critical population size that will ensure genetic diversity in an isolated population?
- How are genes linked to each other on the same chromosome?
- How often do spontaneous mutations occur in a species of yeast?
- What is the Q_{10} temperature coefficient[1] for invertebrates in the Arctic?
- How does a change in ambient temperature affect the rate of transpiration in plants?
- How can the efficiency of transformation be calculated in bacteria exposed to plasmids containing a gene for antibiotic resistance?

Problem solving involves a complex interplay among observation, theory, and inference. For example, say that for one of your investigations you explore a typical dihybrid genetic cross like one you may have studied earlier in an introductory biology course. This time, however, you collect data from the F2 generation, and note four different phenotype combinations (observation). You count the number of each combination. Using your understanding of the role of chromosomes in inheritance, you work to make a theoretical prediction of what your results might be assuming independent assortment of genes (hypothesis). However, you find that the observed results don't quite match your expected results. Now what? You've been using quantitative thinking, and now it is time to extend the thinking into possible solutions to this problem.

1 Q_{10} temperature coefficient: a measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10 °C.

In this case, the deviation from expected may be due to random chance, or it may be due to a phenomenon known as linkage, where two genes are located close together on the same chromosome instead of on separate chromosomes. There is not enough space here to fully explore the strategies for solving such a problem, but realize that the challenge requires a different level of commitment on your part to work through the problem and solve it. Instead of the instructions for each lab investigation walking you through such problems step by step, this manual provides you with opportunities to explore problems you can solve on your own, which will give you a deeper learning experience.

Analysis

When you start to design your own investigations to answer your own questions, you may find that appropriate and adequate data analysis is a challenge. This is the result of having done too many investigations that have the analysis scripted for you. From the very first inkling of the question that you plan to investigate, you also should consider how you plan to analyze your data. Data analysis describes your data quantitatively. Descriptive statistics help to paint the picture of the variation in your data; the central tendencies, standard error, best-fit functions, and the confidence that you have collected enough data. Analysis helps you to make your case when arguing for your conclusion that your data meet accepted standards for reliability and validity. Data analysis is complex. Obviously, there is not enough space in this overview to do the topic justice, but do not let this deter you. Data analysis is an essential component of each investigation in this manual, and is integral to the communication process. Your teacher will be a valuable guide in this process.

Hypothesis Testing

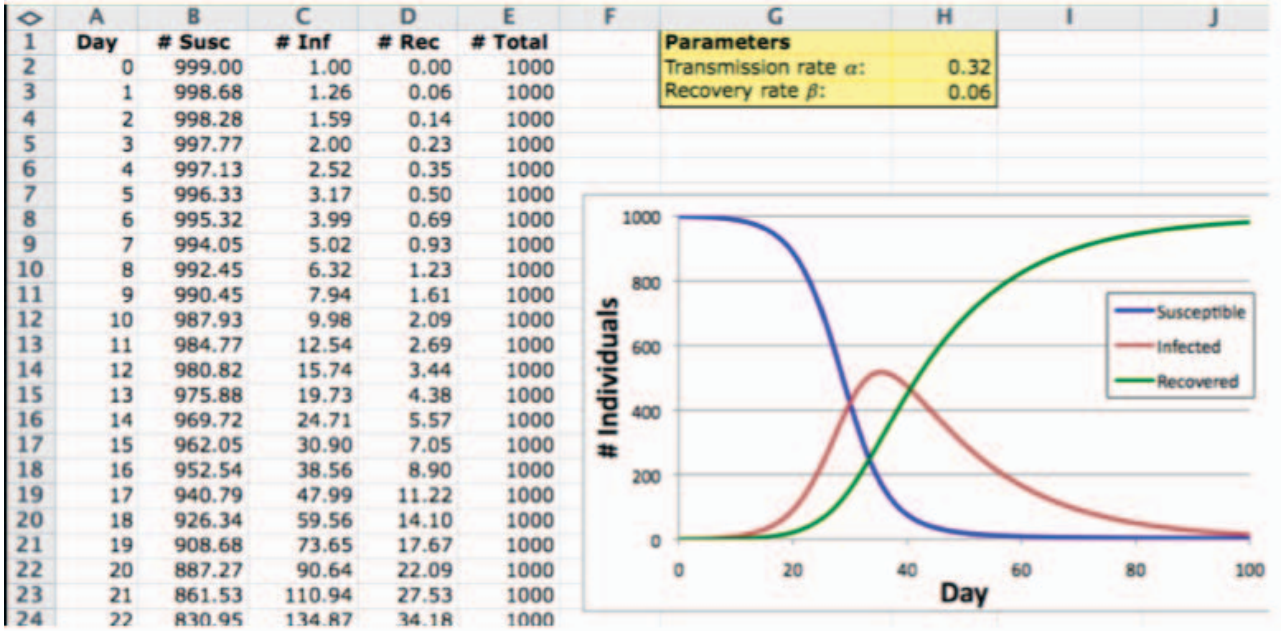
In the investigations in this manual, you are asked to modify your question into an appropriate hypothesis. Your experimental design should provide evidence that will help you to conclude whether or not your hypothesis should be accepted. Part of the evidence needed to produce such a conclusion is based on a number of statistical tests that are designed for specific situations. You may be familiar with a statistical hypothesis test, such as a chi-square test or a T-test. These tests can help you to determine probability that the data you have sampled are significantly different from a theoretical population. You've undoubtedly read about such tests, as they are applied when testing new drug treatments or medical procedures. Your teacher can help guide you as you select the methods appropriate to your study. Deciding on the appropriate methods for hypothesis testing (statistical tests) before you carry out your experiment will greatly facilitate your experimental design.

Modeling

Not all biological research involves wet lab investigations[2]. Investigations also can involve a quantitative model. Quantitative models are often computer based. Thinking about and developing computer models may seem to be a new way of thinking and doing biology, but actually you've been constructing mental models of biological phenomena since you first began your study of biology. Models are simplifications of complex phenomena, and are important tools to help drive prediction and identify the important factors that are largely responsible for particular phenomena.

To develop a mathematical model, you must first define the relevant parameters or variables. For example, if you were creating a model of disease in a population, you might divide the population into three components: the part of the population that is susceptible but not infected, the part of the population that is infected, and the part of the population that has recovered from the disease. The probability of transmitting the infection and the probability for recovery are important parameters to define as well. The next step would be to graphically define these parameters and their relation to one another, as you did previously (see Figure 3).

With this graphic, you can imagine word equations that step through the process of a disease cycle in a population. These word equations can then be interpreted into the language of a spreadsheet to get something like Figure 4.



	A	B	C	D	E
1	Day	# Susc	# Inf	# Rec	# Total
2	0	999.00	1.00	0.00	1000
3	1	998.68	1.26	0.06	1000
4	2	998.28	1.59	0.14	1000
5	3	997.77	2.00	0.23	1000
6	4	997.13	2.52	0.35	1000
7	5	996.33	3.17	0.50	1000
8	6	995.32	3.99	0.69	1000
9	7	994.05	5.02	0.93	1000
10	8	992.45	6.32	1.23	1000
11	9	990.45	7.94	1.61	1000
12	10	987.93	9.98	2.09	1000
13	11	984.77	12.54	2.69	1000
14	12	980.82	15.74	3.44	1000
15	13	975.88	19.73	4.38	1000
16	14	969.72	24.71	5.57	1000
17	15	962.05	30.90	7.05	1000
18	16	952.54	38.56	8.90	1000
19	17	940.79	47.99	11.22	1000
20	18	926.34	59.56	14.10	1000
21	19	908.68	73.65	17.67	1000
22	20	887.27	90.64	22.09	1000
23	21	861.53	110.94	27.53	1000
24	22	830.95	134.87	34.18	1000

Source: Shodor/Project SUCCEED workshops

Figure 4. A Disease Cycle in a Population

2 Wet lab investigation: laboratories in which chemicals, drugs, or other material or biological matter are tested and analyzed requiring water, direct ventilation, and specialized piped utilities, as opposed to a computer-based lab.

Models help to provide insight and guidance for an investigation. They help to focus the investigation on parameters that are most influential. Models have to be checked against real data. The assumptions and the limitations of any model should be explicitly articulated. Building models is a challenge, but it is a challenge that, when met, pays very large dividends in learning.

REFERENCES

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http://shodor.org/succeed/curriculum/apprenticeship/Modeling/Excel/LessonPlan2/ Microsoft Excell II activity, Project Succeed workshops, Shodor.

Waterman, M., and E. Stanley. 2008. *Biological Inquiry: A Workbook of Investigative Cases for Campbell and Reece Biology*. 8th ed. San Francisco, CA: Pearson/Benjamin Cummings.

INVESTIGATION 1

ARTIFICIAL SELECTION

Can extreme selection change expression of a quantitative trait in a population in one generation?

BACKGROUND

Evolution is a process that has existed throughout the history of life on Earth. One of the key driving forces of evolution is natural selection, which is differential reproduction in a population — some organisms in a population may reproduce more than others and leave more viable offspring in the next population or generation. Differential reproduction results in a population with a genetic makeup that is different from that of the previous population. Thus, populations may change over time. This process of change is evolution. With natural selection, environmental factors play a key role in determining which organisms reproduce and how many of their offspring survive. In artificial selection, humans determine which organisms reproduce, allowing some individuals to reproduce more than others. What will happen to a population of these organisms over time when exposed to artificial selection?

For the first part of this investigation, you and your classmates will perform one round of artificial selection on a population of Wisconsin Fast Plants®. First, you will identify and quantify several traits that vary in the population and that you can quantify easily. You will then perform artificial selection by cross-pollinating only selected plants. You'll collect the seeds, plant them, and then sample the second-generation population and see if it is different from the previous one. Your results will generate questions, and you then will have a chance to test your own ideas about how selection works.

Learning Objectives

- To investigate natural selection as a major mechanism of evolution
- To convert a data set from a table of numbers that reflects a change in the genetic makeup of a population over time and to apply mathematical methods and conceptual understandings to investigate the cause(s) and effect(s) of this change
- To apply mathematical methods to data from a real population to predict what will happen to the population in the future
- To investigate how natural selection acts on phenotypic variations in populations
- To evaluate data-based evidence that describes evolutionary changes in the genetic makeup of a population over time due to changes in the environment
- To design an investigation based on your observations and questions related to the importance of a single trait in the life history of a plant

General Safety Precautions

When growing plants under lights, be careful to avoid any situation where water or fertilizer could come in contact with the electrical wires.

THE INVESTIGATIONS

Getting Started

In *On the Origin of Species*, Charles Darwin used artificial selection — the kind of selection that is used to develop domestic breeds of animals and plants — as a way to understand and explain natural selection. Like natural selection, artificial selection requires variation in the population under selection. For selection to work, the variations must be inheritable. To conduct artificial selection, humans decide on a specific trait of a plant or animal to enhance or diminish and then select which individuals with that desired trait will breed, producing the next generation and the next population.

Materials

- Lighting: light box systems (grow lights)
- Growing system: recycled plastic soda or water bottles
- Wicking: mason twine
- Fertilizer: Miracle-Gro Nursery Select All Purpose Water-Soluble Plant Food or Peters Professional with micronutrients
- Soil: Jiffy-Mix (soil mix, not potting soil)
- Vermiculite
- Fast Plant seed (C1-122 works well and provides some additional options; it is heterozygous for two Mendelian traits, green/light green leaves and with anthocyanin [purple stems] and without anthocyanin. Other seed stocks, such as standard Fast Plant seeds, work as well.)
- Bee sticks for pollination
- Digital cameras to record the investigation
- Plastic magnifiers
- Laboratory notebook

Procedure

How will you know if artificial selection has changed the genetic makeup of your population? That is one of the questions you will be trying to answer. You then will have a chance to test your own ideas about how selection works.

Plant Cultivation: First-Generation Plants

Step 1 Prepare growing containers. Go to the Wisconsin Fast Plants website and find the instructions for converting small soda bottles into planting containers (***http://www.fastplants.org/grow.lighting.bottle.php***). Plan to use one-liter bottles or smaller. You can raise up to 6 plants per container.

Figure 1. Notice that the scissors are cutting along the bottom of the bottle curve. This provides better control.

Figure 2. Feed mason twine through a small hole in the lid.

Figure 3. The growing systems are ready for planting.

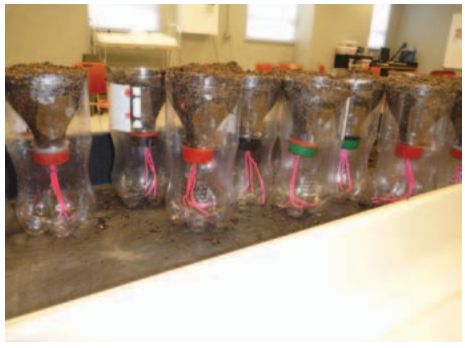


Figure 4. Soil is in place along with the wicking.

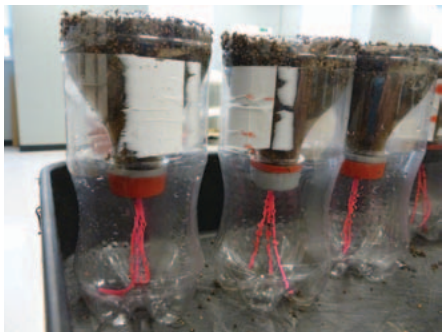


Figure 5. Mix fertilizer — one bottle cap of fertilizer in eight liters of water. Wet the soil gently until water drips from the wicks. Then fill the reservoirs with the dilute fertilizer solution. Plant the seeds carefully — about six to a bottle, uniformly spaced on the surface, not buried in the soil.

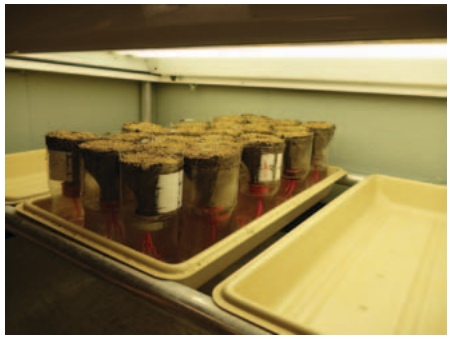


Figure 6. Cover with a light layer of vermiculite. Place the reservoirs — with fertilizer water, seeds on the surface of the soil, and a light layer of vermiculite on the soil — under the lights.

Step 2 Each day, check your plants and make sure that the reservoirs are full, especially on Fridays. These reservoirs have enough volume to last a three-day weekend for small plants.

As your plants grow, record your observations daily. Also try to identify a trait that you could measure or observe reliably. Look for variation in the plants you are growing and describe any you see in your notebook. Observe your classmates' plants as well. Are there also variations in their plants?

Note: Carefully read Steps 3–7 *before* the plants begin to flower.

Step 3 When the plants are about 7 to 12 days old (Figure 7), the class needs to choose 1–2 variable traits for artificial selection. Several variable traits can work for this. Compare your observations with those of other students. You want a trait that varies between plants in a single bottle but also varies between containers. The trait should not be something that is Yes or No, but rather something that varies within a range. That is, look for traits that you can score on a continuum (length, width, number, and so on).

If you and your classmates cannot identify a trait on your own, your teacher will provide additional guidance.

Figure 7. The plants here are 7–12 days old.

Step 4 Score each of your plants for the trait that your class chose to evaluate. You may need a magnifier to do this accurately. Don't be surprised if some plants are not very different from one another.

Step 5 In your lab notebook, compile a list of all the possible traits your class identified. Calculate appropriate descriptive statistics for the class data for the first generation: mean, median, range, standard deviation, etc. Create a histogram that shows the frequency distribution of the trait that you have selected. You can find help for this in Chapter 3.

Step 6 You are now ready to make selection decisions. Directional selection tends to move the variability of a trait in one direction or the other (increase or decrease the trait in the next population). As a class, pick a trait you want to try to affect. Find the top (or bottom) 10% of plants with that trait in the entire class's population (e.g., out of a population of 150 plants, the 15 hairiest plants), and mark any that are in your plant bottle container. Using scissors, cut off the tops of the remaining plants in your container (those not in the top 10%).

Step 7 Just as you did in Step 5, construct a new histogram and calculate descriptive statistics for the selected population of plants. Record the data in your lab notebook. Once you have finished, isolate these selected plants from the rest of the population. Move the bottles of selected plants to another light system so that the plants can finish out their life cycle in isolation. This population will serve as the parents for a new generation.

Step 8 On about day 14–16, when several flowers are present on each of the selected plants, cross-pollinate the selected plants with a single bee stick or pollinating device. Fast Plants® are self-incompatible — each plant must be fertilized by pollen from

another plant. Collect and distribute pollen from every flower on every plant in the selected population. Reserve this bee stick for only the selected population. Avoid contaminating with the pollen from the remaining Fast Plants. Pollinate flowers in the selected population for the next three days with the same bee stick. Be sure to record observations about pollination in your lab notebook. Likewise, with separate bee sticks you can pollinate the plants from the larger population, but be careful to keep them separate from the selected population.

Step 9 Maintain the plants through the rest of their life cycle. As the seedpods form be sure to limit each of the plants to 8 to 10 seedpods. Any more will likely result in poor seed quality. Once the seedpods start to turn yellow (about day 28–36), remove the fertilizer water from the reservoirs and allow the plants to dry for several days. After the plants and seedpods have dried (about a week later), harvest the seedpods from the selected population into a small paper bag for further drying. Be sure to record observations about the plants' life cycle in your lab notebook.

Step 10 Continue to monitor, pollinate, and maintain your control plants throughout the rest of their life cycle. Just be careful to keep the original population and the selected population separate.

Plant Cultivation: Second-Generation Plants

Step 11 You should now have two populations of second-generation seeds: (1) a population that is the offspring of the selected plants from generation one and (2) a population that is the offspring of the remaining plants from generation one. Take seeds from the selected population and plant them to grow the second generation of plants under conditions that are identical to those you used for generation one. Use new bottle containers or, if you choose to use the previous bottle systems, make sure that you thoroughly clean the systems and sterilize with a dilute (10%) bleach solution. Use new wicking cord and new soil. To get your seed, break open the seedpods into a small plastic petri dish lid.

Step 12 When the second-generation plants are about seven to 12 days old, reexamine the plants and score for the trait you selected. Score the plants at the same life history stage using the same method.

Step 13 Unless you plan on growing these plants for another generation (maybe another round of selection), you do not have to save these plants. You can discard them and clean up your growing equipment at this point.

Step 14 Compile, analyze, and graph the class data as you did for the first generation. What is the outcome of your artificial selection? Be sure to record this preliminary analysis in your notebook.

Analyzing and Evaluating Results

Up to this point of the investigation, your analysis has largely been descriptive, but your data should raise some questions.

- Are the two populations/generations before and after selection actually different?
- Are the means significantly different?
- Should you use median or mean as a measure of central tendencies at this point in the investigation?
- Compare your two graphs from the two populations. The chapter on quantitative methods in this lab manual (Chapter 3) provides some guidance here. Consider constructing a bar graph to compare the mean number of hairs per generation. Include error bars, but first determine what is appropriate.
- What statistical test could you apply to help you define your confidence about whether these two populations are different?
- Compare the second population to the parent subpopulation of generation one. How do these two populations compare? How does this comparison differ from your other comparison?

As you carry out your analysis, be sure to include your rationale for the quantitative methods you have chosen in your discussion. Did evolution occur in your Fast Plant population? Justify your conclusion in your laboratory notebook.

Designing and Conducting Your Investigation

In the previous steps, you quantified a variable trait and then selected about 10% of the plants in the population that strongly expressed that trait. You isolated this subpopulation from the larger population during pollination and the rest of the life cycle. You then planted the resulting second generation of seeds, raised the plants to a similar life stage as the previous population, and scored the variation in the second-generation plants. During this long process, you recorded your observations, reflections, and perhaps some questions in your laboratory notebook.

As you worked, you likely started to think about questions of your own. You might want to know why the trait you tested is even variable to start with. How does it help the plants grow and survive? You might also have identified some other trait that you want to explore instead of the one the class chose.

Does one form or another of the trait offer an advantage in the natural world? How could you test this? Phenotypic variation is the result of the interaction of the genotypic variation with the variables in the environment. How much of the variation that you studied could be the result of environmental differences?

You and your class may decide to do this work as a class (to distribute the work involved) or work in small groups. You will report your work to the class and possibly to other AP® Biology classes in a manner agreed upon by you and your instructor. Posters,

lab reports, online reports, and oral presentations are all possible effective means of submitting your work for review.

Where Can You Go from Here?

An essential component of this investigation is to take it beyond the simple selection experiment. With the skills and knowledge gained in the selection experiment, you should be able to design new experiments to investigate the adaptive characteristics of the trait you studied.

Start with a question of your own regarding hairs or some other variable quantitative trait, such as plant height, stem color, or flower number. For instance, in a closely related plant, one investigation demonstrated that herbivore damage early in the plant's development led to increased trichome numbers in later leaves. Could herbivore damage influence the hairy trait expression? Design and carry out an investigation to answer your question.

INVESTIGATION 2

MATHEMATICAL MODELING: HARDY-WEINBERG*

How can mathematical models be used to investigate the relationship between allele frequencies in populations of organisms and evolutionary change?

BACKGROUND

Evolution occurs in populations of organisms and involves variation in the population, heredity, and differential survival. One way to study evolution is to study how the frequency of alleles in a population changes from generation to generation. In other words, you can ask *What are the inheritance patterns of alleles, not just from two parental organisms, but also in a population?* You can then explore how allele frequencies change in populations and how these changes might predict what will happen to a population in the future.

Mathematical models and computer simulations are tools used to explore the complexity of biological systems that might otherwise be difficult or impossible to study. Several models can be applied to questions about evolution. In this investigation, you will build a spreadsheet that models how a hypothetical gene pool changes from one generation to the next. This model will let you explore parameters that affect allele frequencies, such as selection, mutation, and migration.

The second part of the investigation asks you to generate your own questions regarding the evolution of allele frequencies in a population. Then you are asked to explore possible answers to those questions by applying more sophisticated computer models. These models are available for free.

This investigation also provides an opportunity for you to review concepts you might have studied previously, including natural selection as the major mechanism of evolution; the relationship among genotype, phenotype, and natural selection; and fundamentals of classic Mendelian genetics.

* Transitioned from the *AP Biology Lab Manual* (2001)

Learning Objectives

- To use a data set that reflects a change in the genetic makeup of a population over time and to apply mathematical methods and conceptual understandings to investigate the cause(s) and effect(s) of this change
- To apply mathematical methods to data from a real or simulated population to predict what will happen to the population in the future
- To evaluate data-based evidence that describes evolutionary changes in the genetic makeup of a population over time
- To use data from mathematical models based on the Hardy-Weinberg equilibrium to analyze genetic drift and the effect of selection in the evolution of specific populations
- To justify data from mathematical models based on the Hardy-Weinberg equilibrium to analyze genetic drift and the effects of selection in the evolution of specific populations
- To describe a model that represents evolution within a population
- To evaluate data sets that illustrate evolution as an ongoing process

General Safety Precautions

There are some important things to remember when computer modeling in the classroom. To avoid frustration, periodically save your work. When developing and working out models, save each new version of the model with a different file name. That way, if a particular strategy doesn't work, you will not necessarily have to start over completely but can bring up a file that had the beginnings of a working model.

If you have difficulty refining your spreadsheet, consider using the spreadsheet to generate the random samples and using pencil and paper to archive and graph the results.

As you work through building this spreadsheet you may encounter spreadsheet tools and functions that are not familiar to you. Today, there are many Web-based tutorials, some text based and some video, to help you learn these skills. For instance, typing "How to use the SUM tool in Excel video" will bring up several videos that will walk you through using the SUM tool.

THE INVESTIGATIONS

Getting Started

This particular investigation provides a lab environment, guidance, and a problem designed to help you understand and develop the skill of modeling biological phenomena with computers. There are dozens of computer models already built and available for free. The idea for this laboratory is for you to build your own from scratch. To obtain the maximum benefit from this exercise, you should not do too much background preparation. As you build your model and explore it, you should develop a more thorough understanding of how genes behave in population.

To help you begin, you might want to work with physical models of population genetics, such as simulations that your teacher can share with you. With these pencil-and-paper simulations, you can obtain some insights that may help you develop your computer model.

Procedure

It is easy to understand how microscopes opened up an entire new world of biological understanding. For some, it is not as easy to see the value of mathematics to the study of biology, but, like the microscope, math and computers provide tools to explore the complexity of biology and biological systems — providing deeper insights and understanding of what makes living systems work.

To explore how allele frequencies change in populations of organisms, you will first build a computer spreadsheet that models the changes in a hypothetical gene pool from one generation to the next. You need a basic familiarity with spreadsheet operations to complete this lab successfully. You may have taken a course that introduced you to spreadsheets before. If so, that will be helpful, and you may want to try to design and build your model on your own after establishing some guidelines and assumptions. Otherwise, you may need more specific guidance from your teacher. You can use almost any spreadsheet program available, including free online spreadsheet software such as Google Docs or Zoho (***http://www.zoho.com***), to complete the first section of your investigation.

In the second part of the investigation, you will use more sophisticated spreadsheet models or computer models to explore various aspects of evolution and alleles in populations. To understand how these complex tools work and their limitations, you first need to build a model of your own.

Building a Simple Mathematical Model

The real world is infinitely complicated. To penetrate that complexity using model building, you must learn to make reasonable, simplifying assumptions about complex processes. For example, climate change models or weather forecasting models are simplifications of very complex processes — more than can be accounted for with even the most powerful computer. These models allow us to make predictions and test hypotheses about climate change and weather.

By definition, any model is a simplification of the real world. For that reason, you need to constantly evaluate the assumptions you make as you build a model, as well as evaluate the results of the model with a critical eye. This is actually one of the powerful benefits of a model — it forces you to think deeply about an idea.

There are many approaches to model building; in their book on mathematical modeling in biology, Otto and Day (2007) suggest the following steps:

1. Formulate the question.
2. Determine the basic ingredients.
3. Qualitatively describe the biological system.
4. Quantitatively describe the biological system.
5. Analyze the equations.
6. Perform checks and balances.
7. Relate the results back to the question.

As you work through the next section, record your thoughts, assumptions, and strategies on modeling in your laboratory notebook.

Step 1 Formulate the question.

Think about a recessive Mendelian trait such as cystic fibrosis. Why do recessive alleles like cystic fibrosis stay in the human population? Why don't they gradually disappear?

Now think about a dominant Mendelian trait such as polydactyly (more than five fingers on a single hand or toes on a foot) in humans. Polydactyly is a dominant trait, but it is not a *common* trait in most human populations. Why not?

How do inheritance patterns or allele frequencies change in a population? Our investigation begins with an exploration of answers to these simple questions.

Step 2 Determine the basic ingredients.

Let's try to simplify the question *How do inheritance patterns or allele frequencies change in a population?* with some basic assumptions. For this model, assume that all the organisms in our hypothetical population are diploid. This organism has a gene locus with two alleles — *A* and *B*. (We could use *A* and *a* to represent the alleles, but *A* and *B* are easier to work with in the spreadsheet you'll be developing.) So far, this imaginary population is much like any sexually reproducing population.

How else can you simplify the question? Consider that the population has an infinite gene pool (all the alleles in the population at this particular locus). Gametes for the next generation are selected totally at random. What does that mean? Focus on answering that question in your lab notebook for a moment — it is key to our model. For now let's consider that our model is going to look only at how allele frequencies might change from generation to generation. To do that we need to describe the system.

Step 3 Qualitatively describe the biological system.

Imagine for a minute the life cycle of our hypothetical organism. See if you can draw a diagram of the cycle; be sure to include the life stages of the organism. Your life cycle might look like Figure 1.

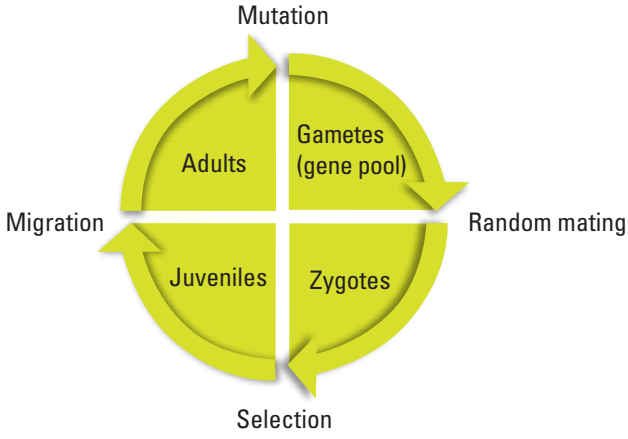


Figure 1. Life Stages of a Population of Organisms

To make this initial exploration into a model of inheritance patterns in a population, you need to make some important assumptions — all the gametes go into one infinite pool, and all have an equal chance of taking part in fertilization or formation of a zygote. For now, all zygotes live to be juveniles, all juveniles live to be adults, and no individuals enter or leave the population; there is also no mutation. Make sure to record these assumptions in your notebook; later, you will need to explore how your model responds as you change or modify these assumptions.

Step 4 Quantitatively describe the biological system.

Spreadsheets are valuable tools that allow us to ask *What if?* questions. They can repeatedly make a calculation based on the results of another calculation. They can also model the randomness of everyday events. Our goal is to model how allele frequencies change through one life cycle of this imaginary population in the spreadsheet. Use the diagram in Figure 1 as a guide to help you design the sequence and nature of your spreadsheet calculation. The first step is to randomly draw gametes from the gene pool to form a number of zygotes that will make up the next generation.

To begin this model, let's define a couple of variables.

Let

p = the frequency of the *A* allele
and let q = the frequency of the *B* allele

Bring up the spreadsheet on your computer. The examples here are based on Microsoft® Excel, but almost any modern spreadsheet can work, including Google's online Google Docs (***https://docs.google.com***) and Zoho's online spreadsheet (***http://www.zoho.com***).

Hint: If you are familiar with spreadsheets, the RAND function, and using IF statements to create formulas in spreadsheets, you may want to skip ahead and try to build a model on your own. If these are not familiar to you, proceed with the following tutorial.

Somewhere in the upper left corner (in this case, cell D2), enter a value for the frequency of the *A* allele. This value should be between 0 and 1. Go ahead and type in labels in your other cells and, if you wish, shade the cells as well. This blue area will represent the gene pool for your model. (Highlight the area you wish to format with color, and right-click with your mouse in Excel to format.) This is a spreadsheet, so you can enter the value for the frequency of the *B* allele; however, when making a model it is best to have the spreadsheet do as many of the calculations as possible. All of the alleles in the gene pool are either *A* or *B*; therefore $p + q = 1$ and $1 - p = q$. In cell D3, enter the formula to calculate the value of q.

In spreadsheet lingo it is

=1-D2

Your spreadsheet now should look something like Figure 2.

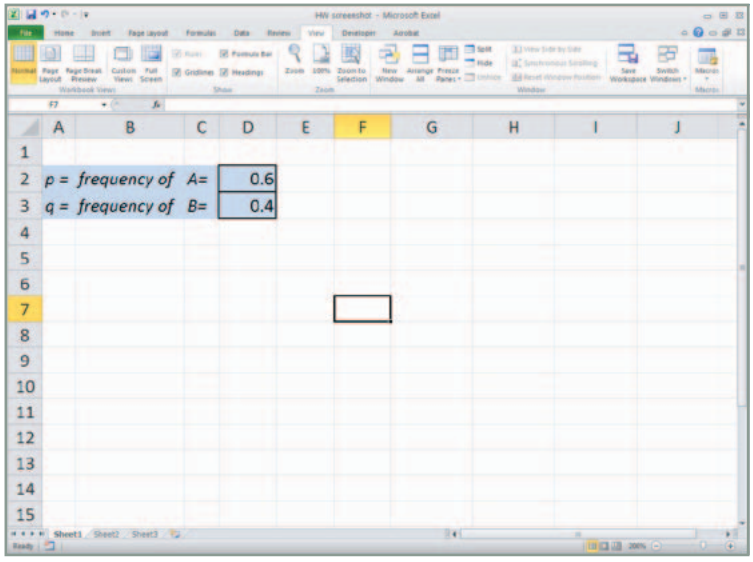


Figure 2

Let's explore how one important spreadsheet function works before we incorporate it into our model. In a nearby empty cell, enter the function (we will remove it later).

=Rand()

Note that the parentheses have nothing between them. After hitting *return*, what do you find in the cell? If you are on a PC, try hitting the F9 key several times to force recalculation. On a Mac, enter *cmd* + or *cmd* =. What happens to the value in the cell? Describe your results in your lab notebook.

The RAND function returns random numbers between 0 and 1 in decimal format. This is a powerful feature of spreadsheets. It allows us to enter a sense of randomness to our calculations if it is appropriate — and here it is when we are "randomly" choosing gametes from a gene pool. Go ahead and delete the RAND function in the cell.

Let's select two gametes from the gene pool. In cell E5, let's generate a random number, compare it to the value of *p*, and then place either an *A* gamete or a *B* gamete in the cell. We'll need two functions to do this, the RAND function and the IF function. Check the help menu if necessary.

Note that the function entered in cell E5 is

=IF(RAND()<=D$2,"A", "B")

Be sure to include the *$* in front of the *2* in the cell address D2. It will save time later when you build onto this spreadsheet.

The formula in this cell basically says that if a random number between 0 and 1 is less than or equal to the value of *p*, then put an *A* gamete in this cell, or if it is not less than or equal to the value of *p*, put a *B* gamete in this cell. IF functions and RAND functions are very powerful tools when you try to build models for biology.

Now create the same formula in cell F5, making sure that it is formatted exactly like E5. When you have this completed, press the recalculate key to force a recalculation of your spreadsheet. If you have entered the functions correctly in the two cells, you should see changing values in the two cells. (This is part of the testing and retesting that you have to do while model building.) Your spreadsheet should look like Figure 3.

Try recalculating 10–20 times. Are your results consistent with what you expect? Do both cells (E5 and F5) change to *A* or *B* in the ratios you'd expect from your *p* value? Try changing your *p* value to 0.8 or 0.9. Does the spreadsheet still work as expected? Try lower *p* values. If you don't get approximately the expected numbers, check and recheck your formulas now, while it is early in the process.

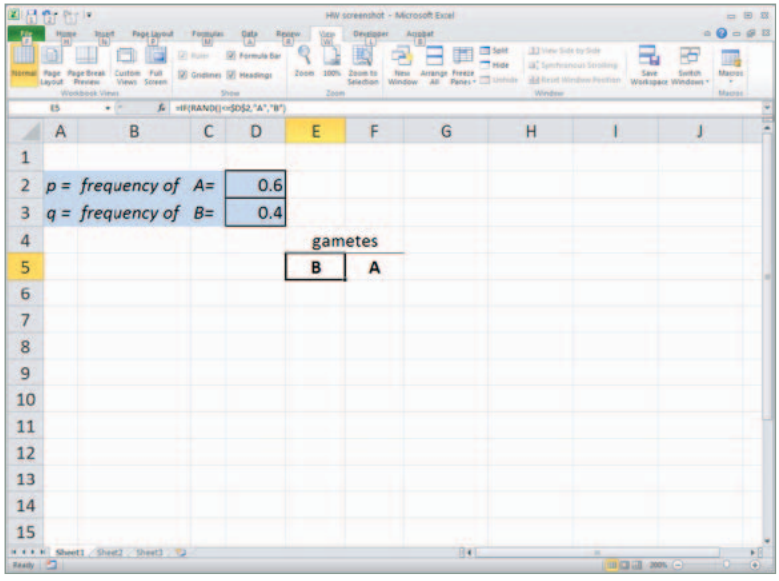


Figure 3

You could stop here and just have the computer recalculate over and over — similar to tossing a coin. However, with just a few more steps, you can have a model that will create a small number or large number of gametes for the next generation, count the different genotypes of the zygotes, and graph the results.

Copy these two formulas in E5 and F5 down for about 16 rows to represent gametes that will form 16 offspring for the next generation, as in Figure 4. (To copy the formulas, click on the bottom right-hand corner of the cell and, with your finger pressed down on the mouse, drag the cell downward.)

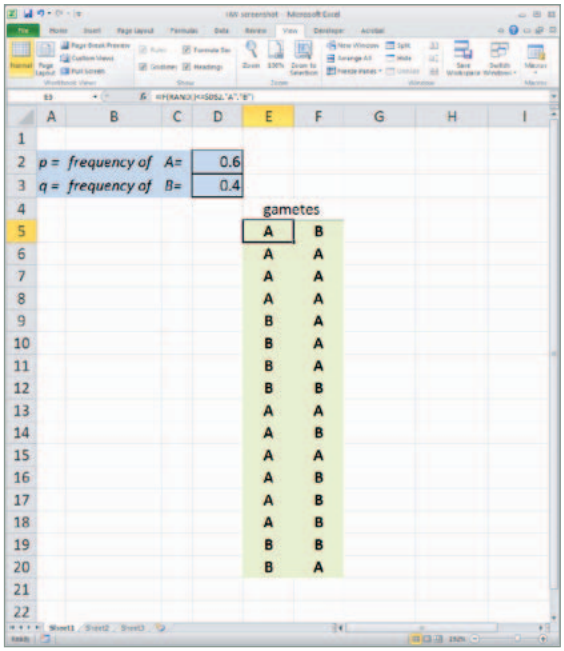


Figure 4

We'll put the zygotes in cell G5. The zygote is a combination of the two randomly selected gametes. In spreadsheet vernacular, you want to concatenate the values in the two cells. In cell G5 enter the function =CONCATENATE(E5,F5), and then copy this formula down as far down as you have gametes, as in Figure 5 on the next page.

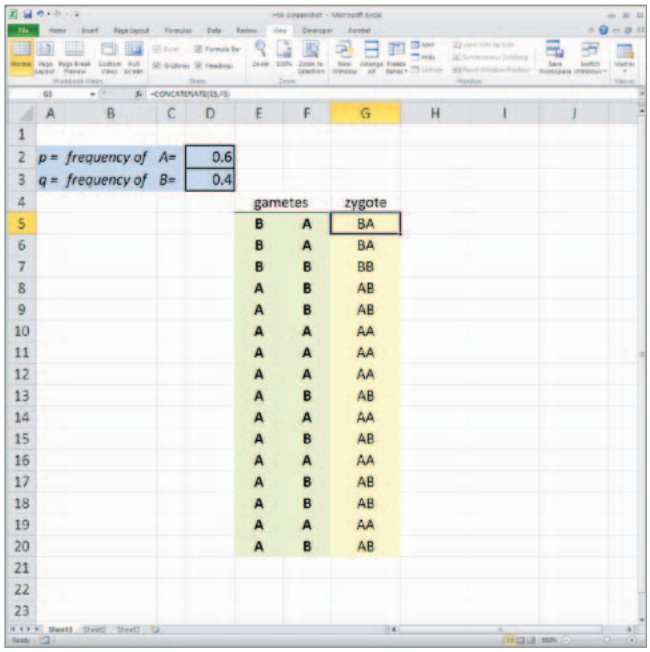


Figure 5

The next columns on the sheet, H, I, and J, are used for bookkeeping — that is, keeping track of the numbers of each zygote's genotype. They are rather complex functions that use IF functions to help us count the different genotypes of the zygotes.

The function in cell H5 is =IF(G5="AA",1,0), which basically means that if the value in cell G5 is AA, then put a 1 in this cell; if not, then put a 0.

Enter the following very similar function in cell J5: =IF(G5="BB",1,0)

- Can you interpret this formula?
- What does it say in English?

Your spreadsheet now should resemble Figure 6.

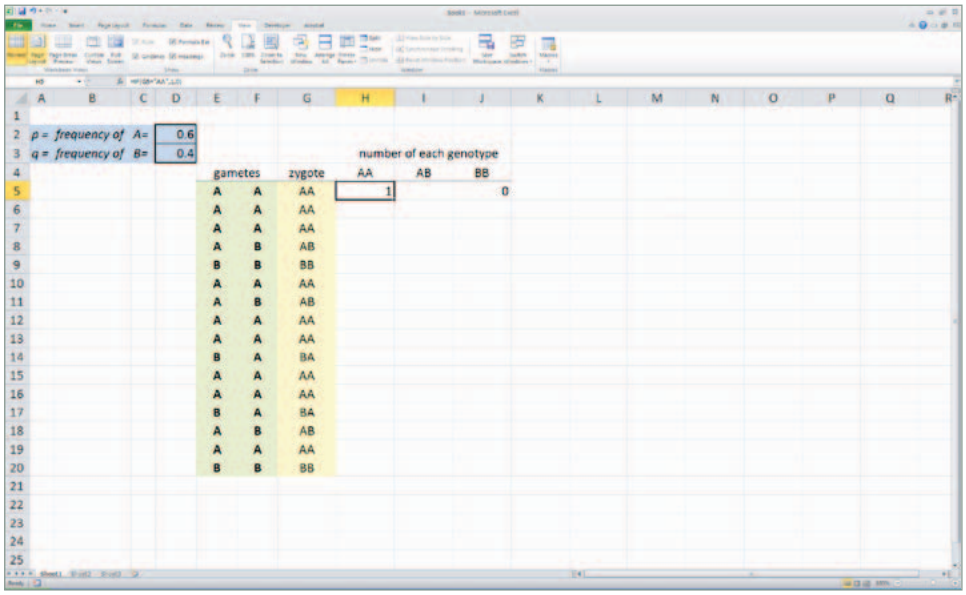


Figure 6

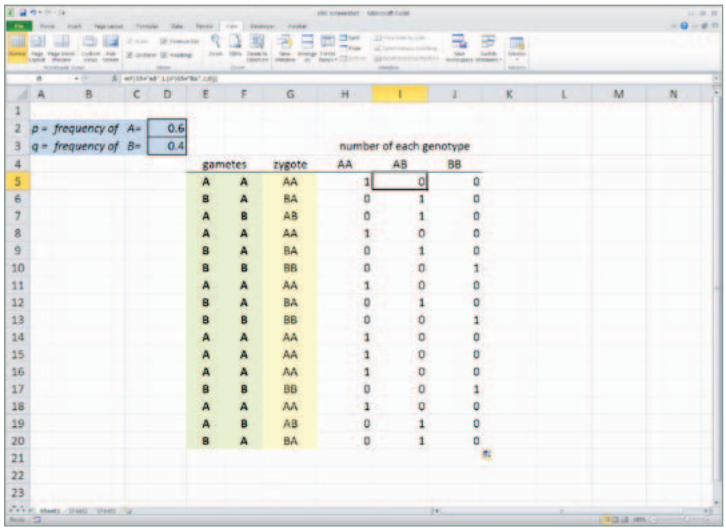
Now let's tackle the nested IF function. This is needed to test for either *AB* or *BA*.

In cell I5, enter the nested function:

=IF(G5="AB",1,(IF(G5="BA",1,0))).

This example requires an extra set of parentheses, which is necessary to nest functions. This function basically says that if the value in cell G5 is exactly equal to *AB*, then put a 1; if not, then if the value in cell G5 is exactly *BA*, put a 1; if it is neither, then put a 0 in this cell. Copy these three formulas down for all the rows in which you have produced gametes.

Enter the labels for the columns you've been working on — *gametes* in cell E4, *zygote* in cell G5, *AA* in cell H4, *AB* in cell I4, and *BB* in cell J4, as shown in Figure 7 on the next page.



	A	B	C	D	E	F	G	H	I	J
1										
2	p = frequency of	A=		0.6						
3	q = frequency of	B=		0.4						
4					gametes		zygote	number of each genotype AA	AB	BB
5					A	A	AA	1	0	0
6					B	A	BA	0	1	0
7					A	B	AB	0	1	0
8					A	A	AA	1	0	0
9					B	A	BA	0	1	0
10					B	B	BB	0	0	1
11					A	A	AA	1	0	0
12					B	A	BA	0	1	0
13					B	B	BB	0	0	1
14					A	A	AA	1	0	0
15					A	A	AA	1	0	0
16					A	A	AA	1	0	0
17					B	B	BB	0	0	1
18					A	A	AA	1	0	0
19					A	B	AB	0	1	0
20					B	A	BA	0	1	0
21										
22										
23										

Figure 7

As before, try recalculating a number of times to make sure everything is working as expected. What is expected? If you aren't sure yet, keep this question in mind as you complete the sheet. You could use a *p* value of 0.5, and then you'd see numbers similar to the ratios you would get from flipping two coins at once. Don't go on until you are sure the spreadsheet is making correct calculations. Try out different values for *p*. Make sure that the number of zygotes adds up. Describe your thinking and procedure for checking the spreadsheet in your lab notebook.

Now, copy the cells E5 through J5 down for as many zygotes as you'd like in the first generation. Use the SUM function to calculate the numbers of each genotype in the H, I, and J columns. Use the genotype frequencies to calculate new allele frequencies and to recalculate new *p* and *q* values. Make a bar graph of the genotypes using the chart tool. Your spreadsheet should resemble Figure 8.

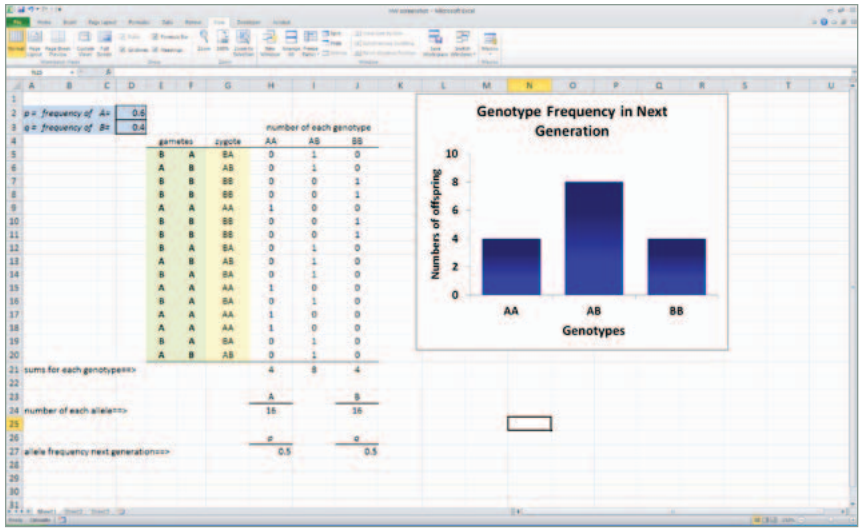


Figure 8

Testing Your Mathematical Model

You now have a model with which you can explore how allele frequencies behave and change from generation to generation. Working with a partner, develop a plan to answer this general question: *How do inheritance patterns or allele frequencies change in a population over one generation?* As you work, think about the following more specific questions:

- What can you change in your model? If you change something, what does the change tell you about how alleles behave?
- Do alleles behave the same way if you make a particular variable more extreme? Less extreme?
- Do alleles behave the same way no matter what the population size is? To answer this question, you can insert rows of data somewhere between the first row of data and the last row and then copy the formulas down to fill in the space.

Try out different starting allele frequencies in the model. Look for and describe the patterns that you find as you try out different allele frequencies. Develop and use a pattern to select your values to test and organize your exploration. In particular, test your model with extreme values and intermediate values. In your lab notebook, describe your observations and conclusions about the population inheritance patterns you discover.

Try adding additional generations to your model to look at how allele frequencies change in multiple generations. To do this, use your newly recalculated p and q values to seed the next generation. Once you've included the second generation, you should be able to copy additional generations so that your model looks something like Figure 9, with each new generation determining the new p and q values for the next.

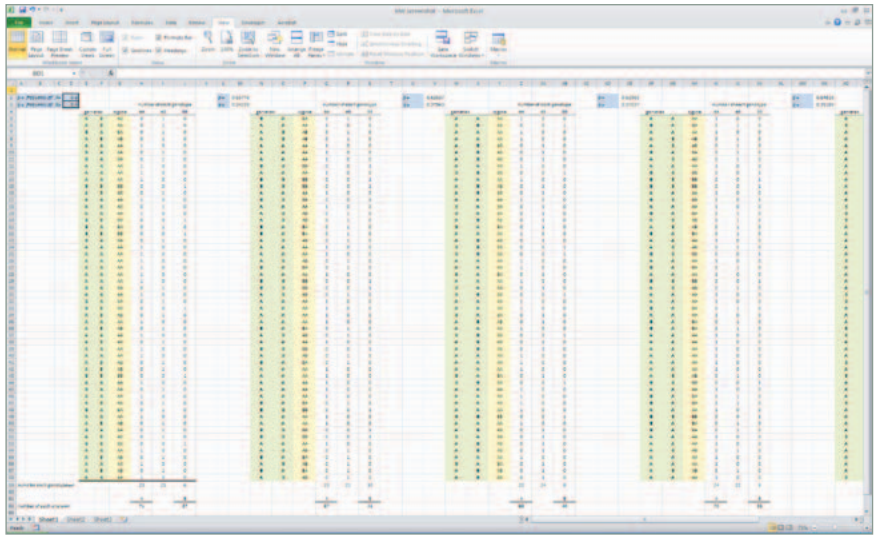


Figure 9

Try to create a graph of p values over several generations, for different-sized populations. See if you can detect a pattern of how population size affects the inheritance pattern. Be sure to try out both large and small populations of offspring.

This model relies on the RAND function to randomly select gametes from an infinite gene pool.

- What would happen if there were no randomness to this selection?
- What kind of pattern of genotypes would you expect in the next generation?

Creating a Formula that Predicts the Genotypes of the Next Generation

Here are two approaches to develop the formula. You might first try a graphical approach. Create a Punnet square, like Figure 10 and similar to what you might use to solve a Mendelian genetics problem. In this case, however, plot the values of p and q. Scale each side of the square based on the magnitude of the p or q values. Place this diagram in your lab notebook, and fill in the squares with variables and values, as in Figure 10.

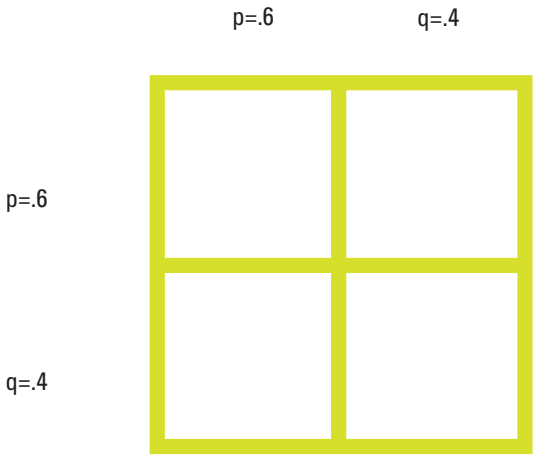


Figure 10

Of course, you could also calculate the expected results for the next generation.

Remember that $p + q = 1$

The probability of two A alleles combining in one organism in the next generation is p^2. The probability of two B alleles combining is q^2. The probability of a combination of AB is $p * q$, as is the probability of combination of BA alleles, for a total of $2pq$.

For the next generation, the formula that predicts genotypes is

$$(p + q)^2 = 1, \text{ which works out to: } p^2 + 2pq + q^2.$$

Based on the calculations you made while testing your model, how would you answer the following questions?

- In the absence of random events (an infinitely large population), are the allele frequencies of the original population expected to change from generation to generation?
- How does this compare to a population that has random gamete selection but is small?
- What happens to allele frequencies in such a population? Is it predictable?

This mathematical model can predict allele frequencies from generation to generation. In fact, it is a *null* model. That is, in the absence of random events or other real-life factors that affect populations, the allele frequencies do not change from generation to generation. This is known as the Hardy-Weinberg equilibrium (H-W equilibrium). The H-W equilibrium is a valuable tool for population biologists because it serves as a baseline to measure changes in allele frequencies in a population. If a population is not in H-W equilibrium, then something else is happening that is making the allele frequencies change.

What factors can cause allele frequencies to change in a population? (Hint: There are many.) How could you model these factors using your spreadsheet?

Designing and Conducting Your Investigation

By this point you've been able to use your model to explore how random chance affects the inheritance patterns of alleles in large and small populations. Perhaps you've also been able to find some interesting patterns in how alleles behave across generations.

At the end of the last section you were asked what factors can cause allele frequencies to change in a population and how you would model them. Choose one of your answers, and try it out using your spreadsheet. This may involve adding multiple columns or rows along with a few extra operations. Keep the life cycle of your hypothetical population in mind as you develop additional strategies.

With your new spreadsheet model, generate your own questions regarding the evolution of allele frequencies in a population. From these questions (noted in your lab notebook), you need to develop hypotheses that you can test — those that allow you to easily manipulate the parameters of population size, number of generations, selection (fitness), mutation, migration, and genetic drift. Collect sufficient data by running your model repeatedly. Analyze your data. Formulate your conclusions and present a miniposter that supports your claim with sound reasoning and evidence to the class. Your teacher may have some ideas for questions to investigate.

Where Can You Go from Here?

An excellent extension to this laboratory is the following investigation:

McMahon, K. A. 2008. Supertasters—Updating the Taste Test for the A & P Laboratory. Pages 398–405, in Tested Studies for Laboratory Teaching, Volume 29 (K.L. Clase, Editor). Proceedings of the 29th Workshop/Conference of the Association for Biology Laboratory Education (ABLE).

Your teacher will provide the lab, or you can google "ABLE proceedings + supertaster" to access the lab.

There are few human traits that express the intermediate dominance necessary for testing for the null hypothesis. The supertaster trait described in this laboratory does express an intermediate phenotype; therefore, it creates an exemplary investigative population genetics laboratory.

REFERENCE

Otto, S. P. and T. Day (2007). A Biologist's Guide to Mathematical Modeling in Ecology and Evolution. Princeton University Press.
http://www.zoology.ubc.ca/biomath/

INVESTIGATION 3

COMPARING DNA SEQUENCES TO UNDERSTAND EVOLUTIONARY RELATIONSHIPS WITH BLAST

How can bioinformatics be used as a tool to determine evolutionary relationships and to better understand genetic diseases?

BACKGROUND

Between 1990–2003, scientists working on an international research project known as the Human Genome Project were able to identify and map the 20,000–25,000 genes that define a human being. The project also successfully mapped the genomes of other species, including the fruit fly, mouse, and *Escherichia coli*. The location and complete sequence of the genes in each of these species are available for anyone in the world to access via the Internet.

Why is this information important? Being able to identify the precise location and sequence of human genes will allow us to better understand genetic diseases. In addition, learning about the sequence of genes in other species helps us understand evolutionary relationships among organisms. Many of our genes are identical or similar to those found in other species.

Suppose you identify a single gene that is responsible for a particular disease in fruit flies. Is that same gene found in humans? Does it cause a similar disease? It would take you nearly 10 years to read through the entire human genome to try to locate the same sequence of bases as that in fruit flies. This definitely isn't practical, so a sophisticated technological method is needed.

Bioinformatics is a field that combines statistics, mathematical modeling, and computer science to analyze biological data. Using bioinformatics methods, entire genomes can be quickly compared in order to detect genetic similarities and differences. An extremely powerful bioinformatics tool is BLAST, which stands for Basic Local Alignment Search Tool. Using BLAST, you can input a gene sequence of interest and search entire genomic libraries for identical or similar sequences in a matter of seconds.

In this laboratory investigation, you will use BLAST to compare several genes, and then use the information to construct a *cladogram*. A cladogram (also called a phylogenetic tree) is a visualization of the evolutionary relatedness of species. Figure 1 is a simple cladogram.

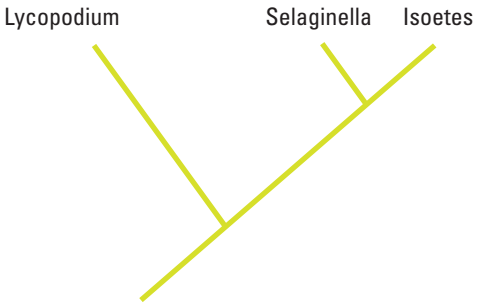


Figure 1. Simple Cladogram Representing Different Plant Species

Note that the cladogram is treelike, with the endpoints of each branch representing a specific species. The closer two species are located to each other, the more recently they share a common ancestor. For example, *Selaginella* (spikemoss) and *Isoetes* (quillwort) share a more recent common ancestor than the common ancestor that is shared by all three organisms.

Figure 2 includes additional details, such as the evolution of particular physical structures called shared derived characters. Note that the placement of the derived characters corresponds to when (in a general, not a specific, sense) that character evolved; every species above the character label possesses that structure. For example, tigers and gorillas have hair, but lampreys, sharks, salamanders, and lizards do not have hair.

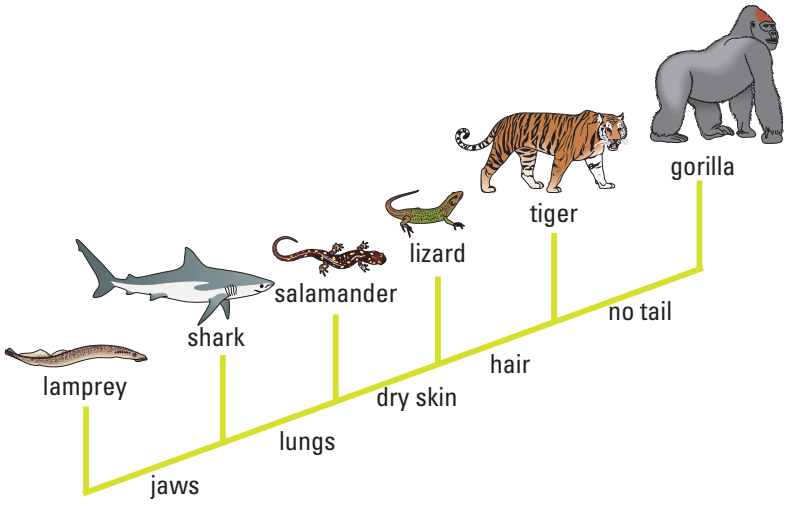


Figure 2. Cladogram of Several Animal Species

The cladogram above can be used to answer several questions. Which organisms have lungs? What three structures do all lizards possess? According to the cladogram, which structure — dry skin or hair — evolved first?

Historically, only physical structures were used to create cladograms; however, modern-day cladistics relies heavily on genetic evidence as well. Chimpanzees and humans share 95%+ of their DNA, which would place them closely together on a cladogram. Humans and fruit flies share approximately 60% of their DNA, which would place them farther apart on a cladogram.

Can you draw a cladogram that depicts the evolutionary relationship among humans, chimpanzees, fruit flies, and mosses?

Learning Objectives

- To create cladograms that depict evolutionary relationships
- To analyze biological data with a sophisticated bioinformatics online tool
- To use cladograms and bioinformatics tools to ask other questions of your own and to test your ability to apply concepts you know relating to genetics and evolution

General Safety Precautions

There are no safety precautions associated with this investigation.

THE INVESTIGATIONS

Getting Started

Your teacher may assign the following questions to see how much you understand concepts related to cladograms before you conduct your investigation:

1. Use the following data to construct a cladogram of the major plant groups:

Table 1. Characteristics of Major Plant Groups

Organisms	Vascular Tissue	Flowers	Seeds
Mosses	0	0	0
Pine trees	1	0	1
Flowering plants	1	1	1
Ferns	1	0	0
Total	3	1	2

2. GAPDH (glyceraldehyde 3-phosphate dehydrogenase) is an enzyme that catalyzes the sixth step in glycolysis, an important reaction that produces molecules used in cellular respiration. The following data table shows the percentage similarity of this gene and the protein it expresses in humans versus other species. For example, according to the table, the GAPDH gene in chimpanzees is 99.6% identical to the gene found in humans, while the protein is identical.

Table 2. Percentage Similarity Between the GAPDH Gene and Protein in Humans and Other Species

Species	Gene Percentage Similarity	Protein Percentage Similarity
Chimpanzee *(Pan troglodytes)*	99.6%	100%
Dog *(Canis lupus familiaris)*	91.3%	95.2%
Fruit fly *(Drosophila melanogaster)*	72.4%	76.7%
Roundworm *(Caenorhabditis elegans)*	68.2%	74.3%

a. Why is the percentage similarity in the gene always lower than the percentage similarity in the protein for each of the species? (Hint: Recall how a gene is expressed to produce a protein.)

b. Draw a cladogram depicting the evolutionary relationships among all five species (including humans) according to their percentage similarity in the GAPDH gene.

Online Activities

You can also prepare for the lab by working through the following online activities:

- "The Evolution of Flight in Birds"
  http://www.ucmp.berkeley.edu/education/explorations/reslab/flight/main.htm
  This activity provides a real-world example of how cladograms are used to understand evolutionary relationships.
- "What did T. rex taste like?"
  http://www.ucmp.berkeley.edu/education/explorations/tours/Trex/index.html
- "Journey into Phylogenetic Systematics"
  http://www.ucmp.berkeley.edu/clad/clad4.html

Figure 3. Fossil Specimen

Procedure

A team of scientists has uncovered the fossil specimen in Figure 3 near Liaoning Province, China. Make some general observations about the morphology (physical structure) of the fossil, and then record your observations in your notebook.

Little is known about the fossil. It appears to be a new species. Upon careful examination of the fossil, small amounts of soft tissue have been discovered. Normally, soft tissue does not survive fossilization; however, rare situations of such preservation do occur. Scientists were able to extract DNA nucleotides from the tissue and use the information to sequence several genes. Your task is to use BLAST to analyze these genes and determine the most likely placement of the fossil species on Figure 4.

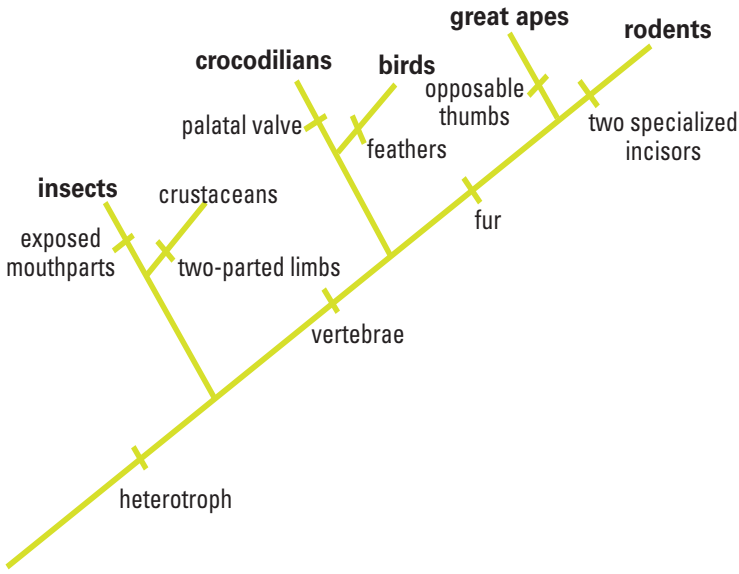


Figure 4. Fossil Cladogram

Step 1 Form an initial hypothesis as to where you believe the fossil specimen should be placed on the cladogram based on the morphological observations you made earlier. Draw your hypothesis on Figure 4.

Step 2 Locate and download gene files. Download three gene files from *http://blogging4biology.edublogs.org/2010/08/28/college-board-lab-files/*.

Step 3 Upload the gene sequence into BLAST by doing the following:

a. Go to the BLAST homepage: ***http://blast.ncbi.nlm.nih.gov/Blast.cgi***

b. Click on "Saved Strategies" from the menu at the top of the page.

Figure 5

c. Under "Upload Search Strategy," click on "Browse" and locate one of the gene files you saved onto your computer.

d. Click "View."

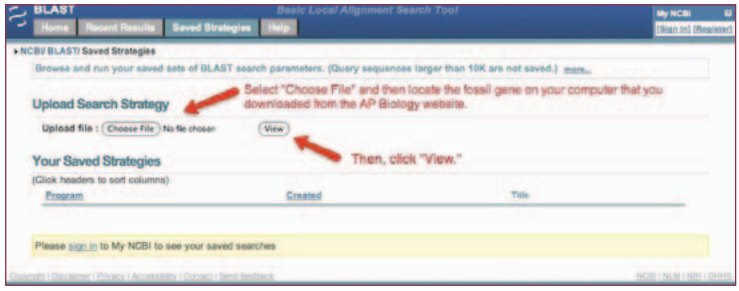


Figure 6

e. A screen will appear with the parameters for your query already configured. NOTE: Do not alter any of the parameters. Scroll down the page and click on the "BLAST" button at the bottom.

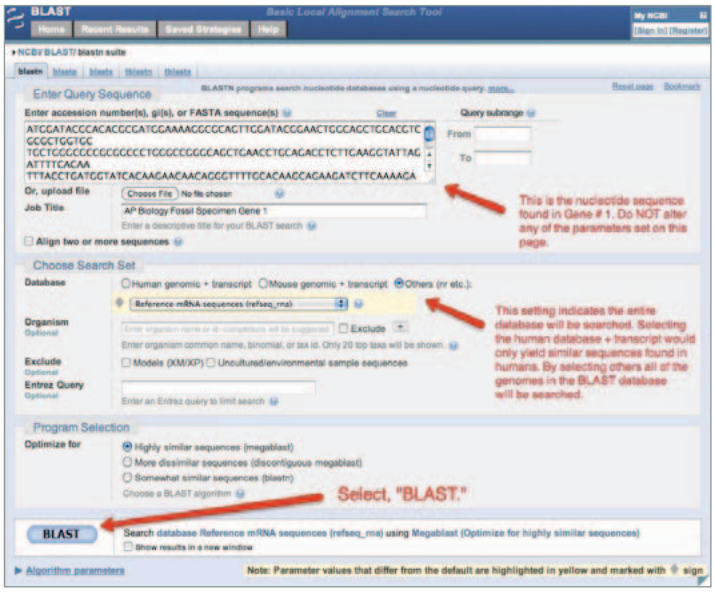


Figure 7

f. After collecting and analyzing all of the data for that particular gene (see instructions below), repeat this procedure for the other two gene sequences.

Step 4 The results page has two sections. The first section is a graphical display of the matching sequences.

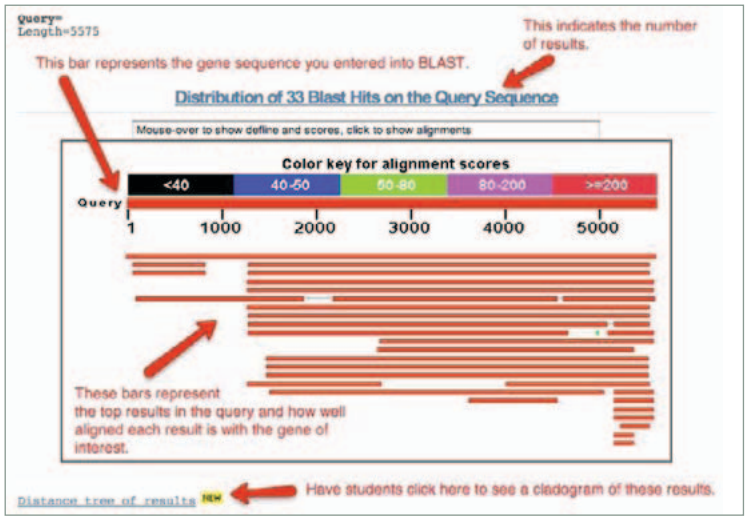


Figure 8

Scroll down to the section titled "Sequences producing significant alignments." The species in the list that appears below this section are those with sequences identical to or most similar to the gene of interest. The most similar sequences are listed first, and as you move down the list, the sequences become less similar to your gene of interest.

Figure 9

If you click on a particular species listed, you'll get a full report that includes the classification scheme of the species, the research journal in which the gene was first reported, and the sequence of bases that appear to align with your gene of interest.

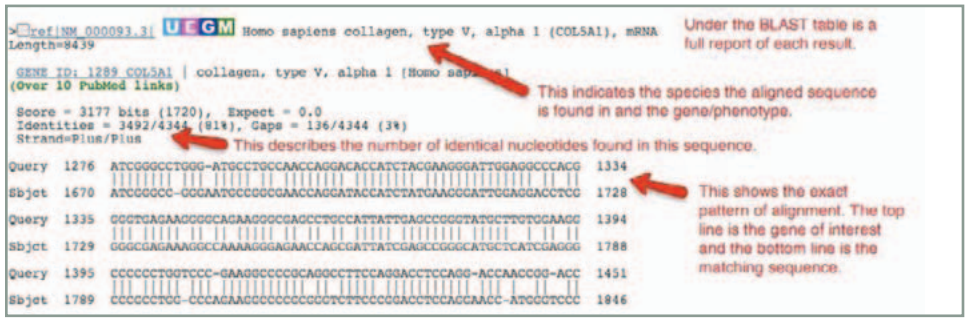


Figure 10

If you click on a particular species listed, you'll get a full report that includes the species' classification scheme, the research journal in which the gene was first reported, and the sequence of bases that appear to align with your gene of interest.

If you click on the link titled "Distance tree of results," you will see a cladogram with the species with similar sequences to your gene of interest placed on the cladogram according to how closely their matched gene aligns with your gene of interest.

Analyzing Results

Recall that species with common ancestry will share similar genes. The more similar genes two species have in common, the more recent their common ancestor and the closer the two species will be located on a cladogram.

As you collect information from BLAST for each of the gene files, you should be thinking about your original hypothesis and whether the data support or cause you to reject your original placement of the fossil species on the cladogram.

For each BLAST query, consider the following:

- The higher the score, the closer the alignment.
- The lower the e value, the closer the alignment.
- Sequences with e values less than 1e-04 (1 x 10-4) can be considered related with an error rate of less than 0.01%.

1. What species in the BLAST result has the most similar gene sequence to the gene of interest?
2. Where is that species located on your cladogram?
3. How similar is that gene sequence?
4. What species has the next most similar gene sequence to the gene of interest?

Based on what you have learned from the sequence analysis and what you know from the structure, decide where the new fossil species belongs on the cladogram with the other organisms. If necessary, redraw the cladogram you created before.

Evaluating Results

Compare and discuss your cladogram with your classmates. Does everyone agree with the placement of the fossil specimen? If not, what is the basis of the disagreement?

On the main page of BLAST, click on the link "List All Genomic Databases." How many genomes are currently available for making comparisons using BLAST? How does this limitation impact the proper analysis of the gene data used in this lab?

What other data could be collected from the fossil specimen to help properly identify its evolutionary history?

Designing and Conducting Your Investigation

Now that you've completed this investigation, you should feel more comfortable using BLAST. The next step is to learn how to find and BLAST your own genes of interest. To locate a gene, you will go to the Entrez Gene website (***http://www.ncbi.nlm.nih.gov/gene***). Once you have found the gene on the website, you can copy the gene sequence and input it into a BLAST query.

Example Procedure

One student's starting question: What is the function of actin in humans? Do other organisms have actin? If so, which ones?

1. Go to the Entrez Gene website (***http://www.ncbi.nlm.nih.gov/gene***) and search for "human actin."
2. Click on the first link that appears and scroll down to the section "NCBI Reference Sequences."
3. Under "mRNA and Proteins," click on the first file name. It will be named "NM 001100.3" or something similar. These standardized numbers make cataloging sequence files easier. Do not worry about the file number for now.
4. Just below the gene title click on "FASTA." This is the name for a particular format for displaying sequences.
5. The nucleotide sequence displayed is that of the actin gene in humans.
6. Copy the entire gene sequence, and then go to the BLAST homepage (***http://blast.ncbi.nlm.nih.gov/Blast.cgi***).
7. Click on "nucleotide blast" under the Basic BLAST menu.
8. Paste the sequence into the box where it says "Enter Query Sequence."
9. Give the query a title in the box provided if you plan on saving it for later.

10. Under "Choose Search Set," select whether you want to search the human genome only, mouse genome only, or all genomes available.

11. Under "Program Selection," choose whether or not you want highly similar sequences or somewhat similar sequences. Choosing somewhat similar sequences will provide you with more results.

12. Click BLAST.

Below is a list of some gene suggestions you could investigate using BLAST. As you look at a particular gene, try to answer the following questions:

- What is the function in humans of the protein produced from that gene?
- Would you expect to find the same protein in other organisms? If so, which ones?
- Is it possible to find the same gene in two different kinds of organisms but not find the protein that is produced from that gene?
- If you found the same gene in all organisms you test, what does this suggest about the evolution of this gene in the history of life on earth?
- Does the use of DNA sequences in the study of evolutionary relationships mean that other characteristics are unimportant in such studies? Explain your answer.

Suggested Genes to Explore	Families or Genes Studied Previously
ATP synthase	Enzymes
Catalase	Parts of ribosomes
GAPDH	Protein channels
Keratin	
Myosin	
Pax1	
Ubiquitin	

INVESTIGATION 4

DIFFUSION AND OSMOSIS

What causes my plants to wilt if I forget to water them?

BACKGROUND

Cells must move materials through membranes and throughout cytoplasm in order to maintain homeostasis. The movement is regulated because cellular membranes, including the plasma and organelle membranes, are selectively permeable. Membranes are phospholipid bilayers containing embedded proteins; the phospholipid fatty acids limit the movement of water because of their hydrophobic characteristics.

The cellular environment is aqueous, meaning that the solvent in which the solutes, such as salts and organic molecules, dissolve is water. Water may pass slowly through the membrane by osmosis or through specialized protein channels called aquaporins. Aquaporins allow the water to move more quickly than it would through osmosis. Most other substances, such as ions, move through protein channels, while larger molecules, including carbohydrates, move through transport proteins.

The simplest form of movement is diffusion, in which solutes move from an area of high concentration to an area of low concentration; diffusion is directly related to molecular kinetic energy. Diffusion does not require energy input by cells. The movement of a solute from an area of low concentration to an area of high concentration requires energy input in the form of ATP and protein carriers called pumps.

Water moves through membranes by diffusion; the movement of water through membranes is called osmosis. Like solutes, water moves down its concentration gradient. Water moves from areas of high potential (high free water concentration) and low solute concentration to areas of low potential (low free water concentration) and high solute concentration. Solutes decrease the concentration of free water, since water molecules surround the solute molecules. The terms *hypertonic, hypotonic,* and *isotonic* are used to describe solutions separated by selectively permeable membranes. A hypertonic solution has a higher solute concentration and a lower water potential as compared to the other solution; therefore, water will move into the hypertonic solution through the membrane by osmosis. A hypotonic solution has a lower solute concentration and a higher water potential than the solution on the other side of the membrane; water will move down its concentration gradient into the other solution. Isotonic solutions have equal water potentials.

In nonwalled cells, such as animal cells, the movement of water into and out of a cell is affected by the relative solute concentration on either side of the plasma membrane. As water moves out of the cell, the cell shrinks; if water moves into the cell, it swells and may eventually burst. In walled cells, including fungal and plant cells, osmosis is affected not only by the solute concentration, but also by the resistance to water movement in the cell by the cell wall. This resistance is called turgor pressure. The presence of a cell wall prevents the cells from bursting as water enters; however, pressure builds up inside the cell and affects the rate of osmosis.

Water movement in plants is important in water transport from the roots into the shoots and leaves. You likely will explore this specialized movement called transpiration in another lab investigation.

Understanding Water Potential

Water potential predicts which way water diffuses through plant tissues and is abbreviated by the Greek letter psi (ψ). Water potential is the free energy per mole of water and is calculated from two major components: (1) the solute potential (ψ_S), which is dependent on solute concentration, and (2) the pressure potential (ψ_P), which results from the exertion of pressure—either positive or negative (tension) — on a solution. The solute potential is also called the osmotic potential.

$$\psi = \psi_P + \psi_S$$

Water Potential = Pressure Potential + Solute Potential

Water moves from an area of higher water potential or higher free energy to an area of lower water potential or lower free energy. Water potential measures the tendency of water to diffuse from one compartment to another compartment.

The water potential of pure water in an open beaker is zero ($\psi = 0$) because both the solute and pressure potentials are zero ($\psi_S = 0$; $\psi_P = 0$). An increase in positive pressure raises the pressure potential and the water potential. The addition of solute to the water lowers the solute potential and therefore decreases the water potential. This means that a solution at atmospheric pressure has a negative water potential due to the solute.

The solute potential (ψ_S) = – iCRT, where i is the ionization constant, C is the molar concentration, R is the pressure constant (R = 0.0831 liter bars/mole-K), and T is the temperature in K (273 + °C).

A 0.15 M solution of sucrose at atmospheric pressure ($\psi_P = 0$) and 25°C has an osmotic potential of -3.7 bars and a water potential of -3.7 bars. A bar is a metric measure of pressure and is the same as 1 atmosphere at sea level. A 0.15 M NaCl solution contains 2 ions, Na^+ and Cl^-; therefore i = 2 and the water potential = -7.4 bars.

When a cell's cytoplasm is separated from pure water by a selectively permeable membrane, water moves from the surrounding area, where the water potential is higher ($\psi = 0$), into the cell, where water potential is lower because of solutes in the cytoplasm

(ψ is negative). It is assumed that the solute is not diffusing (Figure 1a). The movement of water into the cell causes the cell to swell, and the cell membrane pushes against the cell wall to produce an increase in pressure. This pressure, which counteracts the diffusion of water into the cell, is called turgor pressure.

Over time, enough positive turgor pressure builds up to oppose the more negative solute potential of the cell. Eventually, the water potential of the cell equals the water potential of the pure water outside the cell (ψ of cell = ψ of pure water = 0). At this point, a dynamic equilibrium is reached and net water movement ceases (Figure 1b).

Figures 1a-b. Plant cell in pure water. The water potential was calculated at the beginning of the experiment (a) and after water movement reached dynamic equilibrium and the net water movement was zero (b).

If solute is added to the water surrounding the plant cell, the water potential of the solution surrounding the cell decreases. If enough solute is added, the water potential outside the cell is equal to the water potential inside the cell, and there will be no net movement of water. However, the solute concentrations inside and outside the cell are not equal, because the water potential inside the cell results from the combination of both the turgor pressure (ψ_p) and the solute pressure (ψ_s). (See Figure 2.)

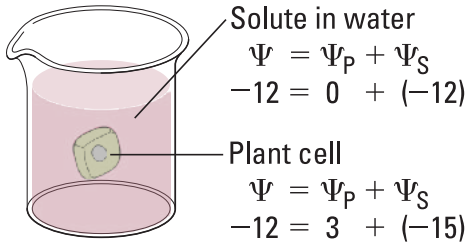


Figure 2. Plant cell in an aqueous solution. The water potential of the cell equals that of surrounding solution at dynamic equilibrium. The cell's water potential equals the sum of the turgor pressure potential plus the solute potential. The solute potentials of the solution and of the cell are not equal.

If more solute is added to the water surrounding the cell, water will leave the cell, moving from an area of higher water potential to an area of lower water potential. The water loss causes the cell to lose turgor. A continued loss of water will cause the cell membrane to shrink away from the cell wall, and the cell will plasmolyze.

- Calculate the solute potential of a 0.1 M NaCl solution at 25°C. If the concentration of NaCl inside the plant cell is 0.15 M, which way will the water diffuse if the cell is placed into the 0.1 M NaCl solutions?
- What must the turgor pressure equal if there is no net diffusion between the solution and the cell?

Learning Objectives

- To investigate the relationship among surface area, volume, and the rate of diffusion
- To design experiments to measure the rate of osmosis in a model system
- To investigate osmosis in plant cells
- To design an experiment to measure water potential in plant cells
- To analyze the data collected in the experiments and make predictions about molecular movement through cellular membranes
- To work collaboratively to design experiments and analyze results
- To connect the concepts of diffusion and osmosis to the cell structure and function

General Safety Precautions

You must wear safety glasses or goggles, aprons, and gloves because you will be working with acids and caustic chemicals. The HCl and NaOH solutions will cause chemical burns, and you should use these solutions in spill-proof trays or pans. Follow your teacher's instructions carefully. Do not work in the laboratory without your teacher's supervision. Talk to your teacher if you have any questions or concerns about the experiments.

THE INVESTIGATIONS

This investigation consists of three parts. In Procedure 1, you use artificial cells to study the relationship of surface area and volume. In Procedure 2, you create models of living cells to explore osmosis and diffusion. You finish by observing osmosis in living cells (Procedure 3). All three sections of the investigation provide opportunities for you to design and conduct your own experiments.

Getting Started

These questions are designed to help you understand kinetic energy, osmosis, and diffusion and to prepare for your investigations.

- What is kinetic energy, and how does it differ from potential energy?
- What environmental factors affect kinetic energy and diffusion?

- How do these factors alter diffusion rates?
- Why are gradients important in diffusion and osmosis?
- What is the explanation for the fact that most cells are small and have cell membranes with many convolutions?
- Will water move into or out of a plant cell if the cell has a higher water potential than the surrounding environment?
- What would happen if you applied saltwater to a plant?
- How does a plant cell control its internal (turgor) pressure?

Procedure 1: Surface Area and Cell Size

Cell size and shape are important factors in determining the rate of diffusion. Think about cells with specialized functions, such as the epithelial cells that line the small intestine or plant root hairs.

- What is the shape of these cells?
- What size are the cells?
- How do small intestinal epithelial and root hair cells function in nutrient procurement?

Materials

- 2% agar containing NaOH and the pH-indicator dye phenolphthalein
- 1% phenolphthalein solution
- 0.1M HCl
- 0.1 M NaOH
- Squares of hard, thin plastic (from disposable plates); unserrated knives; or scalpels from dissection kits
- Metric rulers
- Petri dishes and test tubes
- 2% agar with phenolphthalein preparation

Step 1 Place some phenolphthalein in two test tubes. Add 0.1 M HCl to one test tube, swirl to mix the solutions, and observe the color. Using the same procedure, add 0.1 M NaOH to the other test tube. Remember to record your observations as you were instructed.

- Which solution is an acid?
- Which solution is a base?
- What color is the dye in the base? In the acid?
- What color is the dye when mixed with the base?

Step 2 Using a dull knife or a thin strip of hard plastic, cut three blocks of agar of different sizes.

These three blocks will be your models for cells.

- What is the surface area of each of your three cells?
- What is the total volume of each of your cells?
- If you put each of the blocks into a solution, into which block would that solution diffuse throughout the entire block fastest? Slowest? How do you explain the difference?

Alternative Method

Mix one packet of unflavored gelatin with 237 mL of water: add 2.5 mL 1% phenolphthalein and a few drops of 0.1 M NaOH. The solution should be bright pink. Pour the gelatin mixture into shallow pans and refrigerate overnight.

You may use white vinegar in place of the 0.1 M HCl.

Designing and Conducting Your Investigation

Using the materials listed earlier, design an experiment to test the predictions you just made regarding the relationship of surface area and volume in the artificial cells to the diffusion rate using the phenolphthalein–NaOH agar and the HCl solution. Once you have finished planning your experiment, have your teacher check your design. When you have an approved design, run your experiment and record your results. Do your experimental results support your predictions?

Procedure 2: Modeling Diffusion and Osmosis

You are in the hospital and need intravenous fluids. You read the label on the IV bag, which lists all of the solutes in the water.

- Why is it important for an IV solution to have salts in it?
- What would happen if you were given pure water in an IV?
- How would you determine the best concentration of solutes to give a patient in need of fluids *before* you introduced the fluids into the patient's body?

In this experiment, you will create models of living cells using dialysis tubing. Like cell membranes, dialysis tubing is made from a material that is selectively permeable to water and some solutes. You will fill your model cells with different solutions and determine the rate of diffusion.

Materials

- Distilled or tap water
- 1 M sucrose
- 1 M NaCl
- 1 M glucose
- 5% ovalbumin (egg white protein)
- 20 cm-long dialysis tubing
- Cups
- Balances

- How can you use weights of the filled cell models to determine the rate and direction of diffusion? What would be an appropriate control for the procedure you just described?
- Suppose you could test other things besides weights of the dialysis tubes. How could you determine the rates and directions of diffusion of water, sucrose, NaCl, glucose, and ovalbumin?
- Will protein diffuse? Will it affect the rate of diffusion of other molecules?

Step 1 Choose up to four pairs of different solutions. One solution from each pair will be in the model cell of dialysis tubing, and the other will be outside the cell in the cup. Your fifth model cell will have water inside and outside; this is your control. Before starting, use your knowledge about solute gradients to predict whether the water will diffuse into or out of the cell. Make sure you label the cups to indicate what solution is inside the cell and inside the cup.

Step 2 Make dialysis tubing cells by tying a knot in one end of five pieces of dialysis tubing. Fill each "cell" with 10 mL of the solution you chose for the inside, and knot the other end, leaving enough space for water to diffuse into the cell.

Step 3 Weigh each cell, record the initial weight, and then place it into a cup filled with the second solution for that pair. Weigh the cell after 30 minutes and record the final weight.

Step 4 Calculate the percent change in weight using the following formula: (final – initial)/initial X 100. Record your results.

- Which pair(s) that you tested did not have a change in weight? How can you explain this?
- If you compared 1 M solutions, was a 1 M NaCl solution more or less hypertonic than a 1 M sucrose solution? What is your evidence? What about 1 M NaCl and 1 M glucose and 1 M sucrose?
- Does the protein solution have a high molarity? What is evidence for your conclusion?
- How could you test for the diffusion of glucose?
- Based on what you learned from your experiment, how could you determine the solute concentration inside a living cell?

Designing and Conducting Your Investigation

Living cell membranes are selectively permeable and contain protein channels that permit the passage of water and molecules. In some respects, the dialysis tubing you used is similar to a cell membrane, and you can use it to explore osmosis in greater depth. Think about the questions that came up as you worked through the investigation. What unanswered questions do you still have about osmosis that you could investigate further?

Using the available materials, design an investigation to answer one of your questions. Have your teacher check your design first. Remember to record your results, and be sure to use appropriate controls.

These questions can help jump-start your thinking.

- What factors determine the rate and direction of osmosis?
- What would you predict if you used a starch solution instead of the protein?
- Can you diagram the flow of water based upon the contents of your model cell and the surrounding solution?
- When will the net osmosis rate equal zero in your model cells? Will it ever truly be zero?
- Based upon your observations, can you predict the direction of osmosis in living cells when the cells are placed in various solutions?
- How is the dialysis tubing functionally different from a cellular membrane?

Procedure 3: Observing Osmosis in Living Cells

The interactions between selectively permeable membranes, water, and solutes are important in cellular and organismal functions. For example, water and nutrients move from plant roots to the leaves and shoots because of differences in water potentials. Based upon what you know and what you have learned about osmosis, diffusion, and water potential in the course of your investigations, think about these questions.

- What would happen if you applied saltwater to the roots of a plant? Why?
- What are two different ways a plant could control turgor pressure, a name for internal water potential within its cells? Is this a sufficient definition for turgor pressure?
- Will water move into or out of a plant cell if the cell has a higher water potential than its surrounding environment?

Step 1 Start by looking at a single leaf blade from either *Elodea* (a water plant) or a leaf-like structure from *Mnium hornum* (a moss) under the light microscope. If you need assistance, your teacher will show you how to place specimens on a slide.

- Where is the cell membrane in relation to the cell wall? Can you see the two structures easily? Why or why not?
- What parts of the cell that you see control the water concentration inside the cell?

Back in Procedure 2 you tested diffusion and osmosis properties of several solutions. Now you are going to determine how they affect plant cell turgor pressure.

- What changes do you expect to see when the cells are exposed to the solutions?
- How will you know if a particular treatment is increasing turgor pressure? If it is reducing turgor pressure?
- How could you determine which solution is isotonic to the cells?

Step 2 Test one of the four solutions from Procedure 2 and find out if what you predicted is what happens. When you are done, ask other students what they saw. Be sure to record all of your procedures, calculations, and observations.

Designing and Conducting Your Investigation

Materials

- Potatoes, sweet potatoes, or yams
- Cork borers or french fry cutter
- Balances
- Metric rulers
- Cups
- Color-coded sucrose solutions of different, but unlabeled, concentrations prepared by your teacher

Design an experiment to identify the concentrations of the sucrose solutions and use the solutions to determine the water potential of the plant tissues. (You might want to review the information on water potential described in Understanding Water Potential.) Use the following questions to guide your investigation:

- How can you measure the plant pieces to determine the rate of osmosis?
- How would you calculate the water potential in the cells?
- Which solution had a water potential equal to that of the plant cells? How do you know?
- Was the water potential in the different plants the same?
- How does this compare to your previous determinations in the *Elodea* cells?
- What would your results be if the potato were placed in a dry area for several days before your experiment?
- When potatoes are in the ground, do they swell with water when it rains? If not, how do you explain that, and if so, what would be the advantage or disadvantage?

Analyzing Results

1. Why are most cells small, and why do they have cell membranes with many convolutions?
2. What organelles inside the cell have membranes with many convolutions? Why?
3. Do you think osmosis occurs when a cell is in an isotonic solution? Explain your reasoning.

Where Can You Go from Here?

Do you think that fungal cells have turgor pressure? Design an experiment to test your hypothesis.

INVESTIGATION 5

PHOTOSYNTHESIS

What factors affect the rate of photosynthesis in living leaves?

BACKGROUND

Photosynthesis fuels ecosystems and replenishes the Earth's atmosphere with oxygen. Like all enzyme-driven reactions, the rate of photosynthesis can be measured by either the disappearance of substrate or the accumulation of product (or by-products).

The general summary equation for photosynthesis is

$$2\ H_2O + CO_2 + \text{light} \rightarrow \text{carbohydrate } (CH_2O) + O_2 + H_2O$$

What could you measure to determine the rate of photosynthesis?

- Production of O_2 (How many moles of O_2 are produced for one mole of sugar synthesized?)

  or

- Consumption of CO_2 (How many moles of CO_2 are consumed for every mole of sugar synthesized?)

In this investigation, you will use a system that measures the accumulation of oxygen.

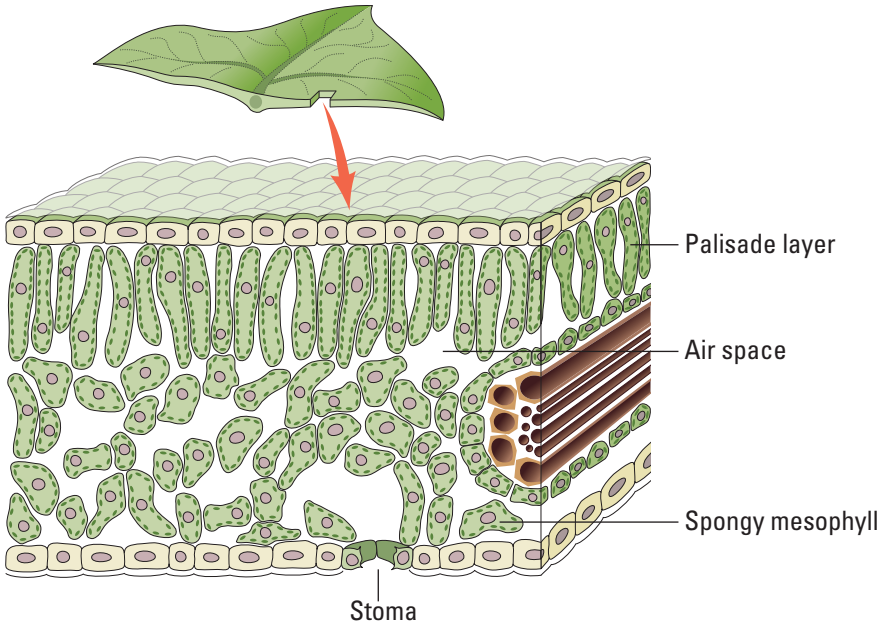


Figure 1. Leaf Anatomy

Because the spongy mesophyll layer of leaves (shown in Figure 1) is normally infused with gases (O_2 and CO_2), leaves — or disks cut from leaves — normally float in water. What would you predict about the density of the leaf disk if the gases are drawn from the spongy mesophyll layer by using a vacuum and replaced with water? How will that affect whether or not the leaf floats? If the leaf disk is placed in a solution with an alternate source of carbon dioxide in the form of bicarbonate ions, then photosynthesis can occur in a sunken leaf disk. As photosynthesis proceeds, oxygen accumulates in the air spaces of the spongy mesophyll, and the leaf disk will once again become buoyant and rise in a column of water. Therefore, the rate of photosynthesis can be *indirectly* measured by the rate of rise of the leaf disks. However, there's more going on in the leaf than that! You must also remember that cellular respiration is taking place at the same time as photosynthesis in plant leaves. (Remember that plant cells have mitochondria, too!) What else could be going on that might affect this process? Aerobic respiration will consume oxygen that has accumulated in spongy mesophyll. Consequently, the two processes counter each other with respect to the accumulation of oxygen in the air spaces of the spongy mesophyll. So now you have a more robust measurement tool — the buoyancy of the leaf disks is actually an indirect measurement of the *net* rate of photosynthesis occurring in the leaf tissue.

Learning Objectives

- To design and conduct an experiment to explore the effect of certain factors, including different environmental variables, on the rate of cellular photosynthesis

- To connect and apply concepts, including the relationship between cell structure and function (chloroplasts); strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; and the physical laws pertaining to the properties and behaviors of gases

General Safety Precautions

You must wear safety goggles or glasses, aprons, and gloves because you will be working in close proximity to exposed lightbulbs that can easily shatter.

Be careful to keep your solutions away from the electrical cord of your light source. Follow your teacher's instructions.

If you investigate temperature as a variable in Designing and Conducting Your Investigation, there is no need to heat any solution beyond 50–60°C.

Most but not all syringes are capable of withstanding the vacuum created in this procedure without failure. However, you should test the syringes beforehand.

THE INVESTIGATIONS

Getting Started

To study photosynthesis, review the properties of light and how it interacts with matter. In addition to your textbook, the Concord Consortium has a Java-based Web activity that will review the properties of light and the ways in which visible light interacts with matter in the process of photosynthesis. This multistep activity uses visualizations, animations, and a molecular modeling engine that does an excellent job of making abstract concepts understandable. To explore this activity, enter these terms in your search engine: "concord consortium molecular workbench photosynthesis."

While going through this activity, record any questions in your laboratory notebook. These questions and others that occur to you while working through the steps in Procedure can serve as a basis for your own investigation in Designing and Conducting Your Investigation.

Procedure

In this part of the lab, you will learn how the floating leaf disk technique can measure the rate of photosynthesis by testing a variable that you know affects photosynthesis. Later, you will apply this technique (or computer-based probes) to test a variable that you choose. It is important for you to develop a few skills during this part of the investigation in order to carry out your own investigation. For the floating disk technique, the most challenging skill is getting the disks to sink. Don't just watch someone do this; make sure you can get the disks to sink as well.

Materials

- Baking soda (sodium bicarbonate)
- Liquid soap (approximately 5 mL of dishwashing soap in 250 mL of water)
- 2 plastic syringes without needle (10 mL or larger)
- Living leaves (spinach, ivy, etc.)
- Hole punch
- 2 clear plastic cups
- Timer
- Light source

Figure 2. Materials

When immersed in water, oxygen bubbles are usually trapped in the air spaces of the spongy mesophyll in the plant leaf. By creating a vacuum in this experimental procedure, the air bubbles can be drawn out of the spongy mesophyll, and the space is refilled by the surrounding solution. This allows the leaf disks to sink in the experimental solution. If the solution has bicarbonate ions and enough light, the leaf disk will begin to produce sugars and oxygen through the process of photosynthesis. Oxygen collects in the leaf as photosynthesis progresses, causing the leaf disks to float again. The length of time it takes for leaf disks to float again is a measure of the net rate of photosynthesis. This process is shown in Figure 3.

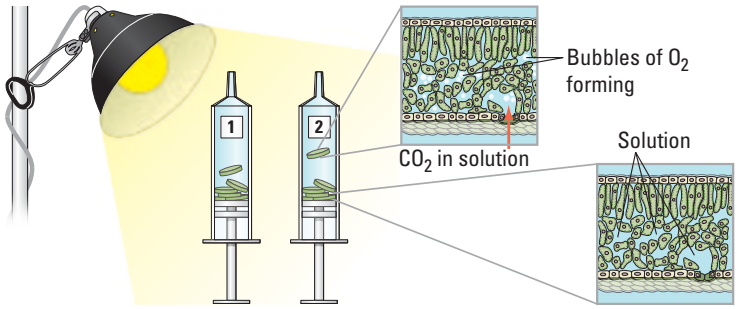


Figure 3. Photosynthesis at Work

Question: If the leaf disks are treated in a way you know increases the net rate of photosynthesis, should they start to float faster or slower? Why?

Step 1 Prepare 300 mL of 0.2% bicarbonate solution for each experiment. The bicarbonate will serve as a source of carbon dioxide for the leaf disks while they are in the solution.

Step 2 Pour the bicarbonate solution into a clear plastic cup to a depth of about 3 cm. Label this cup "With CO_2." Fill a second cup with only water to be used as a control group. Label this cup "Without CO_2." Throughout the rest of the procedure you will be preparing material for both cups, so do everything for both cups simultaneously.

Step 3 Using a pipette, add one drop of a dilute liquid soap solution to the solution in each cup. It is critical to avoid suds. If either solution generates suds, then dilute it with more bicarbonate or water solution. The soap acts as a surfactant or "wetting agent" — it wets the hydrophobic surface of the leaf, allowing the solution to be drawn into the leaf and enabling the leaf disks to sink in the fluid.

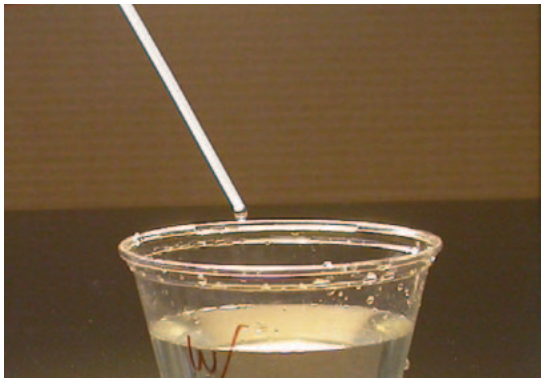


Figure 4. Dilute Liquid Soap Solution Added to Cup

Step 4 Using a hole punch, cut 10 or more uniform leaf disks for each cup. Avoid major leaf veins. (The choice of plant material is perhaps the most critical aspect of this procedure. The leaf surface should be smooth and not too thick.)

Figure 5. Leaf Disks

Step 5 Draw the gases out of the spongy mesophyll tissue and infiltrate the leaves with the sodium bicarbonate solution by performing the following steps:

a. Remove the piston or plunger from both syringes. Place the 10 leaf disks into each syringe barrel.

b. Replace the plunger, but be careful not to crush the leaf disks. Push in the plunger until only a small volume of air and leaf disk remain in the barrel (<10%).

c. Pull a small volume (5 cc) of sodium bicarbonate plus soap solution from your prepared cup into one syringe and a small volume of water plus soap into the other syringe. Tap each syringe to suspend the leaf disks in the solution. Make sure that, with the plunger inverted, the disks are suspended in the solution. Make sure no air remains. Move the plunger to get rid of air from the plunger before you attempt Step d.

d. You now want to create a vacuum in the plunger to draw the air out of the leaf tissue. This is the most difficult step to master. Once you learn to do this, you will be able to complete the entire exercise successfully. Create the vacuum by holding a finger over the narrow syringe opening while drawing back the plunger (see Figure 6a). Hold this vacuum for about 10 seconds. While holding the vacuum, swirl the leaf disks to suspend them in the solution. Now release the vacuum by letting the plunger spring back. The solution will infiltrate the air spaces in the leaf disk, causing the leaf disks to sink in the syringe. If the plunger does not spring back, you did not have a good vacuum, and you may need a different syringe. You may have to repeat this procedure two to three times in order to get the disks to sink. **(If you have any difficulty getting your disks to sink after three tries, it is usually because there is not enough soap in the solution. Try adding a few more drops of soap to the cup and replacing the liquid in the syringe.)** Placing the disks under vacuum more than three times can damage the disks.

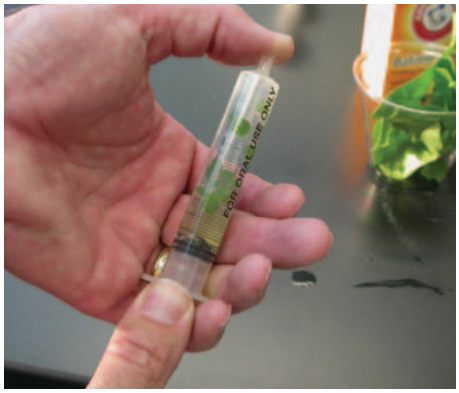


Figure 6a. Creating a Vacuum in the Plunger

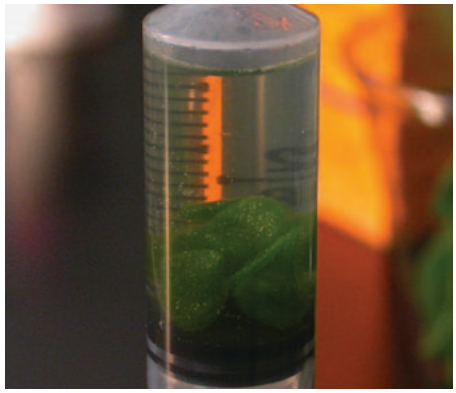


Figure 6b. Sinking Leaf Disks

Step 6 Pour the disks and the solution from the syringe into the appropriate clear plastic cup. Disks infiltrated with the bicarbonate solution go in the "With CO_2" cup, and disks infiltrated with the water go in the "Without CO_2" cup.

Step 7 Place both cups under the light source and start the timer. At the end of each minute, record the number of floating disks. Then swirl the disks to dislodge any that stuck against the side of the cups. Continue until all of the disks are floating in the cup with the bicarbonate solution.

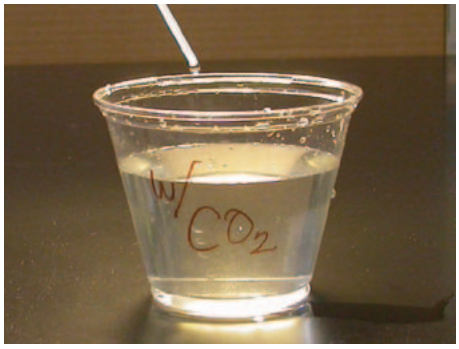


Figure 7a. Cup Under Light Source

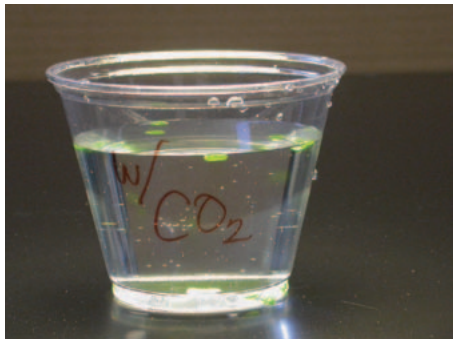


Figure 7b. Disks Floating in Cup with Bicarbonate Solution

Step 8 To make comparisons between experiments, a standard point of reference is needed. Repeated testing of this procedure has shown that the point at which 50% of the leaf disks are floating (the median or ET_{50}, the Estimated Time it takes 50% of the disks to float) is a reliable and repeatable point of reference for this procedure.

Step 9 Record or report findings.

Designing and Conducting Your Investigation

What factors affect the rate of photosynthesis in living plants?

1. Once you have mastered the floating disk technique, you will design an experiment to test another variable that might affect the rate of photosynthesis. Some ideas include the following, but don't limit yourself to just these:

- What environmental variables might affect the net rate of photosynthesis? Why do you think they would affect it? How do you predict they would affect it?
- What features or variables of the plant leaves might affect the net rate of photosynthesis? How and why?
- Could the way you perform the procedure affect the outcome? If the outcome changes, does it mean the net rate of photosynthesis has changed? Why do you think that?

Note: If you are truly stumped, your instructor can give you some guidance. Keep in mind that leaves with hairy surfaces should be avoided. Ivy and spinach work well, but many others do as well. Differences between plants may be one of the ideas that you want to investigate.

2. Use your results to prepare a lab report/mini-poster for a classroom peer review presentation. See Chapter 2 for guidance on this.

Additional Guidelines

1. Consider combining variables as a way to describe differences between different plants. For instance, if you investigate how light intensity affects the rate of photosynthesis, you might generate a "photosynthesis light response curve"—the rate of photosynthesis at different light intensities. The shape of this curve may change for different plants or plants in different light environments. The "light response curve" is a form of measurement itself. How do you think a light response curve (the first variable) for a shade-grown leaf compares to that of a sun-grown leaf? In this situation, sun versus shade is the second variable. Comparing light response curves is a standard research technique in plant physiological ecology.

2. When you compare the ET_{50} across treatments, you will discover that there is an inverse relationship between ET_{50} and the rate of photosynthesis — ET_{50} goes down as rate of photosynthesis goes up, which plots a graph with a negative slope. This creates a seemingly backward graph when plotting your ET_{50} data across treatments, as shown in Figure 8a. To correct this representation and make a graph that shows increasing rates of photosynthesis with a positive slope, the ET_{50} term can be modified by taking its inverse, or $1/ET_{50}$. This creates a more traditional direct relationship graph, as shown in Figure 8b.

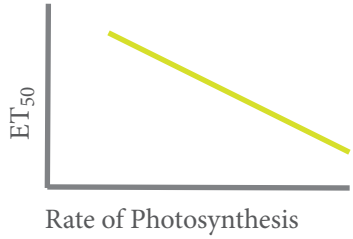


Figure 8a. Inverse Relationship

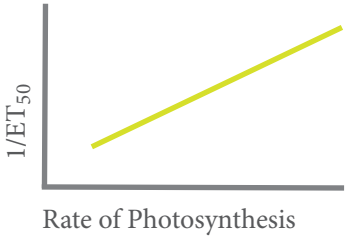


Figure 8b. Direct Relationship

3. Don't forget to include other appropriate data analyses as you prepare and study your discussion and conclusions. In particular for this investigation, you should somehow indicate the variability in your data. The ET_{50} measurement is calculated from the median. To indicate the spread of your data, you could use error bars around the ET_{50} point that express that variation, or you might consider using "box and whisker" plots.

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INVESTIGATION 6

CELLULAR RESPIRATION*

What factors affect the rate of cellular respiration in multicellular organisms?

BACKGROUND

Living systems require free energy and matter to maintain order, to grow, and to reproduce. Energy deficiencies are not only detrimental to individual organisms, but they cause disruptions at the population and ecosystem levels as well. Organisms employ various strategies that have been conserved through evolution to capture, use, and store free energy. Autotrophic organisms capture free energy from the environment through photosynthesis and chemosynthesis, whereas heterotrophic organisms harvest free energy from carbon compounds produced by other organisms. The process of cellular respiration harvests the energy in carbon compounds to produce ATP that powers most of the vital cellular processes. In eukaryotes, respiration occurs in the mitochondria within cells.

If sufficient oxygen is available, glucose may be oxidized completely in a series of enzyme-mediated steps, as summarized by the following reaction:

$$C_6H_{12}O_6 + 6O_{2(g)} \rightarrow 6CO_{2(g)} + 6H_2O + \text{energy}$$

More specifically,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \frac{686 \text{ kilocalories of energy}}{\text{mole of glucose oxidized}}$$

The chemical oxidation of glucose has important implications to the measurement of respiration. From the equation, if glucose is the energy source, then for every molecule of oxygen consumed, one molecule of carbon dioxide is produced.

Suppose you wanted to measure the overall rate of cellular respiration.

- What specific things could you measure?
- Which of these might be easier or harder to measure?

In Procedures, you will learn how to calculate the rate of cellular respiration by using a respirometer system (either microrespirometers or gas pressure sensors with computer interface). These measure relative volume (changes in pressure) as oxygen is consumed by germinating plant seeds. As oxygen gas is consumed during respiration, it is normally

* Transitioned from the *AP Biology Lab Manual* (2001)

replaced by CO_2 gas at a ratio of one molecule of CO_2 for each molecule of O_2. Thus, you would expect no change in gas volume to result from this experiment. However, in the following procedure the CO_2 produced is removed by potassium hydroxide (KOH). KOH reacts with CO_2 to form the solid potassium carbonate (K_2CO_3) through the following reaction:

$$CO_2 + 2KOH \rightarrow K_2CO_3 + H_2O$$

Thus, as O_2 is consumed, the overall gas volume in the respirometer decreases. The change in volume can be used to determine the rate of cellular respiration. Because respirometers are sensitive to changes in gas volume, they are also sensitive to changes in temperature and air pressure; thus, you need to use a control respirometer. What would be a good control for this procedure? Talk with another student for a minute, and come up with at least one possible control you could use.

As you work through Procedures, think about this question: What factors can affect the rate of cellular respiration? In Designing and Conducting Your Investigation, you will design and conduct an experiment(s) to investigate at least one of your responses to this question or some other question you have. Your exploration will likely generate even more questions about cellular respiration.

The investigation also provides an opportunity for you to apply and review concepts that you have studied previously, including the relationship between cell structure and function (mitochondria); enzymatic activity; strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; and the physical laws pertaining to the properties and behaviors of gases.

Learning Objectives

- To learn how a respirometer system can be used to measure respiration rates in plant seeds or small invertebrates, such as insects or earthworms
- To design and conduct an experiment to explore the effect of certain factors, including environmental variables, on the rate of cellular respiration
- To connect and apply concepts, including the relationship between cell structure and function (mitochondria); strategies for capture, storage, and use of free energy; diffusion of gases across cell membranes; and the physical laws pertaining to the properties and behaviors of gases

General Safety Precautions

You must wear safety goggles or glasses, aprons, and gloves during this investigation(s) because KOH (or the alternative, NaOH in Drano) is caustic. Follow your teacher's instructions when using the hot glue gun to seal microrespirometers. Do not work in the laboratory without your teacher's supervision.

THE INVESTIGATIONS

Getting Started

Your teacher may assign the following questions to see how much you understand concepts related to respiration before you design and conduct your own investigation:

1. Why is it necessary to correct the readings of the respirometers containing seeds with the readings taken from respirometers containing only glass beads? Your answer should refer to the concepts derived from the general gas law:

$$PV = nRT$$

Where

P = pressure of the gas

V = volume of the gas

n = number of moles of the gas

R = the gas constant (its value is fixed)

T = temperature of the gas

2. What happens to the volume of the gas being measured (O_2 consumption or CO_2 production) when the temperature or pressure changes during the experiment? If pressure and temperature remain constant, will the volume of gas in the respirometers increase or decrease? Please explain.

Hint: Several tutorials and animations explaining the general gas law are available online (e.g., ***http://www.nclark.net/GasLaws***).

3. Imagine that you are given 25 germinating pea seeds that have been placed in boiling water for five minutes. You place these seeds in a respirometer and collect data. Predict the rate of oxygen consumption (i.e., cellular respiration) for these seeds and explain your reasons.

4. Imagine that you are asked to measure the rate of respiration for a 25 g reptile and a 25 g mammal at 10°C. Predict how the results would compare, and justify your prediction.

5. Imagine that you are asked to repeat the reptile/mammal comparison of oxygen consumption, but at a temperature of 22°C. Predict how these results would differ from the measurements made at 10°C, and explain your prediction in terms of the metabolism of the animals.

6. What difficulties would there be if you used a living green plant in this investigation instead of germinating seeds?

Procedures

The rate of cellular respiration can be measured by several methods, and two reliable methods are detailed below. Your teacher will tell you which method you will use to measure the rate of respiration in germinating plant seeds at room temperature.

Option 1: Using Microrespirometers to Measure the Rate of Cellular Respiration

Materials

- Germinating/nongerminating Wisconsin Fast Plants seeds or seeds of several species of plants, including grasses; small animals, such as crickets or earthworms; small glass beads; or dry, baked seeds
- Safety goggles or glasses, aprons, and gloves
- 1 mL plastic tuberculin syringes without needles
- Thin-stem plastic dropping pipettes
- 40 µL plastic capillary tubes or plastic microhematocrits
- Hot glue gun; absorbent and nonabsorbent cotton
- 3 or 4 one-quarter inch flat metal washers
- Celsius thermometer, centimeter rulers, permanent glass-marking pens
- Constant-temperature water bath
- Manometer fluid (soapy water with red food coloring)
- 15% solution of KOH, potassium hydroxide solution (or NaOH, Drano)

Figure 1. Materials

Figure 2. Microrespirometer Assembly

Constructing a Microrespirometer

Measuring the rate of respiration is more technically challenging than many lab procedures because there are many places for potential error in the assembly and use of equipment. The advantages of the microrespirometer method as described by Richard E. Lee in *American Biology Teacher* include low cost, reliability, simplicity, and rapid response. A modification of the Lee method is described at ***http://www.elbiology.com/labtools/Microrespirometers.html***. However, for the sake of convenience, the procedure is outlined below. **Hint**: Read each step before doing it! You need to assemble two microrespirometers: one for measuring the rate of respiration in germinating seeds and the other for the control.

Step 1 Plug in the hot glue gun and allow it to heat up.

Step 2 Take a tuberculin syringe (without a needle) and make sure that its plunger is pushed all the way in.

Step 3 Carefully insert a 40 µL plastic capillary tube into the syringe where the needle normally would be. Insert it as far as the plunger tip but no farther. This will help prevent the capillary from becoming plugged with glue.

Step 4 While holding the capillary tube straight up, add a small amount of hot glue around its base (where it meets the syringe) to seal the capillary to the syringe. Keep the capillary pointed straight up until the glue cools — this should not take long. If needed, add a bit more glue to ensure an airtight seal between the capillary and syringe. (See Figure 3.)

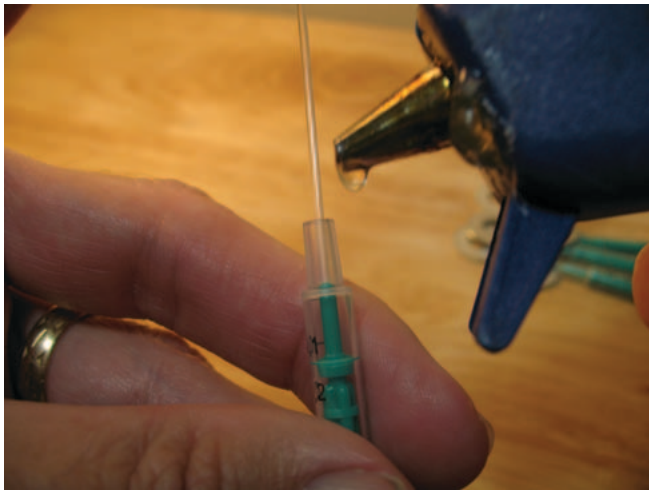

Figure 3. Hot Glue Added to Capillary Tube Base

Step 5 After the glue has cooled, pull back on the plunger and make sure that the glue has not plugged the capillary. If the capillary is plugged, carefully remove the glue and capillary and start over.

Preparing the Microrespirometer

Step 1 Draw a small quantity of manometer fluid (soapy water with red food coloring) into the full length of the microrespirometer's capillary tube. Then eject the fluid back out of the capillary. This coats the inside of the tube with a thin soapy film that helps prevent the manometer fluid from sticking.

Step 2 Carefully insert a small plug of absorbent cotton into the barrel of the microrespirometers, all the way into the 0 mL or cc mark. You can pack this cotton to the end with the barrel of a clean thin-stem pipette. (See Figure 4.)

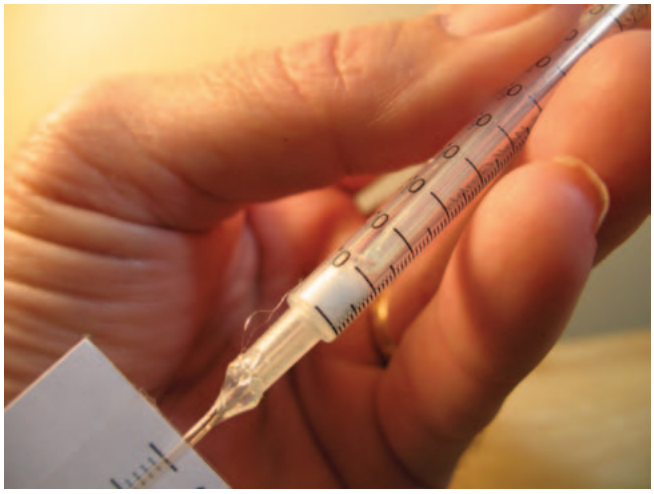

Figure 4. Cotton Inserted into Microrespirometer Barrel

Step 3 Add one small drop of 15% KOH (or NaOH, Drano) to the cotton in the microrespirometers. Do not add too much! **CAUTION: Make sure you are wearing gloves and safety goggles to protect your eyes because KOH is caustic.**

Step 4 Add a small plug of nonabsorbent cotton on top of the absorbent cotton plug already inside the barrel of the microrespirometers. You can pack the cotton to the end with the barrel of a clean thin-stem pipette. (This nonabsorbent plug is needed to protect the seeds from the caustic KOH.)

Step 5 Slowly reinsert the syringe plunger. **CAUTION: Be sure to point the capillary tip into a sink or container**. There may be excess KOH in the syringe that might squirt from the end of the capillary. Push the plunger in until it reaches the cotton so that any excess KOH is removed.

Step 6 Remove the plunger to add seeds.

Step 7 Add 0.5 mL of germinating seeds to the microrespirometers. Push the plunger in to the 1.0 mL mark. This creates a sealed microrespirometer chamber with a 1.0 mL volume.

Step 8 Place three to four washers around the barrel of the microrespirometers. The washers provide weight so that the microrespirometers will sink.

Step 9 Place the microrespirometers in a room temperature (about 20°C) water bath. You must maintain the temperature of the water bath for the experiment. Adjust the level of the water bath so that the capillary tube is sticking out of the water while the barrel of the microrespirometers is completely submerged. You will not be able to read the capillary tube easily unless it is out of the water. Make sure the top end of the capillary tube is open (not sealed).

Setting Up Your Control

Because a microrespirometer is sensitive to changes in gas volume, it is also sensitive to changes in temperature and air pressure. To compensate for any changes, you will use control microrespirometers. The control respirometer is set up just like the microrespirometer except that it contains nonliving matter (e.g., small glass beads or dry, baked seeds) instead of germinating seeds.

Step 1 Add 0.5 mL of beads or baked seeds to the second microrespirometer you assembled. Reinsert the syringe plunger and push it to the 1.0 mL mark. This seals the chamber and creates a chamber that has the same volume as the experimental microrespirometer.

Step 2 Place three to four washers around the barrel of the control.

Step 3 Place the assembled control in the water bath next to the experimental microrespirometer. Adjust the level of the water bath so the capillary tube is sticking out of the water while the barrel of the control is completely submerged. In order to easily read the capillary tube, it must be out of the water. Make sure the top end of the capillary tube is open (not sealed).

The respirometers must be airtight, and they are sensitive to environmental changes, including bumping the lab table. Once the respirometers have reached equilibrium, they should not be touched or moved, nor should anything else be added to or taken out of the water baths (including your hands!).

Collecting Data

Step 1 Prepare a table like Table 1 to record your data and observations in your lab notebook. You will need to record data for both the experimental and control microrespirometers.

Table 1. Results for Option 1, Using Microrespirometers

A Total Time (Min.)	B Water Bath Temperature (20°C)	C Total Distance Fluid Has Moved (cm)	D Change in Fluid Position During Time Interval (cm)
0			
5			
10			
15			
20			
25			

Step 2 Place the experimental and control microrespirometers into the 20°C water bath. Wait 5 minutes to allow the temperature in the microrespirometers to equalize.

Step 3 Use a dropping pipette to add one small drop of manometer fluid to the tip of each capillary tube. If everything is working properly, the drop will be sucked down into the capillary tube. The manometer fluid will seal the chamber of the microrespirometers. (You should use the plunger on the control microrespirometers to get the manometer fluid into the capillary. Pull on the plunger until the manometer drop is about halfway down the capillary. See Figure 5.)

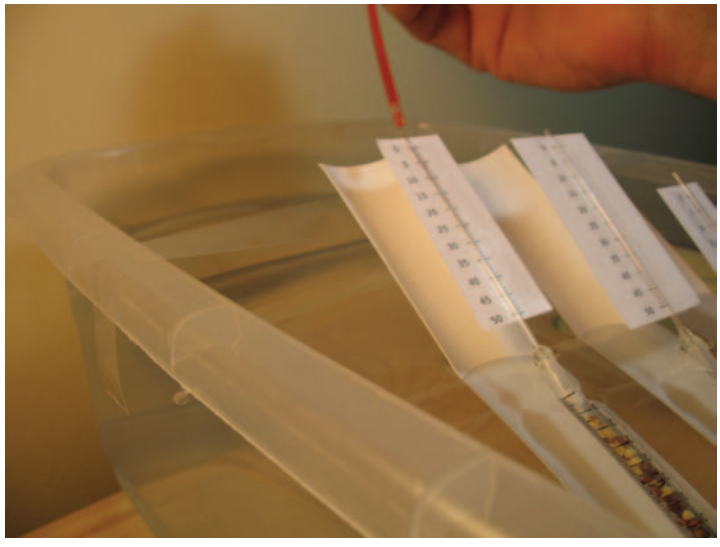

Figure 5. Manometer Fluid Added to Capillary Tube Tip

Step 4 As oxygen is consumed by cellular respiration, the manometer fluid plug will move toward the chamber. Record the starting position of each plug by marking its position on the capillary with a marker. Be sure to mark the bottom edge of the plug. These are your Time 0 marks. Begin timing once you have made the Time 0 marks.

Step 5 At 5-minute intervals, mark the position of the manometer fluid for each capillary tube. Be sure to mark the bottom edge of the fluid plug. Continue marking the positions until the fluid in the microrespirometers has traveled the entire length of the capillary, or until 25 minutes have passed.

Step 6 At the end of 25 minutes, remove the microrespirometers from the water bath. Use a centimeter ruler to measure the distance from the initial mark (Time 0 mark) to each of the 5-minute intervals marked on each capillary tube. Record your measurements in the correct column of your data table.

Step 7 Calculate the change in fluid position during each time interval. To do this, subtract the fluid position at the beginning of the time interval from the fluid position at the end of the time interval. Record your values.

Step 8 Repeat the calculations for your control microrespirometer.

Step 9 Using the values you obtained for the control microrespirometer, correct for any changes in volume that you measure that may be attributed to changes in temperature and air pressure.

Figure 6 shows how the microrespirometer works.

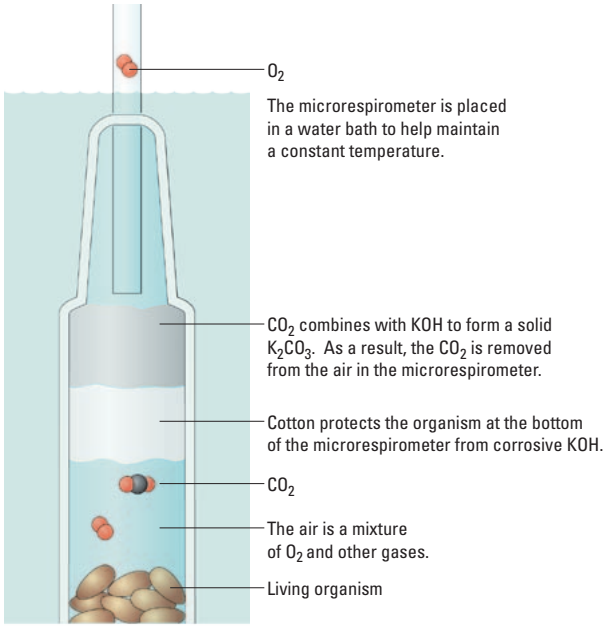

Figure 6. Microrespirometer

Analyzing Results

1. Use your data table to construct a graph. Your goal is to determine respiration rate. How should you plot your data? Which variable will be on the x-axis, and which will be on the y-axis?

2. From the graph, determine the rate of respiration for the germinating seeds at 20°C. **Hint**: Go back and think about what the units of measurement would be for respiration. How can you get a value with those units from your graph?

3. What additional questions can you explore about cellular respiration using the same respirometers from this experiment?

4. In the next part of this investigation, you will design and conduct your own experiments to answer questions that you raised in Procedures. Do you have any suggestions for improving the design of microrespirometers or procedure for measuring oxygen consumption/cellular respiration?

Option 2: Using Gas Pressure Sensors with Computer Interface to Measure the Rate of Cellular Respiration

Gas pressure sensors can be used to measure the rate of cellular respiration by measuring the amount of O_2 consumed, the amount of CO_2 produced, or both simultaneously. Your teacher will provide written instructions or perhaps ask you to download information from the manufacturer's website or another online resource. If you are unfamiliar with the use of probes with a computer interface, you will need to spend time learning how to collect data using the equipment.

General Procedure

1. Use a gas pressure sensor to measure the rate of cellular respiration in germinating seeds at 20°C over a 25-minute time interval or as per instructed by your teacher.

2. What additional questions can you explore about cellular respiration from this experiment?

3. In the next part of this investigation, you will design and conduct your own experiments to answer questions that you raised in the first part of the investigation. Do you have any suggestions for improving the procedure provided for measuring oxygen consumption/cellular respiration using a gas pressure sensor with computer interface?

Designing and Conducting Your Investigation

Now that you have learned how to measure the rate of cellular respiration in germinating seeds, you have a tool for exploring questions on your own. Think about the process of cellular respiration.

- When does it occur? Are there any situations when living cells are not respiring?
- Why might some living cells respire more than others?
- Are there differences between major groups of organisms in how fast they respire?
- What is the difference, if any, in the rate of cellular respiration between germinating seeds and nongerminating seeds?
- Does the temperature of germinating seeds affect the rate of cellular respiration? Do plant seeds consume more oxygen at higher temperatures than at lower temperatures?
- Do germinating seeds just starting to germinate consume oxygen at a greater rate than seeds that have been germinating for several days (age dependence)?
- Do seeds such as Wisconsin Fast Plant seeds (which store energy as oil) respire at a different rate from small grass seeds (which store energy as starch)?
- Do small seeds of spring flowers, weeds, or grasses respire at a different rate from seeds from summer, fall, or winter plants?
- Do seeds from monocot plants respire at different rates from dicot plants?
- Do available nutrients affect the rate of respiration in germinating seeds?
- Can the same respirometer system be used to measure the rate of respiration in small invertebrates, such as insects or earthworms?

Step 1 Design an experiment to investigate one of your own questions about cellular respiration or one of the questions above using microrespirometers or gas pressure sensors. When identifying your design, be sure to address the following:

- What is the essential question being addressed?
- What assumptions are made about the question(s) being addressed? Can those assumptions be verified?
- Will the measurements you choose to make provide the necessary data to answer the question under study?
- Did you include a control in your experiment?
- What are possible sources of error in the experiment(s)?

Step 2 Make a hypothesis, which should include a prediction about the effect of the factor(s) you chose to investigate on the rate of cellular respiration.

Step 3 Conduct your experiment(s) and record data and any answers to your questions in your laboratory notebook or as per instructed by your teacher.

Step 4 Record your data using appropriate methods, such as the example table provided in Procedures. Then graph the results to show the effect of the factors/variables you investigated on the rate of cellular respiration. Calculate the rate(s) of cellular respiration for each factor/variable.

Analyzing Results

1. Your teacher may suggest that you perform statistical analysis of your data, comparing results of the experimental variable(s) to the controls. You should at least express the uncertainty of your measurements with error bars. You may want to review Chapter 3 for more information about statistical analysis.

2. How was the rate of cellular respiration affected by the experimental variable(s) you chose as compared to the control(s)?

3. Compare class data to explain how different variables affect rates of cellular respiration.

Evaluating Results

1. Was your initial hypothesis about the effect of your factor on the rate of cellular respiration supported? Why or why not?

2. What were some challenges you had in performing your experiment? Did you make any incorrect assumptions?

3. Were you able to perform without difficulty the mathematical routines required to analyze your data? If not, what calculations were challenging or required help from your classmates or teacher?

Where Can You Go from Here?

If time is available, ask your teacher if you can extend the investigation to explore answers to other questions that might have been raised as you conducted your experiment(s). For example, if you originally investigated the effect of temperature on metabolic rate in plant seeds, you might want to explore a different aspect, such as the effect of temperature on metabolic rate in small invertebrates, such as insects or earthworms, or the relationship between the mass of an organism and its rate of respiration.

INVESTIGATION 7

CELL DIVISION: MITOSIS AND MEIOSIS

How do eukaryotic cells divide to produce genetically identical cells or to produce gametes with half the normal DNA?

BACKGROUND

One of the characteristics of living things is the ability to replicate and pass on genetic information to the next generation. Cell division in individual bacteria and archaea usually occurs by binary fission. Mitochondria and chloroplasts also replicate by binary fission, which is evidence of the evolutionary relationship between these organelles and prokaryotes.

Cell division in eukaryotes is more complex. It requires the cell to manage a complicated process of duplicating the nucleus, other organelles, and multiple chromosomes. This process, called the cell cycle, is divided into three parts: interphase, mitosis, and cytokinesis (Figure 1). Interphase is separated into three functionally distinct stages. In the first growth phase (G_1), the cell grows and prepares to duplicate its DNA. In synthesis (S), the chromosomes are replicated; this stage is between G_1 and the second growth phase (G_2). In G_2, the cell prepares to divide. In mitosis, the duplicated chromosomes are separated into two nuclei. In most cases, mitosis is followed by cytokinesis, when the cytoplasm divides and organelles separate into daughter cells. This type of cell division is asexual and important for growth, renewal, and repair of multicellular organisms.

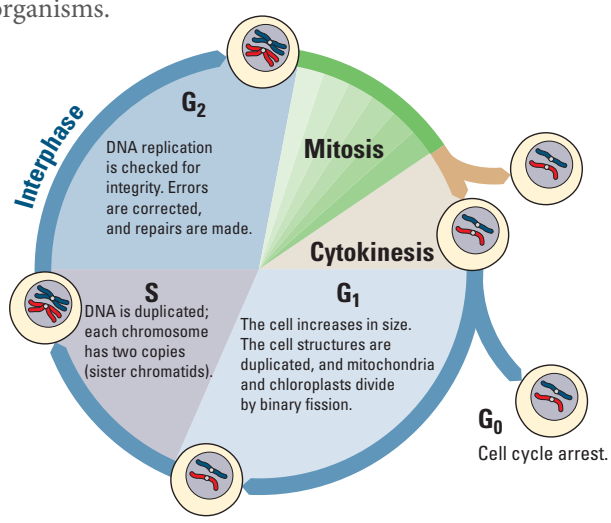

Figure 1. The Cell Cycle Showing G_1, S, and G_2 Phases, Mitosis, and Cytokinesis

Cell division is tightly controlled by complexes made of several specific proteins. These complexes contain enzymes called cyclin-dependent kinases (CDKs), which turn on or off the various processes that take place in cell division. CDK partners with a family of proteins called cyclins. One such complex is mitosis-promoting factor (MPF), sometimes called maturation-promoting factor, which contains cyclin A or B and cyclin-dependent kinase (CDK). (See Figure 2a.) CDK is activated when it is bound to cyclin, interacting with various other proteins that, in this case, allow the cell to proceed from G_2 into mitosis. The levels of cyclin change during the cell cycle (Figure 2b). In most cases, cytokinesis follows mitosis.

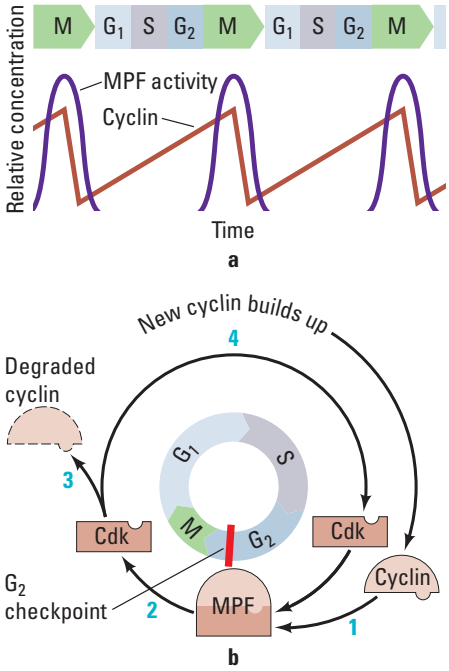

Figure 2. MPF Production During the Cell Cycle

As shown in Figure 3, different CDKs are produced during the phases. The cyclins determine which processes in cell division are turned on or off and in what order by CDK. As each cyclin is turned on or off, CDK causes the cell to move through the stages in the cell cycle.

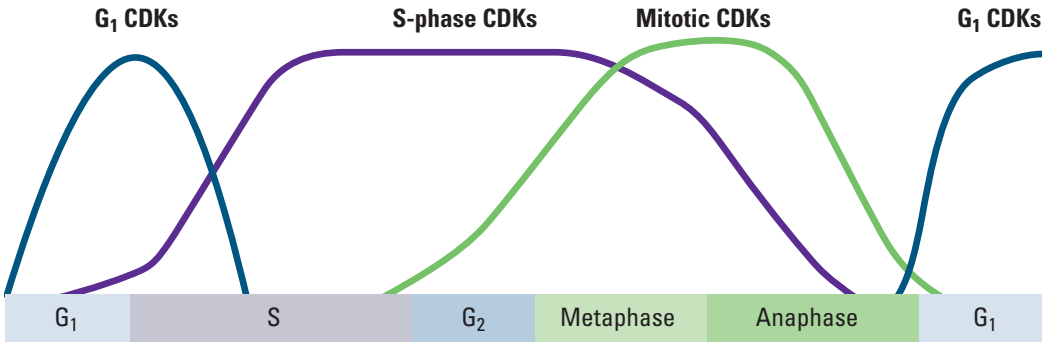

Figure 3. Levels of CDKs During the Cell Cycle

Cyclins and CDKs do not allow the cell to progress through its cycle automatically. There are three checkpoints a cell must pass through: the G_1 checkpoint, G_2 checkpoint, and the M-spindle checkpoint (Figure 4). At each of the checkpoints, the cell checks that it has completed all of the tasks needed and is ready to proceed to the next step in its cycle. Cells pass the G_1 checkpoint when they are stimulated by appropriate external growth factors; for example, platelet-derived growth factor (PDGF) stimulates cells near a wound to divide so that they can repair the injury. The G_2 checkpoint checks for damage after DNA is replicated, and if there is damage, it prevents the cell from going into mitosis. The M-spindle (metaphase) checkpoint assures that the mitotic spindles or microtubules are properly attached to the kinetochores (anchor sites on the chromosomes). If the spindles are not anchored properly, the cell does not continue on through mitosis. The cell cycle is regulated very precisely. Mutations in cell cycle genes that interfere with proper cell cycle control are found very often in cancer cells.

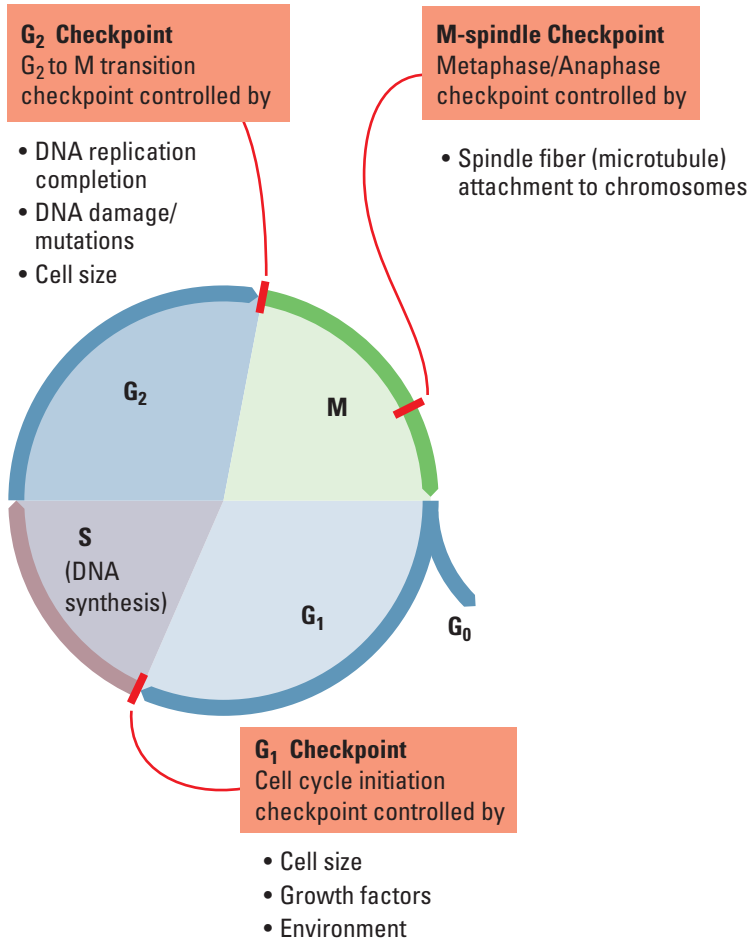

Figure 4. Diagram of the Cell Cycle Indicating the Checkpoints

Learning Objectives

- To describe the events in the cell cycle and how these events are controlled
- To explain how DNA is transmitted to the next generation via mitosis
- To explain how DNA is transmitted to the next generation via meiosis followed by fertilization
- To understand how meiosis and crossing over leads to increased genetic diversity, which is necessary for evolution

General Safety Precautions

You must be careful when preparing specimens for viewing under the compound microscope. Always cover the cover slip with a scientific cleaning wipe, such as a Kimwipe, and press down using a pencil eraser.

You should wear safety goggles or glasses and disposable gloves when handling the chemicals and razor blades in Parts 2 and 5. All materials should be disposed of properly as per your teacher's instructions.

THE INVESTIGATIONS

Getting Started

These questions are designed to see how well you understand and can explain the key concepts related to cell division before you begin your investigations.

1. How did you develop from a single-celled zygote to an organism with trillions of cells? How many mitotic cell divisions would it take for one zygote to grow into an organism with 100 trillion cells?
2. How is cell division important to a single-celled organism?
3. What must happen to ensure successful cell division?
4. How does the genetic information in one of your body cells compare to that found in other body cells?
5. What are some advantages of asexual reproduction in plants?
6. Why is it important for DNA to be replicated prior to cell division?
7. How do chromosomes move inside a cell during cell division?
8. How is the cell cycle controlled? What would happen if the control were defective?

Procedures

Part 1: Modeling Mitosis

You will investigate mitosis using models. Your teacher will give you sockosomes, clay chromosomes, or pipe-cleaner chromosomes.

Review chromosome duplication and movement using these model chromosomes. Think about these questions as you review the cell cycle and mitosis.

- If a cell contains a set of duplicated chromosomes, does it contain any more genetic information than the cell before the chromosomes were duplicated?
- What is the significance of the fact that chromosomes condense before they are moved?
- How are the chromosome copies, called sister chromatids, separated from each other?
- What would happen if the sister chromatids failed to separate?

Part 2: Effects of Environment on Mitosis

Scientists reported that a fungal pathogen, may negatively affect the growth of soybeans *(Glycine max)*. Soybean growth decreased during three years of high rainfall, and the soybean roots were poorly developed. Close relatives of *R. anaerobis* are plant pathogens and grow in the soil. A lectin-like protein was found in the soil around the soybean roots. This protein may have been secreted by the fungus. Lectins induce mitosis in some root apical meristem tissues. In many instances, rapid cell divisions weaken plant tissues.

You have been asked to investigate whether the fungal pathogen lectin affects the number of cells undergoing mitosis in a different plant, using root tips.

- What is your experimental hypothesis? Your null hypothesis? Are these the same?
- How would you design an experiment with onion bulbs to test whether lectins increase the number of cells in mitosis?
- What would you measure, and how would you measure it?
- What would be an appropriate control for your experiment?

Your teacher will provide you with untreated and lectin-exposed roots. You should be comfortable identifying cells in mitosis or in interphase before you begin examining the chromosome squashes.

Preparing Chromosome Squashes

1. Place the onion root tip in 12 M HCl for 4 minutes.
2. Transfer the tip to Carnoy's fixative for 4 minutes.
3. Remove the slide from Coplin jar containing 70% ethanol, dry with a scientific cleaning wipe, and label it.
4. Place the tip on the slide and cut off the distal 2 mm portion of the tip; discard the remainder of the tip.
5. Cover the root tip piece with carbol-fuschin stain for 2 minutes.
6. Blot off excess stain and cover the tip with 1–2 drops of H_2O.
7. Place the cover slip over the tip and cover the cover slip with a scientific cleaning wipe.
8. Firmly press down on the cover slip with the eraser end of a pencil. Do not twist the slide, and be careful not to break the cover slip.

Counting Cells and Analyzing Data

1. Observe the cells at high magnification (400–500 X).
2. Look for well-stained, distinct cells.
3. Within the field of view, count the cells in each phase. Repeat the counts in two other root tips.
4. Collect the class data for each group, and calculate the mean and standard deviation for each group. You must make a table in your notebook for the class data.
5. Compare the number of cells from each group in interphase and in mitosis.

Table 1. Onion Root Tip Cell Phase Data; Treatment Group_____

Tip	Number of Cells		
	Interphase	Mitotic	Total
1			
2			
3			
Total			

1. For this experiment, the number of treated cells in interphase and mitosis will be the observed (o) values.
2. To find out what your expected values are, complete the following steps:
   a. Calculate the percentage of cells in interphase and mitosis in the *control* group from Table 1.
   b. Multiply the percentages by the total number of cells in the *treated* group; this will give the expected numbers (e).
3. Calculate the chi-square (χ^2) value for the test.
4. Compare this value to the critical value in Table 2.

Table 2. Critical Values of the Chi-Square Distribution

Probability	Degrees of Freedom (DF)				
	1	2	3	4	5
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

1. The degrees 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.
2. 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 this critical value, then the null hypothesis is rejected. If the calculated chi-square value is less than this critical value, the null hypothesis is not rejected. In terms of this part of the investigation, what does it mean if your null hypothesis is rejected?

Postlab Review

- What was the importance of collecting the class data?
- Was there a significant difference between the groups?
- Did the fungal pathogen lectin increase the number of root tip cells in mitosis?
- What other experiments should you perform to verify your findings?
- Does an increased number of cells in mitosis mean that these cells are dividing faster than the cells in the roots with a lower number of cells in mitosis?
- What other way could you determine how fast the rate of mitosis is occurring in root tips?

DESIGNING AND CONDUCTING YOUR INVESTIGATION

Now that you have worked with the root tip model system, design and conduct an investigation to determine what biotic or abiotic factors or substances in the environment might increase or decrease the rate of mitosis in roots. For instance, what factors in the soil might affect the rate of root growth and development? Consider, for example, abiotic soil factors such as salinity and pH or biotic factors, including roundworms, that might alter root growth.

Part 3: Loss of Cell Cycle Control in Cancer

Many of us have family members who have or have had cancer. Cancer can occur when cells lose control of their cell cycle and divide abnormally. This happens when tumor-suppressor genes, such as p53 or Rb (retinoblastoma), are mutated. There are many questions you should consider before beginning your investigation.

Review from Part 1

- How is the cell cycle controlled in normal cells?
- What are cyclins and cyclin-dependent kinases? What do these proteins do in a cell?

Prelab Questions for Part 3

- How are normal cells and cancer cells different from each other?
- What are the main causes of cancer?
- What goes wrong during the cell cycle in cancer cells?
- What makes some genes responsible for an increased risk of certain cancers?
- Do you think that the chromosomes might be different between normal and cancer cells?

The last question is the focus of this part of the lab. With your group, form a hypothesis as to how the chromosomes of a cancer cell might appear in comparison to a normal cell and how those differences are related to the behavior of the cancer cell.

For each of the following cases, look at pictures of the chromosomes (karyotype) from normal human cells. Compare them to pictures of the chromosomes from cancer cells. For each case, count the number of chromosomes in each type of cell, and discuss their appearance. Then answer the following questions.

- Do your observations support your hypothesis?
- If not, what type of information might you need to know in order to understand your observations?
- If yes, what type of information can you find that would validate your conclusions?

Case 1: HeLa cells

HeLa cells are cervical cancer cells isolated from a woman named Henrietta Lacks. Her cells have been cultured since 1951 and used in numerous scientific experiments. Henrietta Lacks died from her cancer not long after her cells were isolated. Lacks's cancer cells contain remnants of human papillomavirus (HPV), which we now know increases the risk of cervical cancer.

- From your observations, what went wrong in Henrietta Lacks's cervical cells that made them cancerous?
- How does infection with human papillomavirus virus (HPV) increase the risk of cervical cancer?

Your teacher may ask you to read *The Immortal Life of Henrietta Lacks* by Rebecca Skloot. As you read it, think about the following questions:

- Should tissue be removed from a patient without his or her consent for research?
- How was the HeLa cell line cultured?
- What virus infected Henrietta Lacks and may have caused her cervical cancer? What cellular process is affected by this virus?
- Was there bias in the way Henrietta Lacks was treated at Johns Hopkins?
- Put the use of HeLa cells on trial. Debate what is more important: an individual's rights to his/her own body tissues or the medical knowledge gained by studying a patient's tissues?
- Should Henrietta Lacks's family be compensated for the discoveries made using her cells?
- Do companies or universities have the right to patent discoveries made using a patient's tissues or genes without consulting the patient?
- What other legal and ethical questions are raised in this book?

Case 2: Philadelphia Chromosomes

In normal cells, mitosis usually is blocked if there is DNA damage. Sometimes, though, DNA damage makes cells divide more often. Certain forms of leukemia have a unique feature called a Philadelphia chromosome. Look at the karyotype of leukemia cells in Figure 5, and answer the following questions:

- What happens in a normal cell if the DNA has mutations?
- What would happen if cells with mutated DNA replicated?
- How do cells monitor DNA integrity?
- How are the chromosomes different in the cancer cells compared to normal cells?
- How could these differences lead to cancer?

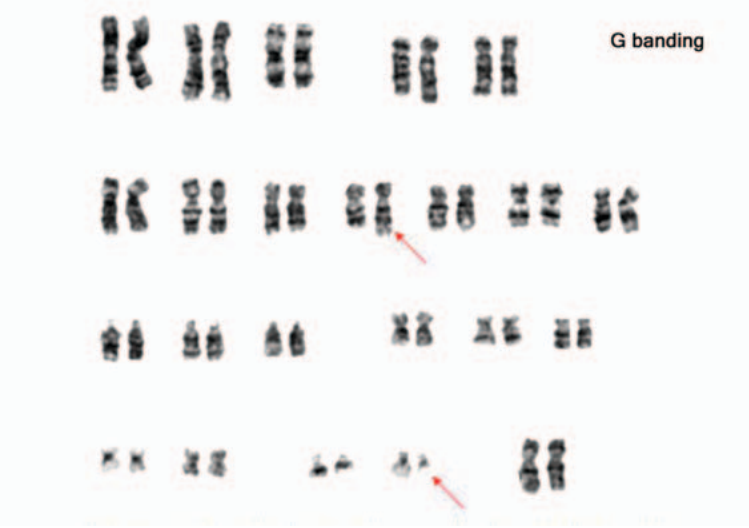

Figure 5. Karyotype of a Patient with Chronic Myelogenous Leukemia Indicating Chromosomal Deformity

Part 4: Modeling Meiosis

Meiosis resembles mitosis but serves a very different purpose. Meiosis is a cell division resulting in the halving, or reduction, of chromosome number in each cell. A diploid organism has two sets of chromosomes (2n), while a haploid cell or organism has one set (1n). Meiosis produces gametes (ova and sperm) in animals and spores in fungi, plants, and protists. Three other important characteristics of meiosis are the exchange of genetic material ("crossing over") between homologous chromosomes, the independent assortment of the chromosomes, and the separation of alleles of the same gene (Figure 6). These characteristics, along with random fertilization, increase the genetic variability in the offspring. These mechanisms are essential to our understanding of genetics and evolution in sexually reproducing organisms.

The hallmark of sexual reproduction is the great diversity seen in the gametes and in the resulting offspring produced by fertilization. Meiosis is integral to this process because this type of cell division produces the sex cells, gametes. Before you begin the modeling exercise, your teacher will ask you to discuss these questions.

- How do sexually reproducing organisms produce gametes from diploid progenitors?
- How does the process increase gamete diversity?
- What are the outcomes from independent assortment and crossing over?
- How does the distance between two genes or a gene and a centromere affect crossover frequencies?

Use the model chromosomes from Part 1 to explain meiosis and crossing-over events. During your investigation, answer the following questions:

- When is the DNA replicated during meiosis?
- Are homologous pairs of chromosomes exact copies of each other?
- What is crossing over?
- What physical constraints control crossover frequencies?
- What is meant by independent assortment?
- How can you calculate the possible number of different kinds of gametes?
- What happens if a homologous pair of chromosomes fails to separate, and how might this contribute to genetic disorders such as Down syndrome and cri du chat syndrome?
- How are mitosis and meiosis fundamentally different?

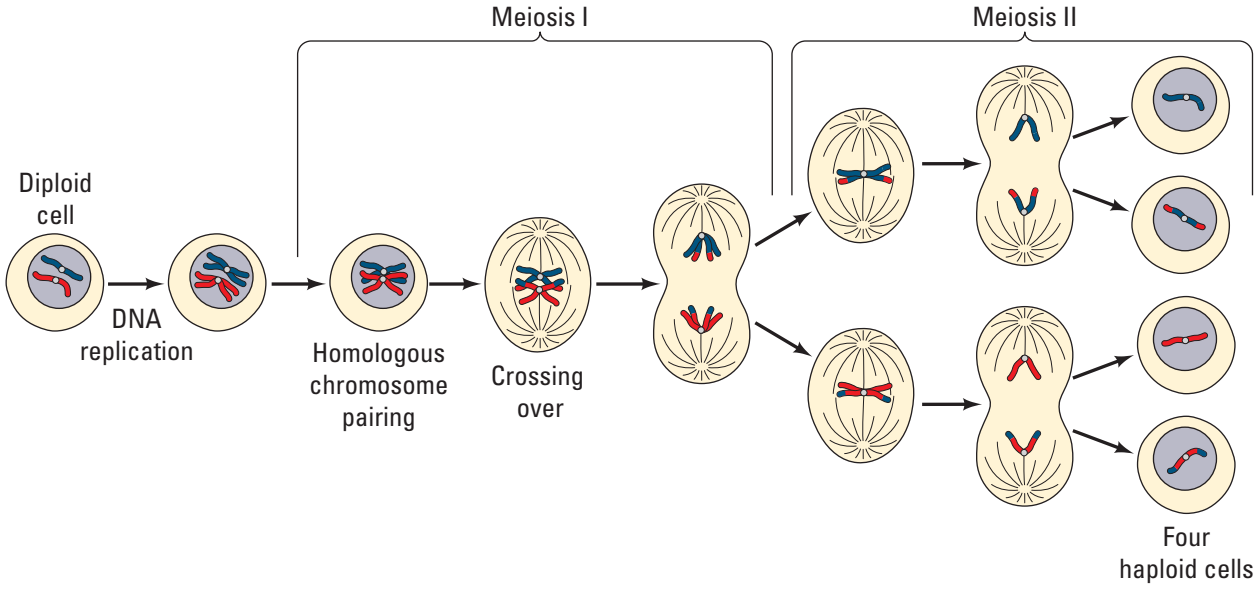

Figure 6. Meiotic Cell Division Emphasizing Chromosome Movement

Part 5: Meiosis and Crossing Over in *Sordaria*

The fungus *Sordaria fimicola* exchanges genetic material when two mycelia meet and fuse. The resulting zygote undergoes meiosis to produce asci; each ascus contains eight haploid spores. A single gene determines the spore color.

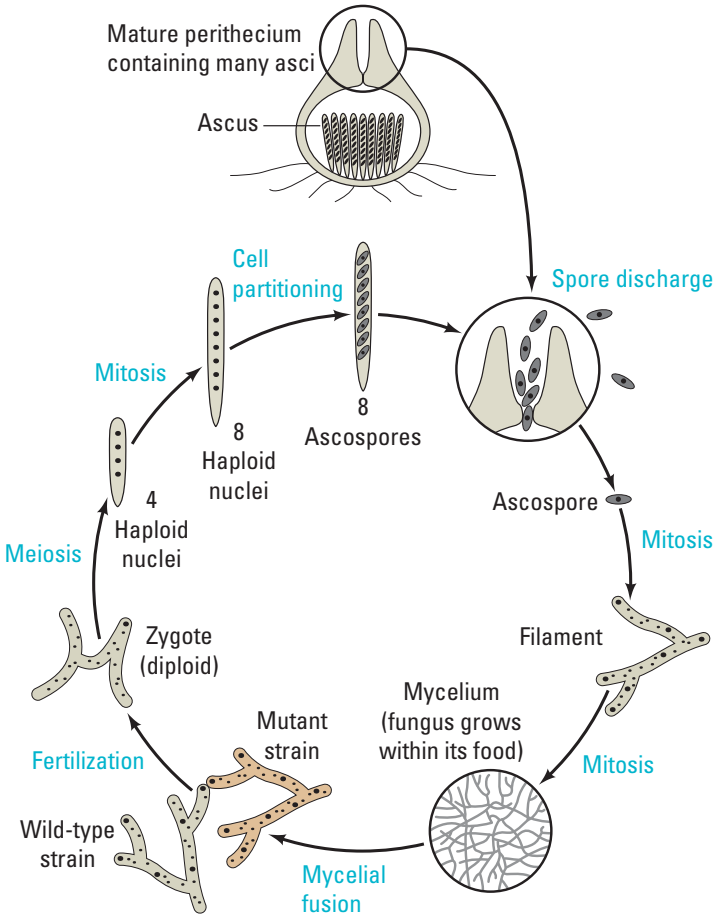

Figure 7. *Sordaria* Life Cycle

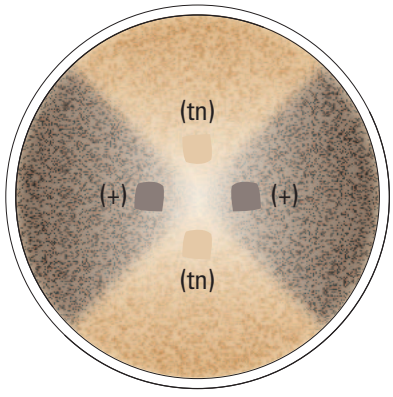

Figure 8: *Sordaria* Cross Plate

A cross was made between wild type (+; black) and tan (tn) strains. The resulting zygote produces either parental type asci, which have four black and four tan spores in a row (4:4 pattern), or recombinant asci, which do not have this pattern.

- How do you explain the differences between the recombinant asci and the parental types?
- What meiotic event can account for this difference?
- Using the model chromosomes from Part 4, predict the possible meiotic outcomes.

1. Place a drop of water onto the microscope slide.
2. Gently scrape some perithecia from the agar plate near where the two strains meet.
3. Place a cover slip over the perithecia and put a scientific cleaning wipe over the cover slip.
4. Gently press down on the cover slip using the eraser end of a pencil.
5. Count at least 50 asci, and score them as either parental or recombinant (crossing over).
6. Enter the data in Table 3 and make the calculations. One map unit equals one recombinant per 100 total events. The percentage of asci showing crossover divided by 2 equals the map units in this activity. This is done because each spore produced by meiosis undergoes a mitotic division.

Table 3. Analysis of Results

Number of Asci Showing 4:4 Pattern	Number of Asci Showing Crossover	Total # of Asci	% Asci Showing Crossover Divided by 2	Gene to Centromere Distance (Map Units)

Evaluating Results

1. Why did you divide the percentage of asci showing crossover (recombinant) by 2?
2. The published map distance between the spore color gene and the centromere is 26 map units. How did the class data compare with this distance?
3. How can you account for any disparities between the class data and the published data?
4. Illustrate what happened during meiosis to produce the results you found.
5. Do you think the Philadelphia chromosome is a result of crossing over as seen in this part of the investigation or some other chromosomal abnormality? Explain your answer.
6. Do you think the cell cycle described for mitosis could be applied to meiosis as well? Explain your answer.

Where Can You Go from Here?

1. Can the same (or any) environmental factors you tested above affect the amount of crossing over that occurs in *Sordaria*? How would you set up an experiment to test this? For example, how does humidity or pH affect the crossover frequency?
2. Revisit the learning objectives stated earlier. Do you better understand mitosis and meiosis? Could you teach this to another class?
3. How do the mechanisms of cell replication affect genetic diversity and evolution? Consider the mechanisms such as crossing over, independent assortment, segregation, nondisjunction, and random fertilization.
4. Prepare a video or write and produce a play about the process of chromosome movement.
5. Investigate how growth factors affect the cell cycle. This will help you review cell communication.
6. Research what tumor suppressors do in the cell cycle and which types of cancers may be caused by mutations in tumor suppressor genes. Specific examples include human papillomavirus (HPV), retinoblastoma protein (Rb), BRCA1 and BRCA2, and p53.

INVESTIGATION 8

BIOTECHNOLOGY: BACTERIAL TRANSFORMATION*

How can we use genetic engineering techniques to manipulate heritable information?

BACKGROUND

Are genetically modified foods safe? There is ongoing debate about whether it is safe to eat fruit and vegetables that are genetically modified to contain toxins that ward off pests. For instance, biotechnologists have succeeded in inserting a gene (Bt) from the bacterium *Bacillus thuringiensis* into the corn genome. When expressed, the Bt toxin kills caterpillars and controls earworms that damage corn, but is the corn safe for human consumption?

Genetic information passed from parent to offspring via DNA provides for continuity of life. In order for information in DNA to direct cellular activities, it must be transcribed into RNA. Some of the RNAs are used immediately for ribosomes or to control other cellular processes. Other RNAs are translated into proteins that have important roles in determining metabolism and development, i.e., cellular activities and phenotypes (traits). When the DNA of a cell changes, the RNAs and proteins they produce often change, which in turn changes how that cell functions.

DNA inside a cell can change several ways. It can be mutated, either spontaneously or after the DNA replication machinery makes an error. Biotechnologists may cause an intentional mutation in a cell's own DNA as a way to change how that cell behaves. The most powerful tool biotechnologists have, though, is the ability to transfer DNA from one organism to another and make it function there. With this tool, they can make cells produce novel protein products the cells did not make previously.

Examples of this powerful tool are all around us. Insulin that people take to control their blood sugar levels is often made from engineered bacteria. Some vaccines, as well as enzymes used for manufacturing denim jeans, are also made using engineered cells. In the near future, engineered bacteria and other cells being developed could help clean up spilled oil or chemicals, produce fuel for cars and trucks, and even store excess carbon dioxide to help slow global climate change. Can you think of other possible applications of genetic engineering? However, biotechnology and human manipulation of DNA raise several ethical, social, and medical issues, such as the safety of genetically modified foods. Can you think of other issues to consider?

* Transitioned from the *AP Biology Lab Manual* (2001)

This biotechnology depends on plasmids, small circles of DNA that were found first in bacteria. Plasmids allow molecular biologists to manipulate genetic information in a laboratory setting to understand more fully how DNA operates. Plasmids also let us move DNA from one bacterium to another easily.

In this investigation, you will learn how to transform *Escherichia coli (E. coli)* bacteria with DNA it has not possessed before so that it expresses new genetic information. Bacterial cells that are able to take up exogenous (external) genetic material are said to be "competent" and are capable of being transformed. You also will calculate transformation efficiency to find out how well the *E. coli* took up the "foreign" DNA. Using these techniques, you will have the opportunity to explore the field of biotechnology further. You might want to explore the following questions:

- What causes mutations in bacteria? Can mutations affect plasmids?
- What is the function of plasmids in bacteria?
- Do cells take up more plasmids in some conditions and less in others?

By learning and applying these fundamental skills, you will acquire the tools to conduct more sophisticated biotechnology investigations, including designing your own experiments to manipulate DNA.

This investigation also provides you with the opportunity to review, connect, and apply concepts that you have studied previously, including cell structure of bacteria; structure and function of cell membranes, enzymes, and DNA and RNA; transcription and translation; the operon model of the regulation of gene expression; evolution and natural selection; and interactions between organisms and their environment.

Interspersed within each investigation are supplemental activities designed to keep you on track and to provide opportunities for you to take a deeper dive into the concepts. Your teacher may assign these activities for homework or ask that you do them as you work through each investigation.

Learning Objectives

- To demonstrate the universality of DNA and its expression
- To explore the concept of phenotype expression in organisms
- To explore how genetic information can be transferred from one organism to another
- To investigate how horizontal gene transfer is a mechanism by which genetic variation is increased in organisms
- To explore the relationship between environmental factors and gene expression
- To investigate the connection between the regulation of gene expression and observed differences between individuals in a population of organisms

General Safety Precautions

Basic Sterile Technique

When working with and culturing bacteria, it is important not to introduce contaminating bacteria or fungi into the experiment. Because these microorganisms are ubiquitous, i.e., you can find them everywhere — on fingertips, bench tops, lab tables, etc. — you must avoid these contaminating surfaces. When working with inoculation loops, bulb pipettes, micropipettes, and agar plates, do not touch the tips of them (or, in the case of agar, the surface itself) or place them directly onto contaminating surfaces. Be sure to wash your hands before beginning the procedure and after — and cover your sneezes. Do not eat, drink, apply cosmetics, or use personal electronic devices in the work area.

Working with *E. coli*

The host *E. coli* used in this investigation, plasmids, and the subsequent transformants created by their combination are *not* pathogenic (disease-causing) bacteria like the *E. coli* O157:H7 strain that has been in the news. However, handling *E. coli* requires appropriate microbiological and safety procedures. Your teacher will provide instructions, but these practices include, but are not limited to, the following:

- Decontaminating work surfaces once a day and after any spill of viable material with a 10% household bleach solution
- Decontaminating all contaminated liquid or solid wastes before disposal [This can be done in an autoclave (20 minutes at 121°C) or in a 10% bleach solution (soaked for 20 minutes).]
- Washing your hands after handling organisms containing recombinant DNA and before leaving the lab
- Wearing protective eyewear and disposable gloves
- Not eating, drinking, applying cosmetics, or using personal electronic devices, such as iPods and cell phones, in the work area

THE INVESTIGATIONS

Getting Started

DNA provides the instructions necessary for the survival, growth, and reproduction of an organism. When genetic information changes, either through natural processes or genetic engineering, the results may be observable in the organism. These changes may be advantageous for the long-term survival and evolution of a species, but it also may be disadvantageous to the individuals who possess the different genetic information.

In bacteria, genetic variation does not happen by mutation alone. It also can be introduced through the lateral (horizontal) transfer of genetic material between cells. Some bacteria undergo conjugation, which is direct cell-to-cell transfer. Other bacteria acquire DNA by transduction (viral transmission of genetic information). The third route is transformation, which is uptake of "naked" DNA from the environment outside the cell.

(You may have previously studied transformation in a different context. In an experiment conducted in 1928, Frederick Griffith, seeking a vaccine against a virulent strain of pneumonia, suggested that bacteria are capable of transferring genetic information through transformation. Little did Griffith know that his work would provide a foundation for genetic engineering and recombinant DNA technology in the 21st century.)

The concept of cell transformation raises the following questions, among others:

- To transform an organism to express new genetic information, do you need to insert the new gene into every cell in a multicellular organism or just one? Which organism is best suited for total genetic transformation — one composed of many cells or one composed of a single cell?
- Can a genetically transformed organism pass its new traits on to its offspring? To get this information, which would be a better candidate for your investigation — an organism in which each new generation develops and reproduces quickly or one that does this more slowly?
- Based on how you answered the first two sets of questions, what organism would be a good choice for investigating genetic transformation — a bacterium, earthworm, fish, or mouse?

If your answer to the last question is "bacterium," you are on the right track. Genetic transformation of bacteria most often occurs when bacteria take up plasmids from their environment. Plasmids are not part of the main DNA of a bacterium. They are small, circular pieces of DNA that usually contain genes for one or more traits that may be beneficial to survival. Many plasmids contain genes that code for resistance to antibiotics like ampicillin and tetracycline. [Antibiotic-resistant bacteria are responsible for a number of human health concerns, such as methicillin-resistant *Staphylococcus aureas* (MRSA) infections.] Other plasmids code for an enzyme, toxin, or other protein that gives bacteria with that plasmid some survival advantage. In nature, bacteria may swap these beneficial plasmids from time to time. This process increases the variation

between bacteria — variation that natural selection can act on. In the laboratory, scientists use plasmids to insert "genes of interest" into an organism to change the organism's phenotype, thus "transforming" the recipient cell. Using restriction enzymes, genes can be cut out of human, animal, or plant DNA and, using plasmids as vectors (carriers of genetic information), inserted into bacteria. If transformation is successful, the recipient bacteria will express the newly acquired genetic information in its phenotype (Figure 1).

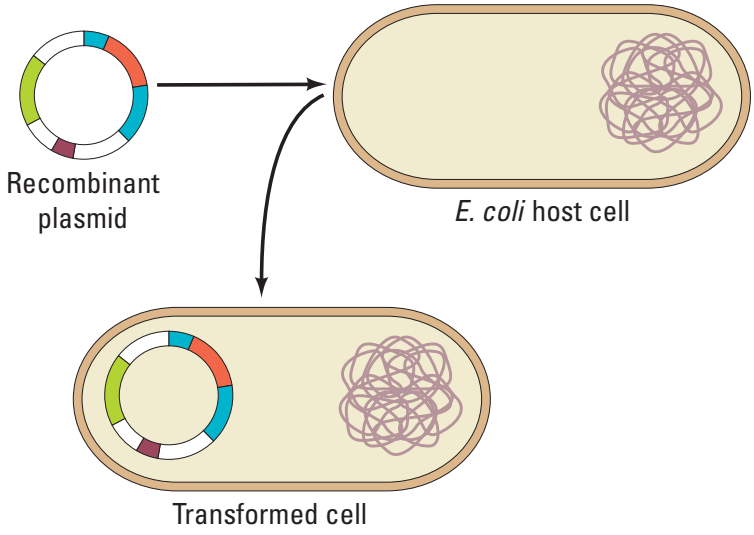

Figure 1. Transformation of Bacteria

In nature, the efficiency of transformation is low and limited to relatively few bacterial strains. Also, bacteria can take up DNA only at the end of logarithmic growth; at this time, the cells are said to be "competent." In the lab, you have discovered several ways to increase the rate of transformation. Now, rather than just a few bacteria taking up a plasmid you want them to use, millions of bacteria can be transformed. The number of bacteria that take up a plasmid successfully is called the "transformation efficiency." This is one of the values you will calculate in this lab unit.

In this investigation, you will use a predefined procedure to transform *E. coli* bacteria with a plasmid carrying a foreign gene. There are several different plasmids your instructor can choose from; you will be instructed about which one to work with for this unit.

E. coli is an ideal organism for the molecular geneticist to manipulate because it naturally inhabits the human colon and easily can be grown in a nutrient medium such as LB broth.

But what is *E. coli's* natural or pre-transformation phenotype?

- Observe the colonies of *E. coli* grown on the starter LB/agar plate provided by your teacher to glean some information before you determine if any genetic transformation has occurred. What traits do you observe in pre-transformed bacteria? Record your observations in your laboratory notebook.

- Some bacteria are naturally resistant to antibiotics, but others are not. How could you use two LB/agar plates, some *E. coli*, and some ampicillin (an antibiotic) to determine how *E. coli* cells are affected by ampicillin?
- What would you expect your experimental results to indicate about the effect of ampicillin on the *E. coli* cells? Do you think that exposure to ampicillin will cause the *E. coli* cells to evolve resistance to ampicillin? Why or why not?
- How will you be able to tell if host *E. coli* cells have been genetically transformed? (**Hint**: You will need some information from your teacher about the plasmid you will be using.)

Procedure

Your teacher will provide you with a plasmid containing one or more genes. The plasmid likely will contain the gene for resistance to ampicillin (pAMP), an antibiotic that is lethal to many bacteria, including *E. coli* cells. This transformation procedure involves the following three main steps to introduce the plasmid DNA into the *E. coli* cells and to provide an environment for the cells to express their newly acquired genes:

1. Adding $CaCl_2$
2. "Heat shocking" the cells
3. Incubating the cells in nutrient broth for a short time before plating them on agar

Materials

Your Workstation

- *E. coli* starter plate prepared by your teacher
- Poured agar plates prepared by your teacher, most likely the following:
  - 2 LB agar plates
  - 2 LB/amp agar (LB agar containing ampicillin) plates
- Transformation solution ($CaCl_2$, pH 6.1) kept *ice cold*
- LB nutrient broth
- Sterile inoculation loops
- 100–1000 µL sterile bulb pipettes
- 1–10 µL micropipettes with sterile tips
- Microcentrifuge tubes
- Microcentrifuge tube holder/float
- Container full of crushed ice
- Marking pen

Common Workstation

- DNA plasmid (most likely pAMP at 0.005 µg/µL)
- 42°C water bath and thermometer
- 37°C incubator
- 20 µL adjustable-volume micropipettes and tips (optional)
- 10% household bleach
- Biohazardous waste disposal bags
- Masking or lab tape

In your lab notebook, record data, answers to questions, and any questions that arise during this part of the activity.

Step 1 Form lab teams, as instructed by your teacher. Familiarize yourself with sterile technique, materials and lab equipment, and safety procedures for handling bacteria and decontaminating the work area.

Step 2 Label one closed microcentrifuge tube (micro test tube) "+ plasmid" and one tube "-plasmid." (What do the "+" and "-" symbols mean?) Label both tubes with your group's number (e.g., G2), and place them in the microcentrifuge tube holder/float.

Step 3 Carefully open the tubes and, using a 100–1000 µL bulb pipette with a sterile tip, transfer 250 µL of the ice cold transformation solution ($CaCl_2$) into each tube. (Note that "µl" and "µL" are alternative symbols for the same volumetric measurement.)

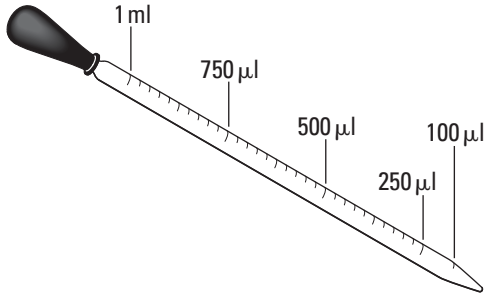

Figure 2. Measuring Volume with a Pipette

Step 4 Place both tubes on (into) the ice.

Step 5 Use a sterile inoculation loop to pick up a single colony of bacteria from your starter plate. Be careful not to scrape off any agar from the plate. Pick up the "+ plasmid" tube and immerse the loop into the $CaCl_2$ solution (transforming solution) at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the solution. Appropriately discard the loop.

Step 6 Use a new sterile 100–1,000 µL micropipette to repeatedly pulse the cells in solution to thoroughly resuspend the cells. (Note that the clear transformation solution will become cloudy as the *E. coli* cells are suspended.) Place the tube back on the ice.

Step 7 Using a new sterile inoculation loop, repeat Steps 5 and 6 for the "- plasmid" tube.

CAUTION: Keep your nose and mouth away from the tip end when pipetting suspension culture to avoid inhaling any aerosol!

Step 8 Using a 1–10 µL micropipette with a sterile tip, transfer 10 µL of the plasmid solution *directly into the* E. coli *suspension* in the "+ plasmid" tube. Tap tube with a finger to mix, but avoid making bubbles in the suspension or splashing the suspension up the sides of the tube. Do not add the plasmid solution into the "- plasmid" tube! (Why not?)

Step 9 Incubate both tubes ("+ plasmid" and "- plasmid") on ice for 10 minutes. Make sure the bottom of the tubes make contact with the ice.

Step 10 While the tubes are sitting on ice, label each of your agar plates on the bottom (not the lid) as directed by your teacher.

Step 11 Following the 10-minute incubation at 0°C, remove the tubes from the ice and "heat shock" the cells in the tubes. It is critical that the cells receive a sharp and distinct shock! Make sure the tubes are closed tightly! Place the tubes into a test tube holder/float, and dunk the tubes into the water bath, set at 42°C, for exactly 50 seconds. Make sure to push the tubes all the way down in the holder so that the bottom of the tubes with the suspension makes contact with the warm water.

Step 12 When the 50 seconds have passed, place both tubes back on ice. For best transformation results, the change from 0°C to 42°C and then back to 0°C must be rapid. Incubate the tubes on ice for an additional two minutes.

Step 13 Remove the holder containing the tubes from the ice and place on the lab counter. Using a 100–1,000 µL micropipette with sterile tip, transfer 250 µL of LB nutrient broth to the "+ plasmid" tube. Close the tube and gently tap with your finger to mix. Repeat with a new sterile micropipette for the "- plasmid" tube.

Step 14 Incubate each tube for 10 minutes at room temperature.

Step 15 Use a 10–1,000 µL micropipette with sterile tip to transfer 100 µL of the transformation ("+ plasmid") and control ("- plasmid") suspensions onto the appropriate LB and LB/Amp plates. Be sure to use a separate pipette for each of the four transfers.

Step 16 Using a new sterile inoculation loop for each plate, spread the suspensions evenly around the surface of the agar by quickly "skating" the flat surface of the sterile loop back and forth across the plate surface (Figure 3). Do not poke or make gashes in the agar! Your teacher might suggest that you use small sterile glass beads to spread the suspensions by gently rocking the beads across the surface of the agar. Allow the plates to set for 10 minutes.

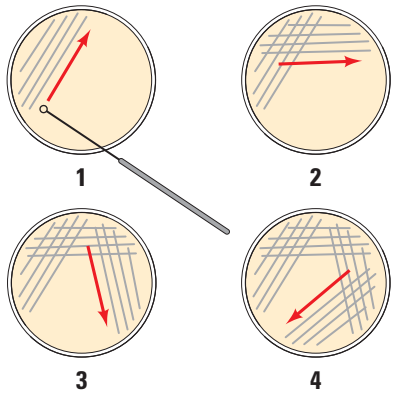

Figure 3. Technique for Plating Bacteria on Agar

Step 17 Stack your plates and tape them together. Place the stack upside down in the 37°C incubator for 24 hours or as per instructed by your teacher.

Analyzing Results

Think about these questions *before* collecting data and analyzing your results. Be sure to record your answers in your laboratory notebook.

1. On which of the plates would you expect to find bacteria most like the original non-transformed *E. coli* colonies you initially observed? Why?
2. If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Again, why?
3. Which plates should be compared to determine if any genetic transformation has occurred? Why?
4. What barriers might hinder the acquisition of plasmids?
5. How can the procedures described above (addition of CI_2 and "heat shocking") help facilitate the introduction of plasmids into the *E. coli* cells?

Consider the amount of bacterial growth you see on each plate. What color are the colonies? How many bacterial colonies are on each plate? Additional questions you might want to consider include the following:

1. Do your results support your original predictions about the "+ plasmid" transformed *E. coli* cells versus "- plasmid" nontransformed cells?
2. Which of the traits that you originally observed for *E. coli* did not seem to become altered? Which traits seem now to be significantly different after performing the transformation procedure?
3. What evidence suggests that the changes were due to the transformation procedures you performed?
4. What advantage would there be for an organism to be able to turn on or off particular genes in response to certain conditions?
5. Was your attempt at performing a genetic transformation successful? If so, *how* successful?

By calculating transformation efficiency, you can measure the success of your transformation quantitatively.

Calculating Transformation Efficiency

Your next task is to learn how to determine the extent to which you genetically transformed *E. coli* cells. This quantitative measurement is referred to as the transformation efficiency. What is the importance of quantifying how many cells have been transformed? In many applications, it is important to transform as many cells as possible. For example, in some forms of gene therapy, cells are collected from the patient, transformed in the laboratory, and then put back into the patient. The more cells that are transformed to produce the needed protein, the more likely the therapy will work.

Calculating transformation efficiency gives you an indication of how effective you were in getting plasmids carrying new information into host bacterial cells. In this example, transformation efficiency is a number that represents the total number of bacterial cells that express the gene for ampicillin resistance divided by the amount of DNA plasmid used in the experiment. The transformation efficiency is calculated using the following formula.

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the agar plate (in } \mu\text{g)}}$$

What two pieces of information will you need to calculate the efficiency of your transformation? Be sure to record all calculations.

1. Calculate the total number of transformed cells.

Observe the number of colonies visible on your LB/amp plate. Do not open the plate! Each colony on the plate can be assumed to be derived from a single cell. As individual cells reproduce, more and more cells are formed and develop into what is termed a colony. Thus, the most direct way to determine the total number of bacteria that were transformed with the plasmid is to count the colonies on the plate.

2. Calculate the amount of plasmid DNA in the bacterial cells spread on the LB/amp plate.

You need two pieces of information to find out the amount of plasmid DNA in the bacterial cells spread on the LB/amp plate: a) the total amount of DNA with which you began the experiment and b) the fraction of the DNA in the bacteria that actually got spread onto the LB/amp plate.

Once you determine this information, you will multiply the total amount of plasmid DNA used in the transformation times the fraction of DNA you spread on the LB/amp plate.

a. Calculate the total amount (mass) of plasmid DNA.

The total amount (mass) of DNA with which you began the experiment is equal to the product of the concentration and the total volume used, or

DNA in µg = (concentration of DNA of µg/µL) x (volume of DNA in µL)

In this example, assume you used 10 µL of plasmid at a concentration of 0.005 pAMP µg/µL.

- Calculate the amount (mass) of plasmid DNA (pAMP) in µg per 1 µL of solution.
- Calculate the total amount of DNA used in this experiment.

How will you use this information?

b. Calculate the fraction of plasmid DNA that actually got spread onto the LB/amp plate.

Since not all the DNA you added to the bacterial cells will be transferred to the agar plate, you need to calculate what fraction of the DNA was actually spread onto the LB/amp plate.

$$\text{Fraction of DNA used} = \frac{\text{Volume spread on the LB/amp plate (µL)}}{\text{Total sample volume in test tube (µL)}}$$

Calculate the fraction of plasmid DNA you spread on the LB/amp plate.

(Hint: Refer to the procedure and your notes. How many microliters of cells containing DNA did you spread onto the plate? What was the total volume of solution in the test tube? Did you add *all* the volumes?)

c. Calculate the micrograms of plasmid DNA that you spread on the LB/amp plate.

To answer this question, you multiply the total mass of plasmid DNA used times the fraction of plasmid DNA you spread on the LB/amp plate.

DNA spread in µg = Total amount of DNA used in µg x fraction of DNA used

What does this number tell you?

3. Calculate transformation efficiency.

Look at your calculations. Fill in the blanks with the correct numbers.

Number of colonies on the LB/amp plate: _____

Micrograms of plasmid DNA spread on the plate: _____

Now calculate the efficiency of the transformation.

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the LB/amp plate (in µg)}}$$

4. What does this mean?

Transformation efficiency calculations result in very large, and very small, numbers. For both very large and very small numbers, scientists often use a mathematical shorthand referred to as scientific notation. For example, if the calculated transformation efficiency is 1,000 bacteria/µg of DNA, they often report this as 10^3 transformants/µg.

How would scientists report 10,000 transformants/µg in scientific notation?

Suppose scientists calculated an efficiency of 5,000 bacteria/µg of DNA. How would they report this in scientific notation?

a. Report your calculated transformation efficiency in scientific notation.

b. What does your calculation of transformation efficiency mean?

c. Biotechnologists generally agree that the transformation protocol that you have just completed has a transformation efficiency of between 8.0×10^2 and 7.0×10^3 transformants per microgram of DNA. How does your transformation efficiency compare? What factors could explain a transformation efficiency that was greater or less than predicted?

Evaluating Results

1. What are some challenges you had in performing your investigation? Did you make any incorrect assumptions?
2. What are some possible sources of error in the transformation procedure? If you had to repeat the procedure, what are ways to minimize potential sources of error?
3. Were you able to perform without difficulty the mathematical routines required to calculate transformation efficiency? Which calculations, if any, were challenging or required help from your classmates or teacher?
4. Can you suggest other preliminary activities that would have better prepared you to tackle the investigation?
5. Does a bacterial cell take in a plasmid with genes the cell already possesses? If so, would this affect your calculations?

Designing and Conducting Your Investigation

Think about these questions again for a minute.

- What causes mutations in bacteria? Can mutations affect plasmids? How would you be able to tell if any observed changes in phenotypes are due to the expression of genes carried on plasmids and are not attributed to a possible mutagen?
- Do bacteria take up more in plasmid in some conditions and less in others? What conditions favor uptake, and which ones inhibit it?
- What other questions do you have about plasmids and transformation?

You can either design an investigation focusing on the information below OR design one based on a question(s) or observation you had as you worked through the genetic transformation you just conducted. Be sure that your experiment applies the science skills you acquired as you worked through this investigation. Make sure that your teacher approves your plan.

You should have noted satellites around the transformed colonies. (Satellites are smaller colonies that grow around the larger transformed colony.) What observations can you make about the satellites? Do they look like transformed bacteria? How can you tell if the satellites contain the plasmid? Design and conduct an experiment to determine if the *E. coli* satellite colonies from your genetic transformation experiment are transformed, too. Available to you are the same chemicals, supplies, and equipment you used in the previous investigation.

Where Can You Go from Here?

The background to this investigation asks you to think about several applications of genetic transformation, including genetically modified food and possible ethical, social, or medical issues raised by the manipulation of DNA by biotechnology. Why are these "issues"? What questions are posed by genetic engineering? In terms of what you have learned about biotechnology, how would you respond to the quote from Michael Crichton's novel and film *Jurassic Park*: "Just because science can do something doesn't mean that it should"?

INVESTIGATION 9

BIOTECHNOLOGY: RESTRICTION ENZYME ANALYSIS OF DNA*

How can we use genetic information to identify and profile individuals?

THE SCENARIO

"OMG! Is that blood?" Laurel nearly broke Marcus's arm as she tried to push past him into the classroom.

Marcus grabbed the sleeve of her cardigan and yanked her back. "Don't! Can't you see the glass?" Laurel tried knocking his hand free, but the 6'4" varsity basketball captain held tight. He made her settle for looking from under his armpit.

Not that what she saw would make any sense. Their AP Biology lab looked like a riot scene. Four chairs and a potted plant were overturned in the center of the room, and broken pieces of glass were scattered across the floor along with several wet red drops. Plink ... plink ... plink. *Marcus's eyes were drawn to the teacher's desk where droplets of brownish liquid fell from a paper cup and collected in a puddle on the linoleum.*

"What happened?" Laurel asked. "Did somebody get hurt?" Laurel and her classmates had gathered in front of the door and strained to see inside Room 102.

Marcus inspected the scene and raised his right arm above his head, his fingers spread apart as if taking a shot from the free throw line. "Stay back!"

"Where's Ms. Mason?" Laurel said. "She told me I could meet her before class to review for the quiz."

"Okay, folks, keep it down." Mr. Gladson, the teacher in the classroom next door, came into the hall. His white lab coat was streaked with several rust-colored stains. The pungent odor of formaldehyde permeated the corridor. "In case you haven't noticed, the bell has rung." He wiped his nose with a tissue and then tossed it into a nearby trash can. A girl's fake shriek from inside the anatomy lab rose above the buzz of Marcus's classmates.

"What's going on?" Bobby's high-pitched whine was unmistakable — and so was the scent of his bubble gum.

"I think something might've happened to Ms. Mason," Marcus said. He dug around in his backpack and pulled out a magnifying glass. "We've got a crime scene to process."

* Transitioned from the *AP Biology Lab Manual* (2001)

"Go figure," Laurel said. "Sherlock Holmes in a varsity jacket."

For the next hour, Marcus and Laurel searched the classroom and discovered several pieces of "evidence" that Marcus described in his biology notebook:

- Ten small drops on floor confirmed by Kastle-Meyer test to be blood
- Shard of glass from a broken 500-mL Erlenmeyer flask, edge smeared with a reddish stain
- Paper cup with lipstick stains, presumed to be Ms. Mason's, found on her desk
- Wad of bubble gum stuck underneath overturned chair
- Mr. Gladson's discarded tissue recovered from trash can in hall outside Room 102
- Bobby's test on photosynthesis with large "F" scrawled in red ink on first page
- Copy of email from Mr. Gladson to Ms. Mason asking her to give up position as department chair

Marcus's new game was afoot!

BACKGROUND

Applications of DNA profiling extend beyond what we see on television crime shows. Are you sure that the hamburger you recently ate at the local fast-food restaurant was actually made from pure beef? DNA typing has revealed that often "hamburger" meat is a mixture of pork and other nonbeef meats, and some fast-food chains admit to adding soybeans to their "meat" products as protein fillers. In addition to confirming what you ate for lunch, DNA technology can be used to determine paternity, diagnose an inherited illness, and solve historical mysteries, such as the identity of the formerly anonymous individual buried at the Tomb of the Unknown Soldier in Washington, D.C.

DNA testing also makes it possible to profile ourselves genetically — which raises questions, including *Who owns your DNA and the information it carries?* This is not just a hypothetical question. The fate of dozens of companies, hundreds of patents, and billions of dollars' worth of research and development money depend on the answer. Biotechnology makes it possible for humans to engineer heritable changes in DNA, and this investigation provides an opportunity for you to explore the ethical, social, and medical issues surrounding the manipulation of genetic information.

Learning Objectives

In this investigation, you will learn how to use restriction enzymes and gel electrophoresis to create genetic profiles. You will use these profiles to help Marcus and Laurel narrow the list of suspects in the disappearance of Ms. Mason.

General Safety Precautions

Never handle gels with your bare hands. An electrophoresis apparatus can be dangerous because it is filled with a highly conductive salt solution and uses DC current at a voltage strong enough to cause a small shock. Always turn the power supply switch

"OFF" and wait 10 seconds before making any connection. Connect BOTH supply leads to the power supply (black to black and red to red, just like when you jump-start a car battery) BEFORE turning on the power supply. Your teacher will tell you for how long and at how many volts (usually 50 volts) you should run your gel. After use, turn off the power supply, and then disconnect BOTH leads from the power supply. *Remember, power supply on last … and off first.*

THE INVESTIGATIONS

Getting Started

Activity I: Restriction Enzymes

The DNA samples collected from the crime scene have been digested with restriction enzymes to generate smaller pieces of DNA, which will then be used to create DNA profiles of suspects.

Restriction enzymes are essential tools for analyzing DNA structure, and more than 200 enzymes are now available commercially. Each restriction enzyme is named for the bacterium in which it was first identified; for example, *Eco*RI was the first enzyme purified from *Escherichia coli,* and *Hin*dIII was the third enzyme isolated from *Haemophilus influenzae*. Scientists have hypothesized that bacteria use these enzymes during DNA repair and as a defense against their infection by bacteriophages. Molecular biologists use restriction enzymes to manipulate and analyze DNA sequences (Johnson 2009).

How do restriction enzymes work? These enzymes digest DNA by cutting the molecule at specific locations called restriction sites. Many restriction enzymes recognize a 4- to 10-nucleotide base pair (bp) palindrome, a sequence of DNA nucleotides that reads the same from either direction. Some restriction enzymes cut (or "cleave") DNA strands exactly in the center of the restriction site (or "cleavage site"), creating blunt ends, whereas others cut the backbone in two places, so that the pieces have single-stranded overhanging or "sticky" ends of unpaired nucleotides.

You have a piece of DNA with the following template strand:

5'-AAAGTCGCTGGAATTCACTGCATCGAATTCCCGGGGCTATATATGGAATTCGA-3'

1. What is the sequence of the complementary DNA strand? Draw it directly below the strand.
2. Assume you cut this fragment with the restriction enzyme *Eco*RI. The restriction site for *Eco*RI is 5'-GAATTC-3', and the enzyme makes a *staggered* ("sticky end") cut between G and A on both strands of the DNA molecule. Based on this information, draw an illustration showing how the DNA fragment is cut by *Eco*RI and the resulting products.

Two pieces of DNA that are cut with the same restriction enzyme, creating either sticky ends or blunt ends, can be "pasted" together using DNA ligase by reconnecting bonds, *even if the segments originated from different organisms.* An example of combining two "sticky end" sequences from different sources is shown in Figure 1. The ability of enzymes to "cut and paste" DNA fragments from different sources to make recombinant DNA molecules is the basis of biotechnology.

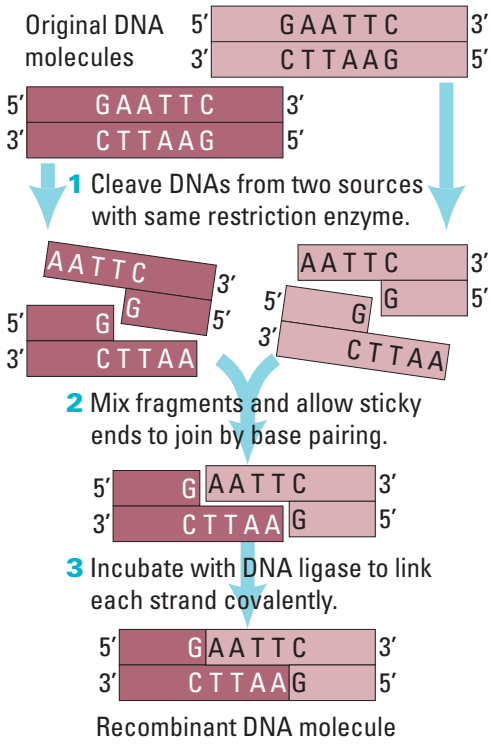

Figure 1. Recombinant DNA Using Restriction Enzymes

Activity II: DNA Mapping Using Restriction Enzymes

One application of restriction enzymes is restriction mapping. Restriction mapping is the process of cutting DNA at specific sequences with restriction enzymes, separating the fragments from each other by a process called gel electrophoresis (without pasting any fragments together), and then estimating the size of those fragments. The size and number of DNA fragments provide information about the structure of the original pieces of DNA from which they were cut.

Restriction mapping enables scientists to create a genetic signature or DNA "fingerprint" that is unique to each organism. The unique fragments, called restriction fragment length polymorphisms (RFLPs), can, for instance, be used to confirm that a mutation is present in one fragment of DNA but not in another, to determine the size of an unknown DNA fragment that was inserted into a plasmid, to compare the genomes of different species and determine evolutionary relationships, and to compare DNA

samples from different individuals within a population. This latter application is widely used in crime scene investigations.

Consider your classmates. More than 99% of your DNA is the same as their DNA. The small difference is attributed to differences in your genetic makeup, with each person having a genetic profile or "fingerprint" as unique as the ridges, arches, loops, and grooves at the ends of his or her fingers.

- Based on this information, can you make a prediction about the products of DNA from different sources cut with the same restriction enzymes? Will the RFLP patterns produced by gel electrophoresis produced by DNA mapping be the same or different if you use just one restriction enzyme? Do you have to use many restriction enzymes to find differences between individuals? Justify your prediction.
- Can you make a prediction about the RFLP patterns of identical twins cut with the same restriction enzymes? How about the RFLP patterns of fraternal twins or triplets?

Now that you understand the basic idea of genetic mapping by using restriction enzymes, let's explore how DNA fragments can be used to make a genetic profile.

Activity III: Basic Principles of Gel Electrophoresis

Creating DNA profiles depends on gel electrophoresis. Gel electrophoresis separates charged molecules, including nucleic acids and amino acids, by how fast they migrate through a porous gel under the influence of an electrical current. Your teacher will likely prepare the gel ahead of time by dissolving agarose powder (a gelatinlike substance purified from seaweed) in a current-carrying buffer. The gel solidifies around a comb placed at one end, forming wells into which you can load DNA fragments. When an electrical current is passed through the gel, the RFLPs (fragments) migrate from one pole to the other. Gel electrophoresis can separate DNA fragments from about 200 to 50,000 base pairs (bp).

- Why do DNA fragments migrate through the gel from the *negatively* charged pole to the *positively* charged pole?

The general process of gel electrophoresis is illustrated in Figure 2.

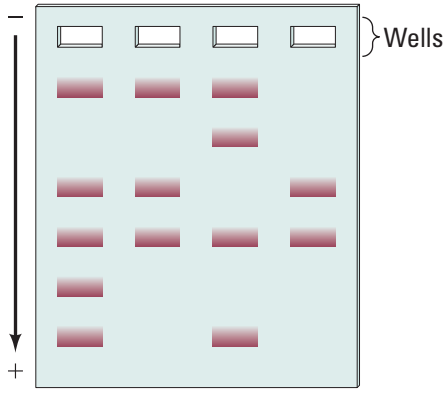

Figure 2. General Process of Gel Electrophoresis

Procedures

Learning to Use Gel Electrophoresis

To determine whose blood was on the classroom floor crime scene, you will need to be familiar with the techniques involved in creating genetic profiles using gel electrophoresis. The steps in the general procedure are described below. After you familiarize yourself with the procedure, you will analyze DNA profiles resulting from an "ideal" or mock gel before using what you have learned to conduct an independent investigation. In Designing and Conducting Your Investigation, you will use these skills to narrow the list of suspects in the disappearance of Ms. Mason based on DNA evidence collected at the crime scene.

Materials

Your Workstation

- 20 µL vials of DNA fragments prepared using restriction enzymes
- Rack for holding samples
- 3 plastic bulb transfer pipettes (or similar devices)
- Permanent marker
- Gel electrophoresis chamber
- Power supply
- Staining tray
- Semi-log graph paper
- Ruler

Common Workstation

- 0.8% agarose solution (or gel, if prepared by teacher)
- 1 X TAE (tris-acetate-EDTA) buffer
- Methylene blue stain

Record data and any answers to questions in your lab notebook.

Casting the Agarose Gel

Before proceeding, your teacher will direct you to short online videos that show how to prepare an agarose gel, load DNA samples into the wells in the gel, and run an electrophoresis.

Step 1 Seal the ends of the gel-casting tray with tape, dams, or any other method appropriate for the gel box that you are using. Insert the well-forming comb. Place the gel-casting tray out of the way on the lab bench so that the agarose poured in the next step can set undisturbed. (Your teacher might cast the gel for you ahead of time.)

Step 2 Carefully pour the liquid gel into the casting tray to a depth of 5–6 mm. The gel should cover only about one-half the height of the comb teeth (Figure 3). While the gel is still liquid, use the tip of a pipette to remove any bubbles.

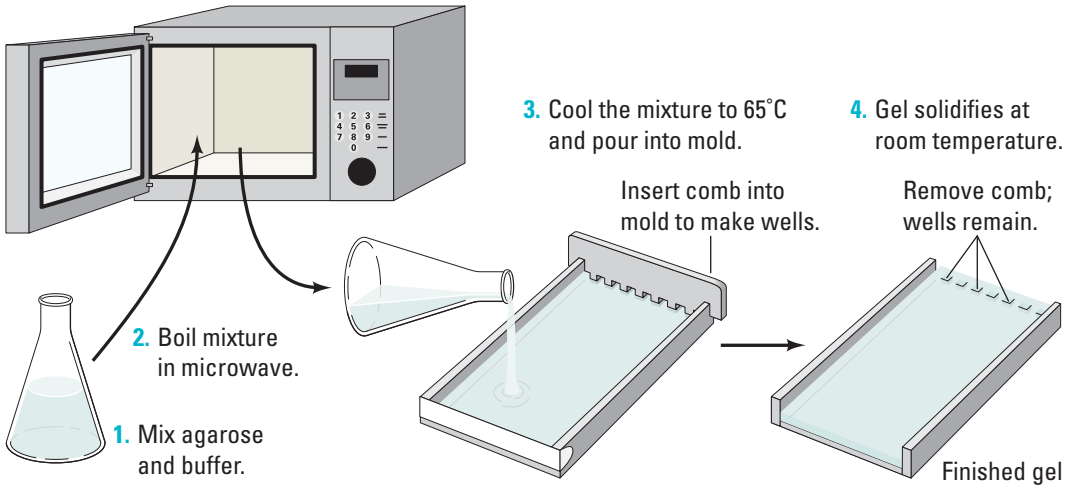

Figure 3. Casting an Agarose Gel

Step 3 The gel will become cloudy as it solidifies (15–20 minutes). Do not disturb or touch the gel while it is solidifying!

Step 4 When the agarose has set, carefully remove the ends of the casting tray and place the tray in the electrophoresis gel box so that the comb is at the negative (black) end.

- Why do you place the wells at the negative end of the gel box?
- What is the chemical nature of DNA? Will the DNA fragments migrate toward the positive end of the gel box or toward the negative end?

Step 5 Fill the box with 1x TAE buffer, to a level that just covers the entire surface of the gel.

Step 6 Gently remove the comb, taking care not to rip the wells. Make sure that the sample wells left by the comb are completely submerged in the buffer.

Step 7 The gel is now ready to be loaded with your DNA samples. (If your teacher says that you will load the gel on another lab day, close the electrophoresis box to prevent drying of the gel.)

Loading the Gel

Before loading your gel with samples of DNA, you should practice using the pipette or other loading device. One easy way to do this is to slowly aspire a sample of buffer and expel it into a "pretend well" on a paper towel ("pretend gel"). Your teacher might suggest another method for practicing how to load gels. Keep practicing until you feel comfortable loading and expelling a sample.

Make sure you record the order in which you load the samples. Be sure to use a fresh loading device (either plastic micropipette or other type of pipette) for each sample. Be sure you know how to use the pipette properly. When in doubt, ask your teacher. Take care not to puncture the bottom of the well with the pipette tip when you load your samples.

Step 1 Load 15–20 µL of each sample of DNA into a separate well in the gel, as shown in Figure 4.

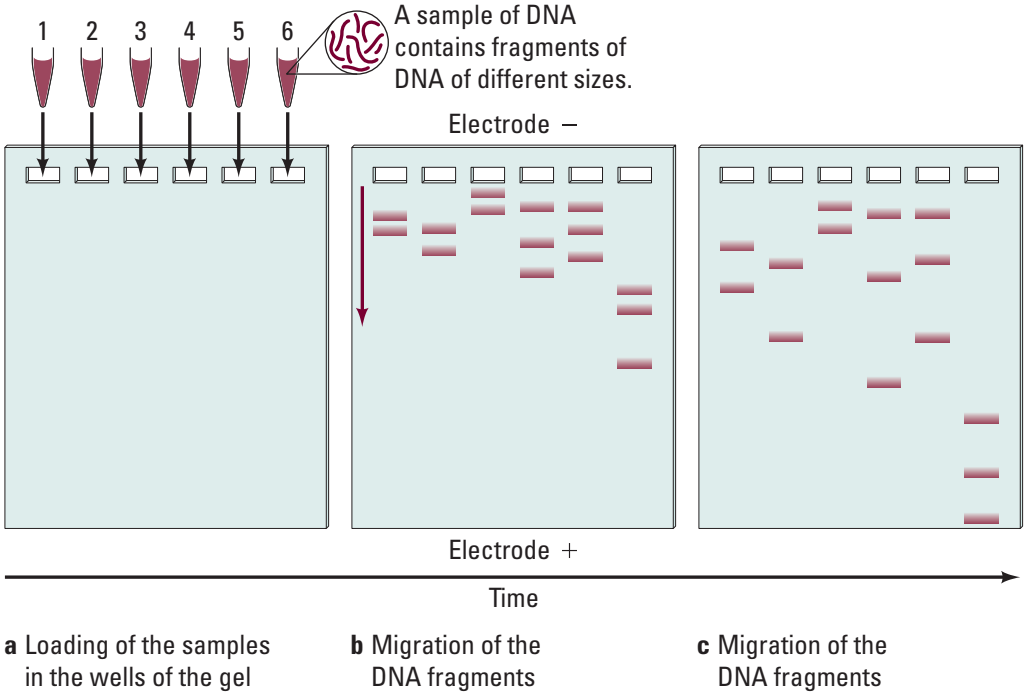

Figure 4. Loading an Agarose Gel and Migrating DNA Fragments Through Time

Step 2 Slowly draw up the contents of the first sample tube into the pipette.

Step 3 Using two hands, steady the pipette over the well you are going to load.

Step 4 Expel any air in the end of the pipette *before* loading the DNA sample.

Step 5 Dip the pipette tip through the surface of the buffer, position it just inside the well, and slowly expel the mixture. Sucrose in the loading dye weighs down the sample, causing it to sink to the bottom of the well. *Be careful not to puncture the bottom of the well with the pipette tip or reaspirate your sample up into the pipette.*

Step 6 Draw the pipette tip out of the buffer.

Step 7 Using a clean loading device for each sample, load the remaining samples into their wells.

Electrophoresis

Step 1 Close the top of the electrophoresis chamber and then connect the electrical leads to an appropriate power supply, positive (+) electrode to positive (+) electrode (black to black) and negative (-) electrode to negative (-) electrode (red to red). Make sure both electrodes are connected to the same channel of the power supply, just as you would connect leads to jump-start a car battery — black to black and red to red.

CAUTION: Be sure to keep the power OFF until you connect all leads!

Step 2 Turn on the power supply and set the voltage as directed by your teacher. (It is recommended that you "run the gel" at 50 volts for approximately 2 hours. If you run the gel at a higher voltage for less time, the fragments migrate too quickly through the gel with less separation. Again, ask your teacher for assistance if needed.)

Step 3 Shortly after the current is applied, you should see loading dye moving through the gel toward the positive pole of the electrophoresis apparatus. (**Note:** The purplish-blue band in the loading dye migrates through the gel at the same rate as a DNA fragment approximately 300 base pairs long.)

Step 4 Allow the DNA to electrophorese until the loading dye band is about 1 cm from the end of the gel. Your teacher may monitor the progress of the electrophoresis in your absence if you have to attend another class.

Step 5 Turn off the power supply, disconnect the leads from the power supply, and remove the lid of the electrophoresis chamber.

Step 6 Carefully remove the casting tray and slide the gel into a staining tray labeled with the name of your group.

- Measure in centimeters the distance that the purplish-blue loading dye has migrated into the gel. Measure from the front edge of the well to the front edge of the dye band (also called the dye front).
- Be sure to record your data (in centimeters).

Step 7 Take your gel to your teacher for further staining instructions. Again, your teacher might monitor the staining procedure.

Analyzing Results

Calculating the Sizes of Restriction Fragment Length Polymorphisms

Mathematical formulas have been developed for describing the relationship between the molecular weight of a DNA fragment and its mobility (i.e., how far it migrates in the gel). In general, DNA fragments, like the ones in your evidence samples, migrate at rates inversely proportional to the $\log_{10}$ of their molecular weights. **For simplicity's sake, base pair length (bp) is substituted for molecular weight when determining the size of DNA fragments.** Thus, the size in base pair length of a DNA fragment can be calculated using the distance the fragment travels through the gel. To calculate the base pair length, a DNA standard, composed of DNA fragments of *known* base pair length, is run on the same gel as the unknown fragments and is then used to create a standard curve. The standard curve, in this case a straight line, is created by graphing the distance each fragment traveled through the gel versus the $\log_{10}$ of its base pair length.

Creating the Standard Curve

As explained above, base pair (bp) length is substituted for molecular weight. Note that in plotting the standard curve, calculating the $\log_{10}$ of the base pair length of each fragment is unnecessary because the base pair size is plotted on the logarithmic axis of semi-log paper. Examine your stained gel on a light box or other surface that helps visualize the bands.

- What observations can you make?
- What quantitative measurements can you make?

1. Examine the "ideal" or mock gel shown in Figure 5 that includes DNA samples that have been cut with three restriction enzymes, *Bam*HI, *Eco*RI, and *Hin*dIII, to produce RFLPs (fragments). Sample D is DNA that has not been cut with enzyme(s). DNA cut with *Hin*dIII provides a set of fragments of known size and serves as a standard for comparison.

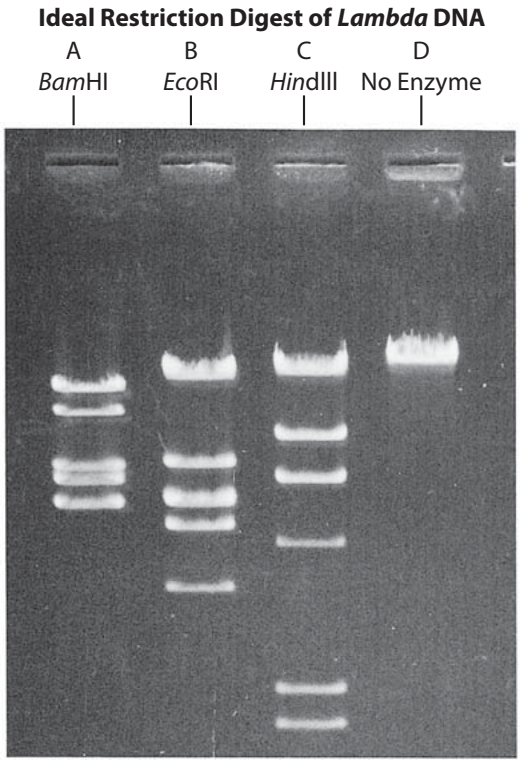

Figure 5. Ideal Gel

2. Using the ideal gel shown in Figure 5, measure the distance (in cm) that each fragment migrated *from* the origin (the well). (**Hint:** For consistency, measure from the front end of each well to the front edge of each band, i.e., the edge farthest from the well.). Enter the measured distances into Table 1. (See * and ** notes below the table for an explanation for why there are only six bands seen but more fragments.)

Table 1. DNA Fragment Migration Distance

*HIN*DIII		*BAM*HI		*ECO*RI	
Distance Traveled	BP Length	Distance Traveled	BP Length	Distance Traveled	BP Length
	*27,491				
	*23,130				
	9,416				
	6,557				
	4,361				
	2,322				
	2,027				
	**564				
	**125				

*For this "ideal" gel, assume that these two bands appear as a single band instead of resolving into separate bands.

** These bands do not appear on the ideal gel and likely will not be seen.

3. Plot the standard curve using the data from the DNA sample cut with *Hin*dIII. To do this, your teacher might ask you to graph the data directly using Excel with distance traveled as the (arithmetic) x-axis and the base pair (bp) length as the (logarithmic) y-axis. Based on this graph, why must the data be plotted using the log scale? You might want to plot the data again using semi-log paper.

   Connect the data point with a best-fit line. However, you should ignore the point plotted for the 27,491bp/23,130 doublet. When using 0.8% agarose gel, these fragments appear as one. Congratulations! Your best-fit line is the standard curve.

4. Now use the standard curve to calculate the approximate sizes of the *Eco*RI and *Bam*HI fragments. Using a ruler, how can you use the standard curve to calculate the sizes of unknown fragments?

Designing and Conducting Your Investigation

Now that you've learned about the techniques used to create DNA profiles or "fingerprints," it's time to apply the techniques as you investigate the disappearance of Ms. Mason. Your task is to design and conduct a procedure *based on DNA evidence* to determine whose blood is spattered on the classroom floor. The chief investigator (your teacher) will provide you with DNA evidence collected at the crime scene from the blood, Ms. Mason (saliva on her coffee cup), Mr. Gladson (tissue with which he wiped his nose), and Bobby (bubble gum). In addition, you will be given a sample of DNA cut with *Hin*dIII. Remember from your analysis of the "ideal" or mock gel that DNA cut with *Hin*dIII serves as a marker, providing a set of RFLPs of known sizes (standard).

Analyzing Results

Evaluate your crime scene samples to determine whose blood was on the classroom floor. Because this case likely will go to trial, visual analysis (qualitative data) of the DNA profiles is not sufficient to identify a perpetrator. Based on your results, write the conclusion to the scenario to reveal "whodunit" based on motive, means, opportunity, and DNA evidence.

Evaluating Results

1. What are some possible challenges you had in performing your investigation?
2. What are some possible sources of error in the electrophoresis procedure? How can you minimize any potential sources of error?

Thinking About Your Results

1. There are important social and ethical implications of DNA analysis. Already, DNA testing can reveal the presence of markers of certain genetic diseases, such as Huntington's. So, who should have access to your genetic profile? Health insurance companies? College admissions offices? Employers? What issues about confidentiality are raised by genetic testing? Who owns your DNA and its information?

2. Suppose a DNA test that predicted your chances of getting a disease, such as cancer, were available. You take the test for cancer, and the results say you have a two in three chance of getting cancer sometime in the next 20 years. Who should have access to this information? Your doctor? Health insurance companies? Employers? Would *you* want to know this information?

3. The Innocence Project (IP) is an international litigation and public policy organization dedicated to exonerating wrongfully convicted individuals through DNA testing. Three-quarters of DNA exoneration cases involve misidentification by witnesses. To date, nearly 300 people previously convicted of serious crimes in the U.S. have been exonerated by DNA testing. However, not everyone is in favor of the IP. One United States Supreme Court justice expressed concern that DNA testing poses risks to the criminal justice system, in which a person is judged by a jury of peers. What social and ethical issues are raised by using DNA evidence to re-examine old court decisions? What other arguments can you make (or find) against using DNA evidence for court cases?

4. With genetic engineering, biotechnicians can clip out beneficial genes from native plants in foreign countries and insert them into their crop plant relatives here in the United States, with great benefits to the latter — to prevent attack by insects, to increase productivity, or to allow the crops to be grown in colder climates. These benefits can be worth billions of dollars, but if the crops are grown in the United States, should countries where the native plants are located benefit from the bioengineering? Who owns the information in DNA? Who can profit from that information? Investigate this controversy on the Internet with examples drawn from different crops grown here in the U.S.

Where Can You Go from Here?

The following are suggestions for expanding your study of biotechnology.

1. Do you remember earlier when you read that more than 99% of your DNA is the same as another person's DNA, and that the 1% difference is attributed to small differences in genetic code? Conduct independent research on how these small differences can be detected by molecular biologists. Begin by investigating unique repeat DNA sequences called variable tandem repeats (VNTRs), short tandem repeats (STRs), and single nucleotide polymorphisms (SNPs). Prepare a mini-poster presentation for your classmates illustrating how these small differences can be used to individualize DNA from different organisms, including humans. Are the differences between you and other individuals in the genes themselves? If so, how do you account for the fact that everyone needs the same genes to produce your cell components and your organs, such as your liver and lungs?

2. Often scientists have only a small amount of DNA available for analysis. The polymerase chain reaction (PCR) is another key technique that molecular biologists use to amplify a specific sequence of DNA. Developed by Kary Mullis in 1983, PCR produces millions of copies of a DNA sequence in a few hours, with the original sequence serving as the template for replication. PCR has a variety of applications, including DNA cloning, determining DNA-based phylogeny, diagnosing hereditary diseases, and identifying genetic fingerprints. Ask your teacher if you can learn to perform PCR. PCR usually requires a relatively expensive piece of equipment, a DNA thermocycler; however, you can investigate less expensive methods of PCR.

3. Select an episode of one of your favorite TV crime investigation shows that focuses on DNA as evidence. Compare TV science with *real* science.

INVESTIGATION 10

ENERGY DYNAMICS

What factors govern energy capture, allocation, storage, and transfer between producers and consumers in a terrestrial ecosystem?

BACKGROUND

Almost all life on this planet is powered, either directly or indirectly, by sunlight. Energy captured from sunlight drives the production of energy-rich organic compounds during the process of photosynthesis. These organic compounds create biomass. The net amount of energy captured and stored by the producers in a system is the system's net productivity. Gross productivity is a measure of the total energy captured. In terrestrial systems, plants play the role of producers. Plants allocate that biomass (energy) to power their life processes or to store energy. Different plants have different strategies of energy allocation that reflect their role in various ecosystems. For example, annual weedy plants allocate a larger percentage of their biomass production to reproductive processes and seeds than do slower growing perennials. As plants, the producers are consumed or decomposed, and their stored chemical energy powers additional individuals, the consumers, or trophic levels of the biotic community. Biotic systems run on energy much as economic systems run on money. Energy is generally in limited supply in most communities. Energy dynamics in a biotic community is fundamental to understanding ecological interactions.

To model ecosystem energy dynamics, you will estimate the net primary productivity (NPP) of Wisconsin Fast Plants (the producers) growing under lights and the flow of energy from plants to cabbage white butterfly larvae (the consumers) as the larvae eat cabbage-family plants.

The following exercises describe skills and methods for estimating energy flow in a terrestrial ecosystem. Note and record any questions that occur to you as you work through this activity.

Questions might include the following:

- What kinds of things affect plant productivity, the growth of cabbage white butterfly larvae, or the interactions of these organisms?
- How do you keep track of energy as it moves through the biological system? Can the techniques used for tracking energy be improved?
- What is the role of energy in ecosystems?

One or more of these questions will help guide you through the final part of this laboratory, where you are expected to carry out your own research project based on one of your questions.

Learning Objectives

- To design and conduct an experiment to investigate a question about energy capture and flow in an ecosystem
- To explain community/ecosystem energy dynamics, including energy flow, NPP, and primary and secondary producers/consumers
- To predict interspecific ecological interactions and their effects
- To use mathematical analyses in energy accounting and community modeling
- To make the explicit connection between biological content and the investigative experience

General Safety Precautions

- Cabbage white butterflies (*Pieris rapae*) are listed as a pest species by the USDA. Therefore, no butterflies or larvae raised in the laboratory should be released to the wild.
- Euthanize the butterflies or larvae by freezing them when your investigation is complete. The plants and soil can simply be discarded.
- Disease outbreaks are common in cultured populations of organisms. Although the diseases associated with the organisms in this investigation are not dangerous to humans, it is important to maintain cleanliness in the laboratory and of your experimental equipment to minimize possible impacts on the study caused by disease.
- Long-term culturing for plants or butterflies requires cleanliness. Be sure to clean all culturing chambers and wipe them down with dilute Clorox (and dry completely) before starting another generation of plants or butterflies. Use new materials if you have any doubts.
- Cultures involve artificial light sources and liquids; caution should be exercised to keep the two separate.

THE INVESTIGATIONS

Getting Started

These questions and tasks are designed to help you understand energy dynamics and prepare for your investigations.

1. The economy of a business or household is somewhat like the energetics of a biological community. A well-run household or business creates a budget based on a careful accounting of money coming in and money going out. Likewise, the energy dynamics of a biological community can be modeled by accounting for the energy coming in and going out through different members of the community. Keeping track of money is relatively straightforward — you count it. You count how much money is coming in and how much is going to various expenses and savings.

How do you keep track of energy in living organisms? It is a challenge. Producers capture light energy and convert it into chemical energy stored in energy-rich molecules. These molecules have mass, so the energy in biological systems can be indirectly measured by mass — biomass. With your lab team, take a moment to brainstorm how you can account for energy use and, in a biological community, transfer it in a manner similar to the ways in which people account for money.

2. This investigation requires you to take care of and maintain healthy populations of living organisms — plants and animals. In fact, before you begin this investigation, you will need to start both plant and animal cultures. Wisconsin Fast Plants and cabbage white butterflies are both easily raised in the classroom or laboratory. Neither takes up much time or equipment, but they both need to be tended regularly. As a lab team, discuss the care and maintenance of the organisms you use in this lab. Prepare a schedule and divide up responsibilities for long-term care and maintenance. (This includes taking care of animal wastes.) Check out online information on care and maintenance of the organisms you and your teacher select for this investigation at ***http://fastplants.ning.com/video/2038532:Video:13*** and ***http://fastplants.ning.com/video/juan-enriquez-wants-to-grow***. See the butterfly life cycle in Figure 1.

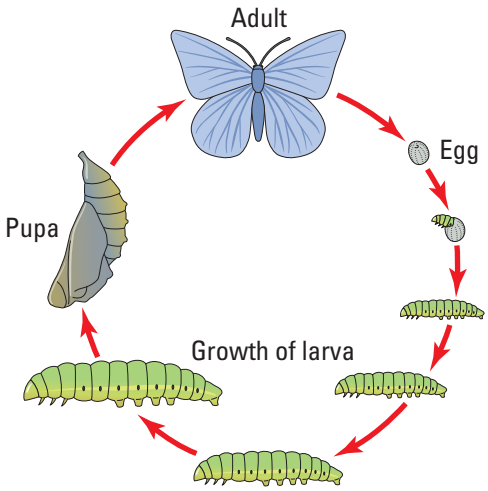

Figure 1. Butterfly Life Cycle

Procedures

Estimating Net Primary Productivity (NPP) of Fast Plants

Remember as you work through the first part of this investigation to think about and record questions you ask while working with these organisms and the system.

Primary productivity is a rate — energy captured by photosynthetic organisms in a given area per unit of time. Based on the second law of thermodynamics, when energy is converted from one form to another, some energy will be lost as heat. When light energy is converted to chemical energy in photosynthesis or transferred from one organism (a plant or producer) to its consumer (e.g., an herbivorous insect), some energy will be lost as heat during each transfer.

In terrestrial ecosystems, productivity (or energy capture) is generally estimated by the change in biomass of plants produced over a specific time period. Measuring biomass or changes in biomass is relatively straightforward: simply mass the organism(s) on an appropriate scale and record the mass over various time intervals. The complicating factor is that a large percentage of the mass of a living organism is water — not the energy-rich organic compounds of biomass. Therefore, to determine the biomass at a particular point in time accurately, you must dry the organism. Obviously, this creates a problem if you wish to take multiple measurements on the same living organism. Another issue is that different organic compounds store different amounts of energy; in proteins and carbohydrates it is about 4 kcal/g dry weight and in fats it is 9 kcal/g of dry weight). As you plan your own investigation, take into consideration all the above information.

You and your teacher will select a model organism for this lab depending on time of year, availability, and cost. The following steps assume that you and your lab team are culturing about 30 to 40 Wisconsin Fast Plants as a model organism. Other plants can be used instead, including wild or native plants, but check with your teacher first.

Step 1 In your lab notebook, design and construct a systems diagram to model energy capture and flow through a plant. Use annotations to help explain your reasoning. Before taking any measurements, think about the input and output of energy in a plant. For instance, what do you predict about the quantity of energy the plants take in compared to the quantity of energy that goes out? What do you think are various ways that a plant (or a number of plants) could lose energy, and how could you estimate the amount of energy lost through these various pathways? Enter your predictions in your lab notebook by constructing an annotated system diagram, such as Figure 2, of the flow of energy into and out of a plant.

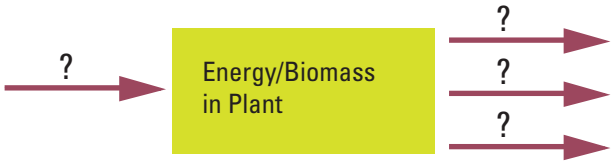

Figure 2. Energy Flow into and out of a Plant

Step 2 Your energy diagram will help you and your lab team design a data collection procedure that helps you measure energy capture and flow in a plant. As a team, design your investigation to sample the biomass of an adequate number of plants early in the life cycle and then again later in the life cycle. Remember, biomass is only the mass of the DRY plant materials, not of the water in the plant. Make sure your procedure accounts for this. Describe this procedure in your lab notebook and then check with your teacher for approval and suggestions. Be sure to record your observations, questions, reflections, and measurements daily in your notebook. In your lab notebook, record information about the size and overall appearance of your Fast Plants.

Step 3 In your notebook, graphically present a comparison of the biomass/energy of plants early in their life cycle versus older plants.

Step 4 Determine the average (mean) grams of biomass added per plant over the period of growth. Each gram of plant biomass represents about 4.35 kcal of energy. Convert grams of biomass/day to NPP (kcal)/day. Show this work in your lab notebook. Explain why this is *net* primary productivity and not *gross* productivity.

Step 5 Explain in your notebook why the mass of dry plants is a better measure of primary productivity and biomass than is the mass of living plants (containing water). What percentage of the living plants is biomass? (Use this calculation in Analyzing and Evaluating Results, Step 4.)

Step 6 Now reconstruct your energy flow diagram with actual data that you have collected in your notebook. Be sure to include an explanation, supported by evidence, as to why you feel your diagram represents energy flow in Fast Plants. Your explanation should also include a description of the uncertainties of your data and your conclusions; put boundaries on your conclusions (as you would insert error bars).

Estimating Energy Flow Between Fast Plants Producers and Cabbage Butterfly Larvae

Don't forget to think of and record questions about these organisms and the system as you work through your investigation.

Step 1 Cabbage white butterfly larvae eat plants from the cabbage family. As with Fast Plants, accounting for energy flow into and out of these butterflies can be inferred from biomass gained and lost. In your lab notebook, develop a system diagram, such as Figure 3, to model energy flow from Fast Plants to cabbage butterfly larvae. Before taking any measurements, predict the input and the output of energy in the butterfly larvae you will be growing. Enter these predictions in your lab notebook.

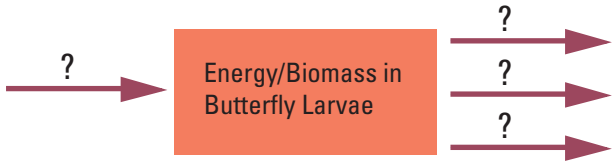

Figure 3. Energy Flow from Fast Plants to Cabbage Butterfly Larvae

Step 2 As butterfly larvae grow toward maturity, they pass through different developmental stages called instars. You will use larvae that are already well along their developmental path through the larval stages (4th or 5th instar). These larvae first grew on young Fast Plants, and they were later transferred to brussels sprouts (another member of the cabbage family) in a Brassica Barn (see Figure 4). For this part of the investigation, you and your lab team need to develop a procedure that will quantify the growth of butterfly larvae over three days. Start with freshly massed brussels sprouts in the Brassica Barn.

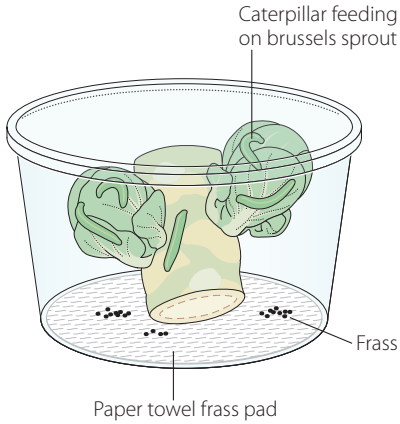

Figure 4. Brassica Barn

Step 3 Create a table in your lab notebook that helps you organize the data collected, including estimates of the energy/biomass flow from plants to butterfly larvae. Develop your procedure keeping in mind your end goal — to measure the biomass consumed by the larvae, the biomass gained by the larvae, and the biomass lost by the larvae. Likely, you'll need to estimate some factors using data from a large sample. Don't forget about the energy in the frass (wastes).

Step 4 Transfer the larvae to another Brassica Barn to finish out their life cycle.

Analyzing and Evaluating Results

Convert biomass measurements (grams) to energy units in kilocalories. Work in small groups to determine how best to complete the following tasks. Make sure that all your units are comparable: per time, mass, and energy.

Step 1 For Fast Plants, assume that one gram of dried biomass contains 4.35 kilocalories of energy. This estimate was determined by burning similar plant material in a bomb calorimeter.

Step 2 You were investigating living butterfly larvae, so you could not dry them or their food supply. Assume that the biomass of 4th instar larvae is 40% of the wet mass. (This estimate may be inaccurate, so you should actually measure this quantity using extra butterfly larvae, if possible.) Calculate the biomass of the larvae. For butterfly larvae, use an average value of 5.5 kcal/g of biomass to calculate energy of each larva.

Step 3 To determine the energy content in the larval frass, use 4.76 kcal of energy/g of frass. Calculate the frass lost per individual larva.

Step 4 To determine the energy content of the brussels sprouts eaten by each larva, convert the wet mass of the sprout to dry mass and multiply by 4.35 kcal/g. Use the estimated percentage of biomass (dry mass) in fresh Fast Plants calculated in estimating the Fast Plants' net primary productivity (NPP), Step 5 on page S130, to estimate the biomass of each brussels sprout.

Step 5 These procedures are similar to an energy audit. Because energy is neither created nor destroyed, you must account for all energy in the system. (That is why you need to determine frass mass.) Combine your two earlier energy flow diagrams into one, and now include all the information that you measured. For those energy pathways that you did not explicitly measure, provide an estimated energy quantity. For instance, the amount of light energy in the system is more difficult to estimate. What other parts of the energy flow diagram could you not actually measure?

Step 6 Graph your results. For the plants and for the butterfly larvae, design and construct appropriate graphs of your results. Enter sketches of these graphs in your lab notebook and prepare more finished copies for your mini-poster presentation when you complete this lab. If you use bar graphs for illustrating the means, standard error bars should be included to display the range of the data. In your notebook (and mini-poster presentation), describe the data and their presentation. Follow that with conclusions that you can support with your data about energy capture and flow in this artificial lab community.

Designing and Conducting Your Investigation

In the previous procedures, you began to develop your skills by applying methods to the problem of energy capture and flow in an ecosystem. You were encouraged to note and record questions about this system as you worked through the investigation. Now it is time to select one of those questions, propose your hypothesis, design your investigation, and carry it out. Be sure to connect your work to your overall understanding of energy and ecosystems.

The following are questions that could be investigated; however, you should have developed your own question(s) and considered a possible investigation(s).

- Do all plants have the same percentage of biomass?
- Is the percentage of biomass the most important characteristic of a plant in terms of its effect on the growth of an animal?
- How do plants with different life strategies allocate biomass in different organs?
- How much is allocated to reproduction?
- How much energy is allocated to plant defense?
- How much energy does it cost an animal to process different plant sources?

Review and consolidate your questions into a list of possibilities. Consult with your teacher and other students. After choosing your question, hypothesis, and design, submit the plan (proposal) for your investigation to your teacher for approval. Be sure to refer to the rubric provided by your teacher that will be used to evaluate your work. Consider working as a team on a single question to reduce your overall workload.

Step 1 Conduct your investigation or construct and test your mathematical model.

Step 2 Prepare a mini-poster that addresses the requirements outlined in the rubric.

Step 3 Present your mini-poster to your peers and invited guests in class. Encourage your peers to review and critique your work based on the rubric guidelines. Use those reviews to improve your mini-poster after the presentation. Your teacher will use the same rubric, along with your lab notebook, to determine your final grade for this investigation.

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INVESTIGATION 11

TRANSPIRATION*

What factors, including environmental variables, affect the rate of transpiration in plants?

BACKGROUND

Cells and organisms must exchange matter with the environment to grow, reproduce, and maintain organization, and the availability of resources influences responses and activities. For example, water and macronutrients are used to synthesize new molecules, and, in plants, water is essential for photosynthesis. Organisms have evolved various mechanisms for accumulating sufficient quantities of water, ions, and other nutrients and for keeping them properly balanced to maintain homeostasis.

Plants absorb and transport water, nutrients, and ions from the surrounding soil via osmosis, diffusion, and active transport. Once water and dissolved nutrients have entered the root xylem, they are transported upward to the stems and leaves as part of the process of transpiration, with a subsequent loss of water due to evaporation from the leaf surface. Too much water loss can be detrimental to plants; they can wilt and die.

The transport of water upward from roots to shoots in the xylem is governed by differences in water (or osmotic) potential, with water molecules moving from an area of high water potential (higher free energy, more water) to an area of low water potential (lower free energy, less water). (You may have studied the concept of water potential in more detail when exploring the processes of osmosis and diffusion in Investigation 4 in this manual.) The movement of water through a plant is facilitated by osmosis, root pressure, and the physical and chemical properties of water. Transpiration creates a lower osmotic potential in the leaf, and the TACT (transpiration, adhesion, cohesion, and tension) mechanism describes the forces that move water and dissolved nutrients up the xylem.

* Transitioned from the *AP Biology Lab Manual* (2001)

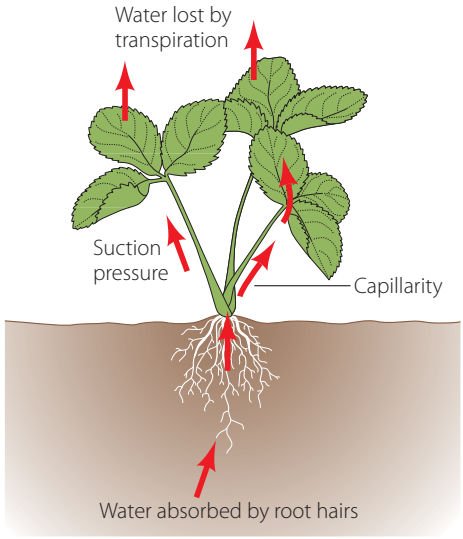

Figure 1. Transpiration Model

During transpiration, water evaporating from the spaces within leaves escapes through small pores called stomata. Although evaporation of water through open stomata is a major route of water loss in plants, the stomata must open to allow for the entry of CO_2 used in photosynthesis. In addition, O_2 produced in photosynthesis exits through open stomata. Consequently, a balance must be maintained between the transport of CO_2 and O_2 and the loss of water. Specialized cells called guard cells help regulate the opening and closing of stomata.

In this laboratory investigation, you will begin by calculating leaf surface area and then determine the average number of stomata per square millimeter. From your data, several questions emerge about the process of transpiration in plants, including the following:

- Do all plants have stomata? Is there any relationship between the number of stomata and the environment in which the plant species evolved?
- Are leaf surface area and the number of stomata related to the rate of transpiration? What might happen to the rate of transpiration if the number of leaves or the size of leaves is reduced?
- Do all parts of a plant transpire?
- Do all plants transpire at the same rate? Is there a relationship between the habitat in which plants evolved and their rate of transpiration?
- What other factors, including environmental variables, might contribute to the rate of transpiration?
- What structural features and/or physiological processes help plants regulate the amount of water lost through transpiration? How do plants maintain the balance between the transport of CO_2 and O_2 and the amount of water lost through transpiration?

You will then design an experiment to investigate one of these questions or a question of your own. As a supplemental activity, you can examine microscopically thin sections of stems, identify xylem and phloem cells, and relate the function of these vascular tissues to observations made about the structure of these cells.

The investigation also provides an opportunity for you to apply and review concepts you have studied previously, including the relationship between cell structure and function; osmosis, diffusion, and active transport; the movement of molecules and ions across cell membranes; the physical and chemical properties of water; photosynthesis; and the exchange of matter between biological systems and the environment.

Learning Objectives

- To investigate the relationship among leaf surface area, number of stomata, and the rate of transpiration
- To design and conduct an experiment to explore other factors, including different environmental variables, on the rate of transpiration
- To investigate the relationship between the structure of vascular tissues (xylem and phloem) and their functions in transporting water and nutrients in plants

General Safety Precautions

If you investigate transpiration rates using a potometer, you should be careful when assembling your equipment and when using a razor blade or scalpel to cut the stem of a plant, cutting to a 45° angle.

When appropriate, you should wear goggles for conducting investigations. Nail polish used in the investigation is toxic by ingestion and inhalation, and you should avoid eye contact. All materials should be disposed of properly as per your teacher's instructions.

THE INVESTIGATIONS

Getting Started

These questions are designed to help you understand concepts related to transpiration in plants before you design and conduct your investigation(s).

1. If a plant cell has a lower water potential than its surrounding environment, make a prediction about the movement of water across the cell membrane. In other words, will the cell gain water or lose water? Explain your answer in the form of a diagram with annotations.

2. In the winter, salt is sometimes spread over icy roads. In the spring, after the ice has melted, grass often dies near these roads. What causes this to happen? Explain your answer in the form of a diagram with annotations.

3. Prepare a thin section of stem from your plant and examine it under the microscope to identify the vascular tissues (xylem and phloem) and the structural differences in their cells. Describe how the observed differences in cellular structure reflect differences in function of the two types of vascular tissue.

4. If you wanted to transplant a tree, would you choose to move the tree in the winter, when it doesn't possess any leaves but it's cold outside, or during the summer, when the tree has leaves and it's warm and sunny? Explain your answer.

Procedure

Materials

- Living representative plant species available in your region/season, such as *Impatiens* (a moisture-loving plant), *Coleus,* oleander (more drought tolerant), *Phaseolus vulgaris* (bean seedlings), pea plants, varieties of *Lycopersicon* (tomato), peppers, and ferns
- Calculator, microscope, microscope slides, clear cellophane tape, clear nail polish, and scissors
- Additional supplies that you might need after you choose a method to determine leaf surface area (Step 1 below). Ask your teacher for advice.

Record data and any answers to questions in your lab notebooks, as instructed by your teacher.

Step 1 Form teams of two or three and investigate methods of calculating leaf surface area. (You will need to calculate leaf surface area when you conduct your experiments.) Think about and formulate answers to the following questions as you work through this activity:

a. How can you calculate the total leaf surface area expressed in cm^2? In mm^2?

b. How can you estimate the leaf surface area of the entire plant without measuring every leaf?

c. What predictions and/or hypotheses can you make about the number of stomata per mm^2 and the rate of transpiration?

d. Is the leaf surface area directly related to the rate of transpiration?

e. What predictions can you make about the rate of transpiration in plants with smaller or fewer leaves?

f. Because most leaves have two sides, do you think you have to double your calculation to obtain the surface area of one leaf? Why or why not?

g. Water is transpired through stomata, but carbon dioxide also must pass through stomata into a leaf for photosynthesis to occur. There is evidence that the level of carbon dioxide in the atmosphere has not always been the same over the history of life on Earth. Explain how the presence of a higher or lower concentration of atmospheric carbon dioxide would impact the evolution of stomata density in plants.

h. Based on the data in the following table, is there a relationship between the habitat (in terms of moisture) to which the plants are adapted and the density of stomata in their leaves? What evidence from the data supports your answer?

Table 1. Average Number of Stomata per Square Millimeter (mm^2) of Leaf Surface Area

PLANT	IN UPPER EPIDERMIS	IN LOWER EPIDERMIS
Anacharis	0	0
Coleus	0	141
Black Walnut	0	160
Kidney Bean	40	176
Nasturtium	0	130
Sunflower	85	156
Oats	25	23
Corn	70	88
Tomato	12	130
Water Lily	460	0

Step 2 Make a wet mount of a nail polish stomatal peel to view leaf epidermis using the following technique:

a. Obtain a leaf. (The leaf may remain on the plant or be removed.)

b. Paint a solid patch of clear nail polish on the leaf surface being studied. Make a patch of at least one square centimeter.

c. Allow the nail polish to dry completely.

d. Press a piece of clean, clear cellophane tape to the dried nail polish patch. Using clear (not opaque) tape is essential here. You might also try pulling the peel away from the leaf without using any tape and then preparing a wet mount of the peel with a drop of water and a cover slip.

e. Gently peel the nail polish patch from the leaf by pulling a corner of the tape and peeling the nail polish off the leaf. This is the leaf impression that you will examine. (Make only one leaf impression on each side of the leaf, especially if the leaf is going to be left on a live plant.)

f. Tape the peeled impression to a clean microscope slide. Use scissors to trim away any excess tape. Label the slide as appropriate for the specimen being examined and label the side of leaf from which the peel was taken.

g. Examine the leaf impression under a light microscope to at least 400X (or highest magnification). Draw and label what you observe. Can you observe any stomata? Search for areas where there are numerous stomata.

h. Count all the stomata in one microscopic field. Record the number.

i. Repeat counts for at least three other distinct microscopic fields and record the number of stomata.

j. Determine an average number of stomata per microscopic field.

k. From the average number per microscopic field, calculate the number of stomata per 1 mm^2. You can estimate the area of the field of view by placing a transparent plastic ruler along its diameter, measuring the field's diameter, and then calculating area by using πr^2. (Most low-power fields have a diameter between 1.5–2.0 mm.)

l. Trade slides with two other lab teams so you examine three different slides under the microscope using the same procedure described above.

Designing and Conducting Your Investigation

The procedure should have raised several questions about factors that relate to the rate of transpiration in plants. Some possible questions are listed below, but you may have others.

- What environmental variables might affect the rate of transpiration?
- Do all parts of a plant transpire?
- Do all plants transpire at the same rate?
- Is there a relationship between the habitat in which plants evolved to their rate of transpiration?

Rate of transpiration can be measured by a variety of methods, including the use of a potometer with or without a gas pressure sensor and computer interface or the use of the whole plant method. These methods are detailed in this investigation, but your teacher may help you substitute another procedure.

If using a gas pressure sensor and computer interface to measure transpiration rate, your teacher likely will provide instructions. If you are unfamiliar with the use of probes with computer interface, it is suggested that you spend about 30 minutes learning how to collect data using the equipment.

Step 1 Design an experiment to investigate one of the aforementioned questions or one of your own questions to determine the effect of an environmental variable(s) on the rate of transpiration in plants. When identifying your design, be sure to address the following questions:

- What is the essential question being addressed?
- What assumptions are made about the questions being addressed?
- Can those assumptions be easily verified?
- Will the measurement(s) provide the necessary data to answer the question under study?
- Did you include a control in your experiment?

Step 2 Make a hypothesis/prediction about which environmental factors will have the greatest effect on transpiration rates. Be sure to explain your hypothesis.

Step 3 Conduct your experiment(s) and record data and any answers to your questions in your lab notebooks or as instructed by your teacher. Write down any additional questions that arose during this study that might lead to *other* investigations that you can conduct.

Option 1: Potometer with or Without Gas Pressure Sensor

Materials

- Representative plant species available in your region/season, such as *Impatiens* (a moisture-loving plant), *Coleus*, oleander (more drought tolerant), *Phaseolus vulgaris* (bean seedlings), pea plants, varieties of *Lycopersicon* (tomato), peppers, and ferns
- Potometer, which you assemble from clear plastic tubing, a ring stand with clamp, and a 0.1-mL or 1.0-mL pipette, depending on the diameter of the stem of the plant you choose. Your teacher will have several different sizes of plastic tubing available. (The tubing can be filled using a water bottle or plastic syringe *without a needle*.) If using a syringe, attach it to the end of the pipette and pull water into the potometer. (Why should the tubing be free of air bubbles? Why must the stem be completely immersed in the water?) If using a gas pressure sensor, the tubing is inserted directly into the device, with no pipettes required. (The potometer assembly is illustrated in Figure 2.)
- Fan, heat lamp, water, small plastic bag, spray bottle with water, salt, and other materials provided by your teacher to simulate an environmental variable
- Petroleum jelly to make an airtight seal between the cut end of stem and tubing filled with water (You can also use small clamps to seal without the "goop.")

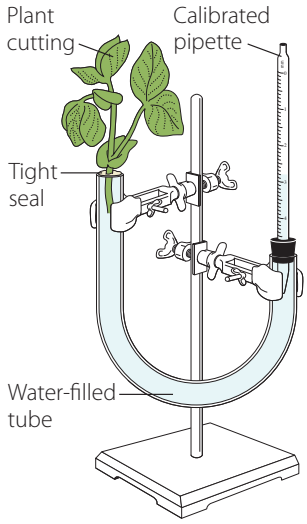

Figure 2. Potometer Assembly

Using a razor blade, carefully cut the plant stem so that its diameter will fit into the piece of plastic tubing in the potometer assembly. Note that it is often helpful to cut the stem while it is submerged under water to prevent air bubbles from being introduced into the xylem. Your teacher will provide additional instructions, if necessary. Please be careful when using the razor blade!

If using the gas pressure sensor to collect data, connect the gas pressure sensor to the computer interface. Prepare the computer for data collection by opening the file "10 Transpiration" from the *Biology with Vernier* folder of Logger*Pro*. If using a pipette to measure water loss, you will have to determine your method of data collection.

Option 2: Whole Plant Method

Materials

- Small potted plant (*Impatiens*, tomato seedling, bean seedling, pea plant, etc.) with many leaves and few flowers
- One-gallon size plastic food storage bag without zipper
- String

Step 1 Saturate the plant with water the day/night before beginning your investigation.

Step 2 Carefully remove a plant from the soil/pot, making sure to retain as much of the root system and keeping soil particles attached to the roots. Wrap the root ball of the plant(s) in a plastic bag and tie the bag around the base so that only the leaves are exposed. (Be sure to remove all flowers and buds.) Do not water your plant any more until you finish your experiment! You can also keep the plant in the plastic pot and place it in the plastic bag.

Step 3 Determine the mass of each plant and then its mass for several days under your environmental condition(s).

Step 4 Record your data in your lab notebook or as instructed by your teacher.

Calculations: Determining Surface Area and Transpiration Rates

Step 1 In the first part of this lab, you were asked to investigate methods to calculate leaf surface area and the surface area of all the leaves on a plant or plant cutting (depending on your experimental setup). Your teacher may suggest a particular method. Determine the total surface area of the leaves in cm^2 and record the value.

Step 2 Calculate the rate of transpiration/surface area. If you are using a gas pressure sensor to collect data, you can express these rate values as $kPa/min/cm^2$, where kPa (kilopascal) is a unit of pressure. Record the rate.

Step 3 After the entire class agrees on an appropriate control, subtract the control rate from the experimental value. Record this adjusted rate.

Step 4 Record the adjusted rate for your experimental test on the board to share with other lab groups. Record the class results for each of the environmental variables investigated.

Step 5 Graph the class results to show the effects of different environmental variables on the rate of transpiration. You may need to convert data to scientific notation with all numbers reported to the same power of 10 for graphing purposes.

Step 6 Your teacher may suggest you perform statistical analysis (e.g., a T-test) of your data, comparing results of experimental variable(s) to controls.

Analyzing Results

1. How was the rate of transpiration affected by your choice of experimental variable as compared to the control?
2. Think of a way you can effectively communicate your results to other lab groups. By comparing results and conclusions, explain how changes or variables in environmental conditions affect transpiration rates.
3. Based on data collected from different lab groups, which environmental variable(s) resulted in the greatest rate of water loss through transpiration? Explain why this factor might increase water loss when compared to other factors.
4. Why did you need to calculate leaf surface area to determine the rate(s) of transpiration?

5. What structural or physiological adaptations enable plants to control water loss? How might each adaptation affect transpiration?

6. Make a prediction about the number of stomata in a leaf and the rate of transpiration. What type(s) of experiments could you conduct to determine the relationship between the number of stomata and the rate of transpiration?

7. Create a diagram with annotation to explain how the TACT (transpiration, adhesion, cohesion, tension) mechanism enables water and nutrients to travel up a 100-ft. tree. Predict how a significant increase in ambient (environmental) temperature might affect the rate of transpiration in this tree. Explain your prediction in terms of TACT and the role of guard cells in regulating the opening and closing of stomata.

Evaluating Results

1. Was your initial hypothesis about the effect of your environmental variable on the rate of transpiration supported by the data you collected? Why or why not?

2. What were some challenges you had in performing your experiment? Did you make any incorrect assumptions about the effect of environmental variables on the rate(s) of transpiration?

3. Were you able to perform without difficulty the mathematical routines required to analyze your data? Which calculations, if any, were challenging or required help from your classmates or teacher?

Where Can You Go from Here?

1. Investigate how guard cells control the opening and closing of stomata, including the role of abscisic acid and K^+.

2. Design an experiment to investigate transpiration in two different types of plants — one that is drought tolerant and one that requires a significant amount of water. What predictions can you make about the rate of transpiration in each?

3. If you had to revise the design of your experiment, what suggestions would you make? Why would you make them?

4. If your investigations generated other questions that you might want to research, ask your teacher if you can conduct other experiments.

INVESTIGATION 12

FRUIT FLY BEHAVIOR

What environmental factors trigger a fruit fly response?

BACKGROUND

Drosophila melanogaster, the common fruit fly, is an organism that has been studied in the scientific community for more than a century. Thomas Hunt Morgan began using it for genetic studies in 1907. The common fruit fly lives throughout the world and feeds on fruit and the fungi growing on rotting fruit. It is a small fly, and one could question why scientists have spent so much time and effort on this tiny insect. It is about the size of President Roosevelt's nose on a dime, but despite its small size, the fly is packed with many interesting physical and behavioral characteristics. Its genome has been sequenced, its physical characteristics have been charted and mutated, its meiotic processes and development have been investigated, and its behavior has been the source of many experiments. Because of its scientific usefulness, *Drosophila* is a model research organism. Its name is based on observations about the fly; the fly follows circadian rhythms that include sleeping during the dark and emerging as an adult from a pupa in the early morning. This latter behavior gave rise to the name *Drosophila*, which means "lover of dew." The explanation for the species name *melanogaster* should be clear after observing the fly's physical features. It has a black "stomach," or abdomen. No doubt the dew-loving, black-bellied fly will continue to make contributions to the scientific community and to student projects.

We begin our investigation with a few simple questions. What do you know about fruit flies? Have you seen fruit flies outside the lab and, if so, where? Describe where and when you have noted fruit flies.

Learning Objectives

- To investigate the relationship between a model organism, *Drosophila*, and its response to different environmental conditions
- To design a controlled experiment to explore environmental factors that either attract or repel *Drosophila* in the laboratory setting
- To analyze data collected in an experiment in order to identify possible patterns and relationships between environmental factors and a living organism
- To work collaboratively with others in the design and analysis of a controlled experiment
- To connect and apply concepts (With the fruit fly as the focal organism, your investigation could pull together many topics, such as genetics, animal behavior, development, plant and animal structures from cells to organs, cell communication, fruit ripening, fermentation, and evolution.)

General Safety Precautions

- Do not add substances to the choice chamber unless your teacher has approved them.
- If the substance you add is flammable, such as ethanol, use precaution and do not conduct your experiment near a heat source or flame.
- Many of the substances used in this experiment are food items, but you should not consume any of them.
- Fruit flies are living organisms that should not be released to the environment. After all the investigations are complete, flies should be tapped into a "morgue" through a funnel. The morgue typically is a 150-mL beaker that contains about 50 mL of salad oil or 70% alcohol.

THE INVESTIGATIONS

Getting Started

This procedure is designed to help you understand how to work with fruit flies. You may start with general information about how to determine the sex of a fruit fly. How do you tell the difference between male and female flies? Is the sex of the fly important to your investigations? Look at the female and male fruit flies in Figure 1. Then look at the fruit flies in Figure 2. Can you identify which ones are female and which ones are male? Focus on the abdomen of the flies to note differences.

Figure 1. Determining the Sex of Fruit Flies

Figure 2. Fruit Flies

Step 1 Using fruit fly cultures, carefully toss 10 to 20 living flies into an empty vial. Be sure to plug the vial as soon as you add the flies. Do not anesthetize the flies before this or any of the behavior experiments.

Step 2 When flies are tossed, they are tapped into an empty vial. Tap a culture vial (push the vial down on a solid surface several times) on the table to move the flies to the bottom of the vial. Quickly remove the foam or cotton top and invert an empty vial over the top of the culture vial. Invert the vials so that the culture vial is on the top and the empty vial is on the bottom, and tap the flies into the empty container by tapping it on a solid surface several times. Be sure to hold the vials tightly to keep them together. You must then separate the vials and cap each separately. Do not try to isolate every fly from the original culture. It is difficult to separate flies, and you may lose a fly or two in the process.

Step 3 After your lab group has the flies in a vial without food, observe the position of the flies in your upright vial.

Step 4 Invert the vial, and observe the position of the flies after 15 seconds and after 30 seconds.

Step 5 What was the flies' response? Did most/all of the flies move in the same general direction? If so, this might be an "orientation movement," which is a movement that is in response to some stimulus. Based on how you manipulated the vial, to what stimulus might the flies be responding? Do you think that they were responding to some chemical change in the vial? Did your observations generate other questions? Explain your answers.

Procedure

Animals move in response to many different stimuli. A chemotaxis is a movement in response to the presence of a chemical stimulus. The organism may move toward or away from the chemical stimulus. What benefit would an organism gain by responding to chemicals in their environment? A phototactic response is a movement in response to light. A geotactic response is a movement in response to gravity.

You will investigate fruit fly movement using a choice chamber that exposes the flies to different substances that you insert into the chamber. Because flies are very common in households (in fact, fruit flies live almost everywhere that humans live), think about using foods or condiments that might result in a positive or a negative chemotactic response from the flies. What foods or condiments do you think would attract or repel flies? Why? Do fruit flies exhibit a response to light or to gravity? How can you alter the chamber to investigate those variables?

Step 1 Prepare a choice chamber by labeling both ends with a marker — one end "A" and the other "B" (see Figure 3). Cut the bottom of the bottles, dry the interior thoroughly, and tape them together. Remove any paper labels.

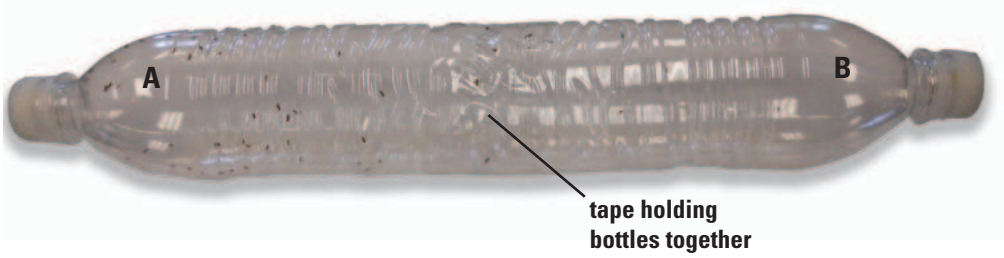

Figure 3. Choice Chamber

Place a cap on one end of a chamber before adding flies. Insert a small funnel in the open end of the chamber and place the chamber upright on the capped end. Tap 20–30 fruit flies into the choice chamber using the funnel.

Step 2 After transfer, quickly cap the other end of the chamber.

Step 3 Begin your study of the choice of flies by placing a few (5–10) drops of distilled water on two cotton balls, and adhere one moist cotton ball to each end of the chamber. (Do not add too much of any chemical to the cotton; too much liquid will drip down into the chamber and affect the experiment by sticking flies to the bottle.) What is the importance of using distilled water at both ends of the chamber?

Step 4 Lay the chamber down on a white surface or on white paper.

Step 5 Give the flies at least 5 minutes of undisturbed time, and then count (or closely approximate) the number of flies at each end of the chamber. Create a table to record the number of flies you find at each end (A and B) of the chamber.

Step 6 List all of the substances that you will be testing, and predict what you think the flies will prefer based on your knowledge of fruit flies.

Step 7 Begin to test each substance you are including in your investigation. Place a few drops of one substance on a cotton ball. Remove cap A, place the cotton ball in the cap, and replace the cap on the chamber. Place a cotton ball with distilled water on the other end. How might you determine which of the substances stimulate a negative chemotaxis and which stimulate a positive chemotaxis?

Step 8 Lay the chamber down on a light colored surface (or on white paper) and observe the flies.

Step 9 Give the flies at least 5 minutes of undisturbed time, and then count the number of flies at each end of the chamber.

Step 10 Change the caps, and give the fruit flies another substance.

Step 11 Gather data for at least four different substances. Which substances do fruit flies prefer? Which do they avoid?

Step 12 Quantify the results and express them graphically. Complete a chi-square analysis of your results. Using data from the entire class, construct a preference table. Were your hypotheses about the preferences of fruit flies supported or not? Did the flies demonstrate a chemotaxis in relation to any of the substances you chose? Can you think of any reasons for their preferences?

Designing and Conducting Your Investigation

Now that you have discovered the preferences for individual substances, design an experiment using the choice chamber to compare the preferences of fruit flies to all test substances or the chemotactic responses of your flies. Create a table that includes the results comparing all of the substances you tested.

The following are questions that you could investigate; however, as you worked through the beginning of this lab, you should have developed your own question and an investigation to answer that question:

- Are all substances equally attractive or repellant to the fruit flies?
- Which substances do fruit flies prefer the most?
- Which substances do fruit flies prefer the least?
- Do preferred substances have any characteristic in common?
- What other factors might affect whether or not the fruit flies moved from one part of your choice chamber to another?
- Do you think that it is the fruit itself that attracts the flies? Should they be called *fruit flies* or something else?
- Some experiments could be designed using fruit fly larvae. Do larvae respond the same way that adults respond? Are there other factors in the environment that affect the choice?
- What factors must be controlled in an experiment about environmental variables and behavior?
- What is the difference among phototaxis, chemotaxis, and geotaxis? Do fruit flies demonstrate all of them?
- Does a phototactic response override a chemotactic response?
- Does the age of the fruit fly change its geotactic response?
- Are there other organisms that respond the same as fruit flies? Are there other organisms that respond differently from fruit flies?

Analyzing Results

Look for patterns in fly behavior based on the number and ratio of fruit flies on different ends of your choice chamber. How will you determine which of the substances stimulate the greatest negative chemotactic response and positive chemotactic response? Do you see any patterns about materials or forces to which fruit flies are attracted?

Develop a method for sharing your results and conclusions to classmates — and then share them!

Evaluating Results

1. Is there anything that was shared by all of the environmental factors to which the flies were attracted?

2. Is there anything that was shared by all of the environmental factors to which the flies were repelled?

3. How do you explain the behavior of fruit flies in someone's kitchen or in nature based on the information you collected? Do your data explain all fruit fly movements? Explain your answers.

Where Can You Go from Here?

One possible extension for this investigation is to identify another organism that behaves similarly to the fruit fly and one that you expect would behave differently. For example, you could substitute ladybugs, houseflies, or mealworms for fruit flies and construct choice chambers using other substances that you think might be attractive to these organisms.

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INVESTIGATION 13

ENZYME ACTIVITY*

How do abiotic or biotic factors influence the rates of enzymatic reactions?

BACKGROUND

Enzymes are the catalysts of biological systems. They speed up chemical reactions in biological systems by lowering the activation energy, the energy needed for molecules to begin reacting with each other. Enzymes do this by forming an enzyme-substrate complex that reduces energy required for the specific reaction to occur. Enzymes have specific shapes and structures that determine their functions. The enzyme's active site is very selective, allowing only certain substances to bind. If the shape of an enzyme is changed in any way, or the protein denatured, then the binding site also changes, thus disrupting enzymatic functions.

Enzymes are fundamental to the survival of any living system and are organized into a number of groups depending on their specific activities. Two common groups are catabolic enzymes ("*cata*" or "*kata-*" from the Greek "to break down") — for instance, amylase breaks complex starches into simple sugars — and anabolic enzymes ("*a-*" or "*an-*" from the Greek "to build up"). (You may know this second word already from stories about athletes who have been caught using anabolic steroids to build muscle.)

Catalytic enzymes, called proteases, break down proteins and are found in many organisms; one example is bromelain, which comes from pineapple and can break down gelatin. Bromelain often is an ingredient in commercial meat marinades. Papain is an enzyme that comes from papaya and is used in some teeth whiteners to break down the bacterial film on teeth. People who are lactose intolerant cannot digest milk sugar (lactose); however, they can take supplements containing lactase, the enzyme they are missing. All of these enzymes hydrolyze large, complex molecules into their simpler components; bromelain and papain break proteins down to amino acids, while lactase breaks lactose down to simpler sugars.

Anabolic enzymes are equally vital to all living systems. One example is ATP synthase, the enzyme that stores cellular energy in ATP by combining ADP and phosphate. Another example is rubisco, an enzyme involved in the anabolic reactions of building sugar molecules in the Calvin cycle of photosynthesis.

* Transitioned from the *AP Biology Lab Manual* (2001)

To begin this investigation, you will focus on the enzyme peroxidase obtained from a turnip, one of numerous sources of this enzyme. Peroxidase is one of several enzymes that break down peroxide, a toxic metabolic waste product of aerobic respiration. Using peroxidase, you will develop essential skills to examine your own questions about enzyme function.

Later, you will have an opportunity to select an enzyme, research its properties and mode of reaction, and then design an experiment to explore its function. The investigation also provides an opportunity for you to apply and review concepts you have studied previously, including the levels of protein structure, energy transfer, abiotic and biotic influences on molecular structure, entropy and enthalpy, and the role of enzymes in maintaining homeostasis.

Learning Objectives

- To understand the relationship between enzyme structure and function
- To make some generalizations about enzymes by studying just one enzyme in particular
- To determine which factors can change the rate of an enzyme reaction
- To determine which factors that affect enzyme activity could be biologically important

General Safety Precautions

Follow general laboratory safety procedures. Wear proper footwear, safety goggles or glasses, a laboratory coat, and gloves. Use proper pipetting techniques, and use pipette pumps, syringes, or rubber bulbs. Never pipette by mouth! Dispose of any broken glass in the proper container. Since the concentrations of the reactive materials in this laboratory are environmentally friendly (0.1% hydrogen peroxide and 0.3% guaiacol), they can be rinsed down a standard laboratory drain. The concentrations used here are deemed to be safe by all chemical standards, but recall that any compound has the potentiality of being detrimental to living things and the environment. When you develop your individual investigations you must always consider the toxicity of materials used.

Key Vocabulary

Baseline is a universal term for most chemical reactions. In this investigation the term is used to establish a standard for a reaction. Thus, when manipulating components of a reaction (in this case, substrate or enzyme), you have a reference to help understand what occurred in the reaction. The baseline may vary with different scenarios pertinent to the design of the experiment, such as altering the environment in which the reaction occurs. In this scenario, different conditions can be compared, and the effects of changing an environmental variable (e.g., pH) can be determined.

Rate can have more than one applicable definition because this lab has two major options of approach, i.e., using a color palette and/or a spectrophotometer to measure percent of light absorbance. When using a color palette to compare the change in a reaction, you can infer increase, decrease, or no change in the rate; this inference is usually called the **relative rate of the reaction**. When using a spectrophotometer (or other measuring devices) to measure the actual percent change in light absorbance, the rate is usually referred to as **absolute rate of the reaction**. In this case, a specific amount of time can be measured, such as 0.083 absorbance/minute.

THE INVESTIGATIONS

Getting Started

Procedure 1: Developing a Method for Measuring Peroxidase in Plant Material and Determining a Baseline

Peroxide (such as hydrogen peroxide) is a toxic byproduct of aerobic metabolism. Peroxidase is an enzyme that breaks down these peroxides. It is produced by most cells in their peroxisomes.

The general reaction can be depicted as follows:

Enzyme + Substrate --> Enzyme-Substrate Complex --> Enzyme + Product(s) + ΔG

For this investigation the specific reaction is as follows:

Peroxidase + Hydrogen Peroxide --> Complex --> Peroxidase + Water + Oxygen

$$2H_2O_2 \rightarrow 2H_2O + O_2 \text{ (gas)}$$

Notice that the peroxidase is present at the start and end of the reaction. Like all catalysts, enzymes are not consumed by the reactions. To determine the rate of an

enzymatic reaction, you must measure a change in the amount of at least one specific substrate or product over time. In a decomposition reaction of peroxide by peroxidase (as noted in the above formula), the easiest molecule to measure would probably be oxygen, a final product. This could be done by measuring the *actual* volume of oxygen gas released or by using an indicator. In this experiment, an indicator for oxygen will be used. The compound guaiacol has a high affinity for oxygen, and in solution, it binds instantly with oxygen to form tetraguaiacol, which is brownish in color. The greater the amount of oxygen gas produced, the darker brown the solution will become.

Qualifying color is a difficult task, but a series of dilutions can be made and then combined on a palette, which can represent the relative changes occurring during the reaction. A color palette/chart ranging from 1 to 10 (Figure 1) is sufficient to compare relative amounts of oxygen produced. Alternatively, the color change can be recorded as a change in absorbency using a variety of available meters, such as a spectrophotometer or a probe system. (Information about the use of spectrophotometers and/or probe systems is found in the Additional Information section of this investigation.)

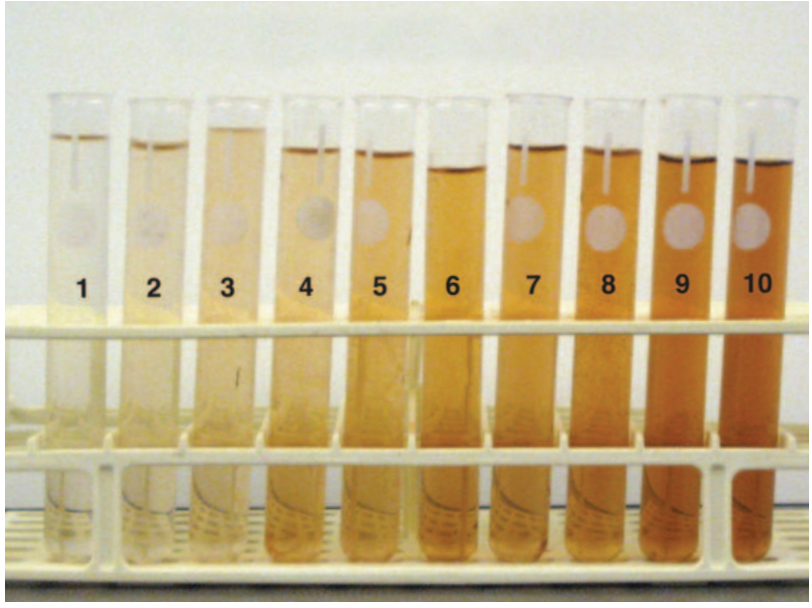

Figure 1. Turnip Peroxidase Color Chart

Materials

- Turnip peroxidase
- 0.1% hydrogen peroxide
- Guaiacol
- Distilled (deionized) water
- 2 test tubes (approximately 16 x 150 mm) and appropriate test tube rack
- Timer
- 1, 5, and 10 mL graduated pipettes, pipette pumps, or syringes (1, 2, 5, and 10 mL)

This investigation is designed to be performed without a spectrophotometer, but your teacher may ask you to use a spectrophotometer or probe system. If so, additional equipment may be required.

Step 1 Using two 16 x 150 mm test tubes, mark one "substrate" and the other tube "enzyme." To the substrate tube, add 7 mL of distilled water, 0.3 mL of 0.1 percent hydrogen peroxide, and 0.2 mL guaiacol for a total volume of 7.5 mL. Cover the test tube with a piece of Parafilm® and gently mix.

Step 2 To the enzyme tube, add 6.0 mL of distilled water and 1.5 mL of peroxidase for a total volume of 7.5 mL. Cover the test tube with a piece of Parafilm and gently mix.

Step 3 Combine the contents of the two tubes (subtrate and enzyme) in another 16 x 150 mL test tube, cover the tube with Parafilm, invert twice to mix, and place the tube in a test tube rack. Immediately begin timing the reaction.

Step 4 Observe the color change for the next 5 minutes. Rotate the tube before each reading. Record the observed color at 0, 1, 2, 3, 4, and 5 minutes. (A cell phone and/or camera are excellent ways to record color change.)

Step 5 Use the color palette/chart (Figure 1) to help you quantify changes in color over time. Graph your data in your laboratory notebook.

Consider the following questions before you proceed to the next experiment:

- You measured the color change at different times. Which time will you use for your later assays? Why? (The time/color change that you select will serve as your baseline for additional investigations.)
- When you use this assay to assess factors that change enzyme activity, which components of the assay will you change? Which will you keep constant?

Procedure 2: Determining the Effect of pH on Enzymatic Activity

Numerous variables can be employed to observe the effects on the rate of an enzymatic reaction and possibly the specific fit of the enzyme with the substrate.

- What do you predict will occur if the pH in the reaction changes? How do you justify your prediction?

Materials

- Turnip peroxidase
- 0.1% hydrogen peroxide
- Guaiacol
- Buffers with range of pH
- Distilled (deionized) water
- 12 test tubes (approximately 16 x 150 mm) and appropriate test tube rack
- Timer
- 1, 5, and 10 mL graduated pipettes, pipette pumps, or syringes (1, 2, 5, and 10 mL)
- Spectrophotometer or probe system

Step 1 Using clean 16 x 150 mL test tubes, make six sets of pairs of original substrate and enzyme tubes for a total of 12 tubes or 6 pairs. This time you will substitute a different pH buffer for the distilled water used in the original enzyme tubes. Prepare the tubes as follows and be sure to label them.

- For each substrate tube in a pair, add 7 mL of distilled water, 0.3 mL of hydrogen peroxide, and 0.2 mL of guaiacol for a total volume of 7. 5 mL.
- For each enzyme tube in the pair, add 6.0 mL of a specific pH solution and 1.5 mL of peroxidase for a total volume of 7.5 mL. For example, in the enzyme tube of the first pair, you can substitute 6.0 mL of buffer solution of pH 3 for the distilled water; in the enzyme tube of the second pair, you can substitute 6.0 mL of buffer solution of pH 5 for the distilled water, and so forth.
- Cover each test tube with a piece of Parafilm, and gently mix.

Step 2 Combine the substrate and enzyme tubes for all six pairs (total volume 15.0 mL per pair), cover with Parafilm, gently mix, and place the tubes back in the test tube rack. Immediately begin timing the reactions.

Step 3 Record the observed color for each tube at 0 minutes and again at the time you chose based on your results in Procedure 1. (Again, a cell phone and/or camera are excellent ways to record color change.)

Step 4 Use the palette/color chart (Figure 1) to help you quantify the changes you observe. Graph your data as color intensity versus pH. What conclusions can you draw from your results?

Designing and Conducting Your Investigation

You now have the basic information and tools needed to explore enzymes in more depth on your own. In this part of the lab, you will do just that. You will have the chance to develop and test your own hypotheses about enzyme activity. To help you get started, read the following questions, and write your answers in your laboratory notebook.

- In Procedure 1, was the limiting factor of your baseline reaction the enzyme or the substrate? How could you modify the procedure you learned to answer this question?
- What are three or four factors that vary in the environment in which organisms live? Which of those factors do you think could affect enzyme activity? How would you modify your basic assay to test your hypothesis?

Design and conduct an experiment to investigate an answer(s) to one of the questions above or another question that might have been raised as you conducted Procedures 1 and 2. Remember, the primary objective of the investigation is to explore how biotic and abiotic factors influence the rate of enzymatic reactions.

Analyzing Results

From the data that you collected from your independent investigation, graph the results. Based on the graph and your observations, compare the effects of biotic and abiotic environmental factors on the rate(s) of enzymatic reactions and explain any differences.

Additional Information

If a spectrophotometer is available, the following information is useful.

The use of measuring devices can better quantify your results. Using a spectrophotometer, you can select a specific wavelength to fit the color/pigment expected in an experiment. The change in the amount or concentration of color/pigment may be measured as absorbance (amount of the wavelength trapped by the pigment) or transmittance (amount of the wavelength that is not trapped by the pigment).

For Procedure 1:

1. Turn on your spectrophotometer approximately 10 to 15 minutes prior to starting the investigation so that it will warm up appropriately.

2. To measure the amount of the compound tetraguaiacol, set the wavelength to 470 nm.

3. Set your machine at zero absorbance using a blank containing all the appropriate materials *except* the substrate (i.e., 13.3 mL of distilled water, 0.2 mL of guaiacol, and 1.5 mL of enzyme extract = 15 mL total).

4. Determine the baseline.

   A. Using two 16 x 150 mm test tubes, label one "substrate" and the other "enzyme." Substrate tube: 7 mL of distilled water, 0.3 mL of hydrogen peroxide, and 0.2 mL guaiacol (total volume 7.5 mL) Enzyme tube: 6 mL of distilled water and 1.5 mL of peroxidase (total volume 7.5 mL)

   B. Combine the materials of the substrate and enzyme tubes. Mix the tubes twice and pour into a cuvette. (When mixing or rotating always cover the opening of the cuvette with Parafilm.)

   C. Place the cuvette into the spectrophometer and record absorbance; this is your initial or "0" time reading. Remove the tube. Repeat recording absorbance at 1, 2, 3, 4, and 5 minutes. Be sure to rotate (use Parafilm to cover) the tube and also clean its surface with a scientific cleaning wipe before each reading.

5. Record and graph your data.

For Procedure 2:

Follow steps 1, 2, and 3 above. In step 4, set up as outlined above. Make an initial reading at time "0" and a second reading at the time you chose as optimal based on results obtained in Procedure 1. Record and graph your data.