Chapter 12

Review of Laboratory Investigations

Review

The College Board requires that 25% of the AP Biology instructional time be devoted to laboratory activity. The laboratory experience is important, as it provides the laboratory experience typical of a first-year college course in biology. The College Board provides 13 laboratory investigations for use in the AP Biology course. In addition, your AP Biology instructor may substitute or supplement these investigations with other labs. Of the 13 investigative labs and instructor supplementary labs, 8 labs are *required*, with 2 labs from each of the Big Ideas: evolution, cellular processes, genetics, and interactions (see Table 12-1).

Questions about lab investigations on the AP exam will either be of a general nature or provide a clear description of all aspects of the lab with enough information to answer the question. However, you will be at a distinct advantage if you have done the lab or are familiar with it. Unfortunately, there is not enough time in the school year to do all 13 labs and another possible half-dozen or so of instructor supplementary labs. To help you gain an advantage for AP lab investigations you have not done, this chapter provides full descriptions of all the AP lab investigations.

The AP free-response part of the exam consists of two long questions and six short questions. At least one of the long questions will address laboratory analysis or design. Other questions, long and short, may also involve laboratory activities or the application of math skills to biological questions. A four-function calculator (with a square root function) is required for the exam. The question types fall into one of the following five categories:

1. **Experimental analysis.** In this type of essay question, you are given some experimental data and asked to interpret or analyze the data. The question usually includes several parts, each requesting specific interpretations of the data. In addition, you are usually asked to prepare a graphic representation of the data. Graph paper is provided. Guidelines for preparing a graph are given in the next section.
2. **Experimental design.** This type of essay question asks you to design an experiment to answer specific questions about given data or an experimental situation. Guidelines for designing an experiment are given in the next section.
3. **Modeling.** This type of question requires you to develop a mathematical expression that explains or can be used to predict a biological process.
4. **Math applications.** In this kind of question, you will explain how a mathematical expression describes a biological process.
5. **Predicting and justifying.** In this type of question, you will be given data, often in tabular or graphical form, that you will use to make predictions or explanations.

If these types of questions all sound the same to you, don't fret. The laboratory investigations that the College Board provides and the ones your teacher uses as supplements will provide you with exposure and familiarity with these kinds of questions. Although the data or situations on the AP exam may differ from your classroom activities, you will be able to draw from lab experiences to answer the questions on the exam.

The material in this chapter summarizes each of the 13 laboratory exercises with a brief description of its experimental design and conclusions. This material is not intended to substitute for an actual laboratory experience, but to provide you with a review that will help you answer the AP Biology questions.

Summary of AP Biology Laboratory Investigations

Table 12-1

Big Idea	Investigation	Description
1. Evolution	1. Artificial Selection	Investigating the effect of selection for trichome number in plants
	2. Modeling Evolution	Investigating evolution in a model population
	3. Comparing DNA Sequences	Using BLAST to find evolutionary relationships
2. Cellular Processes	4. Diffusion and Osmosis	Observing diffusion and osmosis in artificial and living cells
	5. Photosynthesis	Identifying factors that influence the rate of photosynthesis
	6. Cellular Respiration	Identifying factors that influence the rate of cellular respiration
3. Genetics	7. Mitosis and Meiosis	Investigating the cell division processes of mitosis and meiosis
	8. Bacterial Transformation	Transferring foreign DNA into a bacterium
	9. Restriction Enzyme Analysis of DNA	Mapping DNA
4. Interactions	10. Energy Dynamics	Investigating energy flow in an ecosystem
	11. Transpiration	Identifying factors that influence the rate of transpiration
	12. Animal Behavior	Observing behavior of fruit flies in response to stimuli
	13. Enzyme Catalysis	Investigating factors that influence enzyme activity

Graphing and Interpreting Data

The laboratory question in the free-response part of the AP exam will often ask you to create a graph using data provided in the question or ask you to predict and graph data as part of an experiment that you design. Include the following in your graph (Figure 12-1):

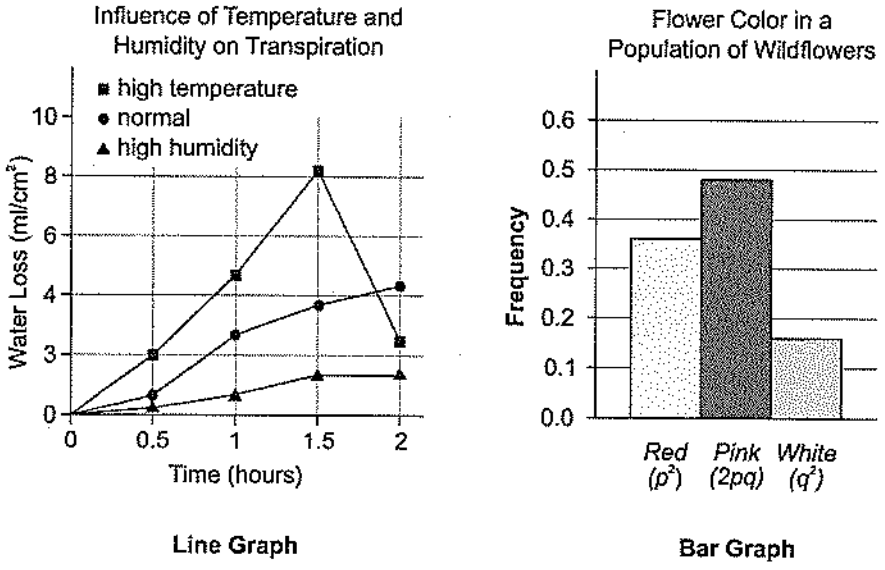

Figure 12-1

1. **Label each axis.** Indicate on each axis what is being measured and in what unit of measurement. For example, "Time (minutes)," "Distance (meters)," or "Water Loss (ml/m^2)" are appropriate labels.
2. **Provide values along each axis at regular intervals.** Select values and spacing that will allow your graph to fill as much of the graphing grid as possible. Be sure that the units on each axis are linear—that is, one unit on the axis must have the same length anywhere on the axis.
3. **Use the *x*-axis for the independent variable and the *y*-axis for the dependent variable.**
   - The **independent variable** is the variable that you choose to manipulate—that is, the variable to which *you* assign values. For example, suppose you wanted to investigate fruit fly longevity as a function of diet. The independent variable is the type of diet. *You* choose to investigate diet and *you* assign different "values" of diet (for example, high protein, high calorie, etc.).
   - The **dependent variable** is how the experiment responds to the independent variable. Its values are the data that you collect. (Think "*d*ata is for the *d*ependent variable.") The dependent variable measures how the experiment is responding to the independent variable that you have chosen to investigate.

   If you are plotting the progress of an event, then you have chosen time as the independent variable and the data you collect that measure changes in the event (such as weight change, distance traveled, or carbon dioxide released) are represented by the dependent variable.
4. **Connect the plotted points.** Either connect the plotted points with straight lines or draw a straight line through the plotted points estimating the line of best fit. Avoid smooth curves, as they usually imply knowledge about intermediate points not plotted or a mathematical equation that fits the experimental results. If the question asks you to make predictions beyond the data actually graphed, extrapolate (extend) the plotted line with a different line form (for example, dotted or dashed).
5. **In graphs with more than one plot, identify each plot.** If you plot more than one set of data on the same graph, identify each plot with a short phrase. Alternately, you can draw the points of each plot with different symbols (for example, circles, squares, or triangles) or connect the plotted points using different kinds of lines (solid line, dashed line, or dash-dot line) and then identify each kind of symbol or line in a legend.
6. **Provide a title for the graph.** Your title should be brief but descriptive.
7. **Use bar graphs for discrete data.** Line graphs, as described above, are appropriate for displaying data when the independent variable is continuous; that is, the independent variable has a countless number of values in an interval. Time, temperature, concentration, and light intensity are examples of continuous variables. For discrete variables, variables that have a limited number of values, a bar graph is more appropriate. A discrete variable may represent drug treatments, diets, genotypes (such as blood type alleles), or phenotypes (such as eye color). To construct a bar graph, draw a vertical column for each value of the independent variable from the *x*-axis to a height on the *y*-axis that reflects the value of its dependent variable. Other aspects of the bar graph, as described above in items 1–3 and 6, are the same.

Designing an Experiment

A laboratory free-response question may ask you to design an experiment to test a given hypothesis or to solve a given problem. In many cases, the question will ask you not only to design an experiment, but also to discuss expected results. Since the form of these questions can vary dramatically, it is not possible to provide a standard formula for preparing your answer. However, the following list provides important elements that you should include in your answer if they are appropriate to the question:

1. **Identify the independent and dependent variables.** The independent variable is the variable you are manipulating to see how the dependent variable changes.
   - You are investigating how the crustacean *Daphnia* responds to changes in temperature. You expose *Daphnia* to temperatures of 5°C, 10°C, 15°C, 20°C, 25°C, and 30°C. You count the number of heartbeats/sec in each case. Temperature is the **independent variable** (you are manipulating it), and the number of heartbeats/sec is the **dependent variable** (you observe how it changes in response to different temperatures).

- You design an experiment to investigate the effect of exercise on pulse rate and blood pressure. The physiological conditions (independent variable, or variable you manipulate) include sitting, exercising, and recovering at various intervals following exercise. You make two kinds of measurements (two dependent variables) to evaluate the effect of the physiological conditions—pulse rate and blood pressure.

2. **Describe the experimental treatment.** The experimental treatment (or treatments) is the various values that you assign to the independent variable. The experimental treatments describe how you are manipulating the independent variable.
   - In the *Daphnia* experiment, the different temperature values (5°C, 10°C, 15°C, 20°C, 25°C, and 30°C) represent six experimental treatments.
   - In the experiment on physiological conditions, the experimental treatments are exercise and recovery at various intervals following exercise.
3. **Identify a control treatment.** The control treatment, or control, is the independent variable at some normal or standard value. The results of the control are used for comparison with the results of the experimental treatments.
   - In the *Daphnia* experiment, you choose the temperature of 20°C as the control because that is the average temperature of the pond where you obtained the culture.
   - In the experiment on physiological conditions, the control is sitting, when the subject is not influenced by exercising.
4. **Use only one independent variable.** Only one independent variable can be tested at a time. If you manipulate two independent variables at the same time, you cannot determine which is responsible for the effect you measure in the dependent variable.
   - In the physiological experiment, if the subject also drinks coffee in addition to exercising, you cannot determine which treatment, coffee or exercise, causes a change in blood pressure.
5. **Random sample of subjects.** You must choose the subjects for your experiments randomly. Since you cannot evaluate every *Daphnia,* you must choose a sample population to study. If you choose only the largest *Daphnia* to study, it is not a random sample and you introduce another variable (size) for which you cannot account.
6. **Describe the procedure.** Describe how you will set up the experiment. Identify equipment and chemicals to be used and why you are choosing to use them. If appropriate, provide a labeled drawing of the setup.
7. **Describe expected results.** Use graphs to illustrate the expected results, if appropriate.
8. **Provide an explanation of the expected results in relation to relevant biological principles.** The results you give are your expected results. Describe the biological principles that led you to make your predictions.
   - In the experiment on physiological conditions, you expect blood pressure and pulse rate to increase during exercise in order to deliver more O_2 to muscles. Muscles use the O_2 for respiration, which generates the ATP necessary for muscle contraction.

Statistical Analyses

When you take the AP exam, you will receive two pages of equations and formulas to use while you answer questions for both sections of the exam. These pages provide you with all of the equations and formulas you will need to solve any questions that evaluate your ability to perform data analysis and solve quantitative problems. You can see these pages at the beginning of Part 3 of this book. Included on these pages are statistical equations and tables that provide you with the tools to perform statistical analysis.

There are two kinds of statistics that you will need to know for the AP exam:

1. **Descriptive statistics** are used to summarize data. In particular, you may need to calculate averages (mean, median, and mode) or measures of variation (range, standard error, and standard deviation). Definitions and formulas for these statistics are provided on the Equations and Formulas pages.

2. The **chi-square (χ^2) test for goodness-of-fit** is used to compare observed results with expected results. The statistic tells you whether the two are significantly different. In particular, the statistic determines whether the difference is due to chance (at an accepted probability) or whether some other factor is responsible. To understand this statistic, consider the following two values of χ^2:
   - $\chi^2 = 0$. In this case, the observed results and the expected results are exactly the same.
   - $\chi^2 = 100$. In most cases, this value of χ^2 is a relatively large value and will indicate that the difference between the observed and expected results did not occur by chance and that they should be considered significantly different. Such a result often indicates that your expectations were wrong or your data collection was faulty.

   More typically, the value you calculate for χ^2 will be between these two extremes. To determine whether the χ^2 value obtained indicates that the difference between the observed data and expected data occurred by chance or because the expectations or data are wrong, a χ^2 statistical table is consulted (provided on the Equations and Formulas pages). If chance explains 95% or more of the deviation of χ^2 from zero, then the experimental results are acceptable. If not, there are errors in the data collection or a problem with the expectations.

   As an example, consider the trait of eye color in fruit flies, where red eyes (*R*) are dominant to brown (sepia) eyes (*r*). A cross between a heterozygous red-eyed fly (*Rr*) and a brown-eyed fly (*rr*) is expected to produce ½ heterozygous red-eyed flies (*Rr*) and ½ brown-eyed flies (*rr*). What can you say about this investigation if you counted 55 red-eyed flies and 45 brown-eyed flies from this cross when you expected 50 of each (½ and ½)? To calculate the χ^2 statistic, create a contingency table of all the values that are needed to calculate the χ^2 statistic, where *O* and *E* represent observed values and expected values, respectively:

	O	E	$O-E$	$(O-E)^2$	$\frac{(O-E)^2}{E}$
Red-eyed flies	55	50	5	25	0.5
Brown-eyed flies	45	50	−5	25	0.5

$$\chi^2 = \Sigma\frac{(O-E)^2}{E} = 0.5 + 0.5 = 1.0$$

Next, you need to determine the degrees of freedom (*df*) of the data using the formula provided on the Equations and Formulas pages:

$$df = \text{number of } E \text{ values} - 1 = 2 - 1 = 1$$

To determine if the observed data values obtained in the fruit fly cross differ from the expected values by chance or for another reason, you compare your calculated value of χ^2 to the table of critical values in the chi-square table provided on the Equations and Formulas pages:

Chi-Square Table								
	Degrees of freedom (*df*)							
p	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.81	9.49	11.07	12.59	14.07	15.51
0.01	6.63	9.21	11.34	13.28	15.09	16.81	18.48	20.09

The table provides critical values for χ^2 for two values of probability, *p*, and at eight different degrees of freedom. A critical value of χ^2 at $p = 0.05$ indicates that 95% of the difference between observed and expected values can be explained by chance. Thus, for the fruit fly calculations, the calculated value of $\chi^2 = 1.0$, at $df = 1$ and $p = 0.05$, is *less* than the critical value. What do you do with this value?

- *Reject the null hypothesis.* The **null hypothesis** is that the observed values are not significantly different from the expected values; that is, the differences are the result of chance. If the calculated value for χ^2 is *more* than the critical value, you reject the null hypothesis.
- *Accept the null hypothesis.* If the calculated value for χ^2 is *less* than the critical value, you do not reject the null hypothesis. "Do not reject the null hypothesis" is not quite the same as saying you accept the null hypothesis because accepting the null hypothesis implies that you proved it to be true. However, proof doesn't happen in statistics (or science). Instead, you were not able to disprove it.

If your calculated value for χ^2 is greater than the critical value, and you rejected the null hypothesis, then something other than chance is influencing your results. Crossing over, deleterious mutations, or nonrandom mating (perhaps some traits produce poor breeders) are factors to consider. High values of χ^2 may also result if the expected values used do not account for sex linkage or are otherwise in error.

Investigation 1: Artificial Selection

In this investigation, you explore the effect of artificial selection. Artificial selection is selection by humans. We have used this kind of selection for thousands of years to breed animals for special purposes and to raise plants to yield larger or more flavorful fruits and vegetables. The investigation suggests using fast-growing plants to examine trichomes (plant hairs) on leaves, but it also encourages using other observable, heritable traits on these plants. Your teacher may substitute other organisms.

In this investigation, you gain experience observing biological phenomena and collecting and analyzing data. You learn firsthand how selection influences the genetic makeup of a population.

Part I: Artificial Selection of a Trait

1. Choose a trait upon which to carry out artificial selection. In order for selection to work—that is, to change the genetic makeup of populations of subsequent generations—the trait must be heritable. If available, examine Wisconsin Fast Plants. Consider trichome density, the color intensity of pigments in leaves, or plant height.
2. Select the plants with the trait of interest. If you choose trichome density, decide whether you want higher or lower density. Select 10% of the class population of plants (12–15 plants) that possess the trait.
3. Among the selected plants, measure the trait. For example, count and record a sample number of trichomes in a specific position on a specific leaf (same leaf from every plant).
4. Allow the selected plants to flower. Cross-pollinate the flowers and collect the seeds when the fruits mature.
5. Plant the seeds from the first generation to start the second generation.
6. When the second-generation plants are mature, score the trait a second time.
7. Compile the data and present them in a graph, such as a histogram.

Part II: Further Investigation of a Trait

Design an experiment to investigate a different trait or further investigate some aspect of the trait you selected for in part I. For example, if you investigated trichomes in part I, consider investigating what, if any, advantage trichomes contribute to the survival or reproductive success of the plant.

Investigation 2: Modeling Evolution

The goal of this investigation is to create a mathematical model of evolution. You enter data into a spreadsheet, make "what if" changes in the data, and observe the results. By doing this, you discover the variables that influence evolution and what kind of influence those variables have. Ultimately, you confirm that Hardy-Weinberg equilibrium describes a population that is *not* evolving; deviations from equilibrium indicate that the population *is* evolving.

Evolution is the change in allele frequencies in a population over time. If a population can be described by Hardy-Weinberg equilibrium, then evolution is not occurring. So that evolution does not occur, there must be:

1. no selection
2. no mutation
3. no gene flow (no migration)
4. large populations (so that genetic drift does not occur)
5. random mating

Part I: A Mathematical Model for Evolution

Depending upon whether you use the AP-provided investigation or another similar activity, the steps you follow will generally look like this:

Step 1: Choose frequencies for *p* and *q*. You begin the activity by choosing values for the frequencies, p and q, of two alleles, A and B. Since there are only two alleles for this gene, the values must sum to 1:

$$p + q = 1$$

Step 2: Mix gametes. An individual can contribute one of these two alleles to a gamete (egg or sperm), either the A allele or the B allele. A zygote, or fertilized egg, will have one of these alleles from each parent and will become AA, AB, BA, or BB. To document Hardy-Weinberg equilibrium, the mixing of the gametes must be random (selection is absent). If you are creating a spreadsheet in this part of the investigation, you can use the random number generator of the spreadsheet (RAND). If you choose $p = 0.6$ and $q = 0.4$, you essentially tell the spreadsheet to randomly assign to each gamete an A or a B allele, except that 60% of the time it needs to be an A and 40% of the time it needs to be a B.

Step 3: Determine frequencies of zygotes. If the union of gametes is a random event, then the chance that any two gametes come together depends upon how many of each gamete is present. The chance that two A alleles come together to form an AA zygote is $p \times p$, or p^2. For the AB and BA zygotes, the chance is $p \times q + q \times p$, or $2pq$. For the BB zygote, the chance is $q \times q$, or q^2. This reasoning results in the Hardy-Weinberg equation:

$$p^2 + 2pq + q^2 = 1$$

The equation is equal to 1 because the terms represent all (100%) of the possible combinations of alleles.

Step 4: Generate multiple generations. Using the allele frequencies of the new generation (the zygotes), repeat steps 2 and 3 for several new generations. The allele frequencies, p and q, for each generation may be slightly different from the previous generation because the mixing of alleles to form zygotes is random. You can reexamine your population with a new set of random combinations of alleles by telling your spreadsheet to recalculate the data. For this, it will select new random numbers.

Part II: Generate "What If" Scenarios

Use your mathematical model of Hardy-Weinberg to generate "what if" scenarios. When you first create your population in your spreadsheet, you will probably begin with a small population (perhaps $N = 16$) spread over

several generations. You can change various attributes of your model to see how it affects your population and how it relates to Hardy-Weinberg equilibrium. For example:

1. **Add additional generations.** If you stretch your population to 10 or more generations, you might find that one of the alleles disappears from that generation (its frequency becomes zero). If one allele disappears, the remaining allele is said to be "fixed." Once an allele is fixed in a population, it remains so until some evolutionary process (mutation or gene flow) reintroduces it.
2. **Increase the population size.** The fluctuations in gene frequencies that you see from generation to generation are examples of **genetic drift,** changes in gene frequencies as a result of chance. The effect of genetic drift decreases as population size increases, so increasing the size of the population should reduce its effect.
3. **Investigate the effect of natural selection.** You can simulate the effect of selection for or against one of the alleles. Selection can influence any of the stages during the life cycle of an individual but ultimately will determine whether that individual will contribute gametes to the next generation. One way to simulate selection is to manipulate the formula in your spreadsheet that determines whether an individual passes its alleles to the next generation. To simulate selection against *BB* individuals, for example, adjust the formula that counts the number of *BB* individuals that contribute gametes to the next generation. In the spreadsheet in Figure 12-2, if no selection occurs, then the number of *BB* individuals is calculated in cell G261 with the following formula:

   2*SUM(G5:G254) + SUM(F5:F254)

   If only 50% of the *BB* individuals survive, the formula becomes the following:

   0.5*2*SUM(G5:G254) + SUM(F5:F254)

   The formulas for all the cells are shown in Figure 12-3.
4. **Investigate the effect of mutation and gene flow.** You can simulate a mutation of *A* to *B* or *B* to *A* in ways similar to an investigation of natural selection, above.

By manipulating your population model, you should make the following conclusions:

1. The frequencies of alleles should remain unchanged over time unless one of the five mechanisms of evolution is active.
2. Small populations are more subject to genetic drift than larger populations.
3. The dominant or recessive characteristic of an allele does not influence its selective value. A dominant or recessive allele has selective value only if the phenotype it generates has selective value. Thus, a recessive trait may have a greater selective value than a dominant trait. Without selection, the allele frequencies remain unchanged, regardless of the dominant or recessive characteristic of the allele.
4. Selection can affect an individual at any stage during its life cycle: gamete, zygote, egg, larva, juvenile, or adult.

Ultimately, you should conclude that any of the five processes listed at the beginning of this investigation can influence the evolution of populations. But which of these has the greatest influence? Although each population is different and although evolution may progress differently in the same population at different times, studies of evolution in populations in the real word (in the "wild") generally demonstrate that natural selection is the strongest driving force for evolution.

	A	B	C	D	E	F	G	H		BN	BO	BP	BQ	BR
1		p =	0.60		Generation				p =	0.84		Generation		
2		q =	0.40		1				q =	0.16		10		
3		gametes		zygote	genotypes				gametes		zygote	genotypes		
4	N				AA	AB	BB					AA	AB	BB
5	1	A	A	AA	1	0	0		A	A	AA	1	0	0
6	2	A	B	AB	0	1	0		A	A	AA	1	0	0
7	3	B	B	BB	0	0	1		A	A	AA	1	0	0
8	4	A	A	AA	1	0	0		B	A	BA	0	1	0
251	247	B	B	BB	0	0	1		A	A	AA	1	0	0
252	248	B	A	BA	0	1	0		A	A	AA	1	0	0
253	249	B	B	BB	0	0	1		B	A	BA	0	1	0
254	250	A	A	AA	1	0	0		A	A	AA	1	0	0
255														
256		p =	q =		p2 =	2pq =	q 2=		p =	q =		p2 =	2pq =	q 2=
257		0.60	0.40		0.36	0.48	0.16		0.83	0.17		0.70	0.27	0.03
258		1.00			1.00				1.00			1.00		
259														
260		Next Generation			A		B		Next Generation			A		B
261		Allele Counts			300		161		Allele Counts			417		75
262		Allele Total				461			Allele Total				492	
263														
264		Next Generation			p		q		Next Generation			p		q
265		Allele Frequencies			0.65		0.35		Allele Frequencies			0.85		0.15
266		Frequency Total				1			Frequency Total				1	
267														
268														
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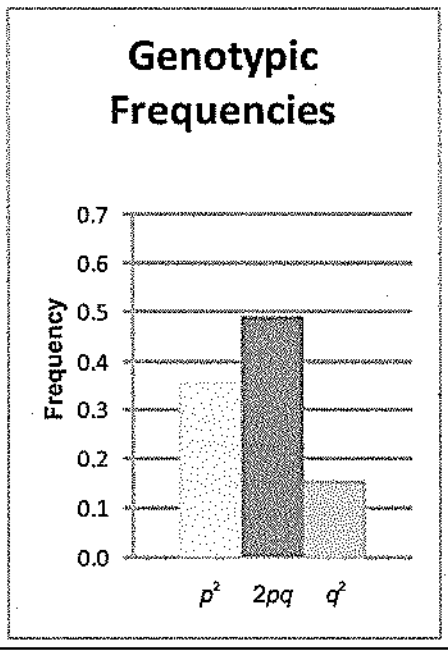
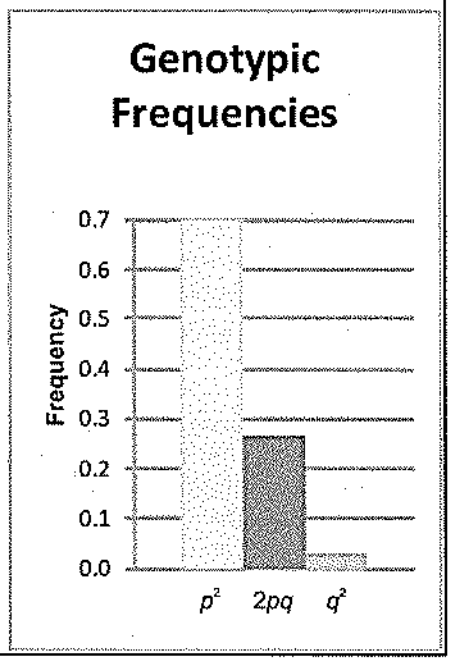




Mathematical Model for Evolution—10 Generations of 50% Selection Against BB Phenotype

Figure 12-2

	A	B	C	D	E	F	G
1		*p* =	0.60 (1)		Generation		
2		*q* =	=1-C1 (2)		1		
3		gametes		zygote	genotypes		
4	*N*				*AA*	*AB*	*BB*
5	1	=IF(RAND()<=C$1, "A","B")	=IF(RAND()<=C$1, "A","B")	=CONCATENATE (B5,C5)	=IF (D5="AA",1,0)	=IF(D5="AA",0, IF(D5="BB",0,1))	=IF (D5="BB",1,0)
6	2	=IF(RAND()<=C$1, "A","B") (3)	=IF(RAND()<=C$1, "A","B")	=CONCATENATE (4) (B6,C6)	(D6=(5)A",1,0)	=IF(D6=(6)A",0, IF(D6=(6)B",0,1))	(D6=(7)B",1,0)
7	3	=IF(RAND()<=C$1, "A","B")	=IF(RAND()<=C$1, "A","B")	=CONCATENATE (B7,C7)	=IF (D7="AA",1,0)	=IF(D7="AA",0, IF(D7="BB",0,1))	=IF (D7="BB",1,0)
252	248	=IF(RAND()<=C$1, "A","B")	=IF(RAND()<=C$1, "A","B")	=CONCATENATE (B252,C252)	=IF (D252="AA",1,0)	=IF(D252="AA",0, IF(D252="BB",0,1))	=IF(D252="BB",1,0)
253	249	=IF(RAND()<=C$1, "A","B")	=IF(RAND()<=C$1, "A","B")	=CONCATENATE (B253,C253)	=IF (D253="AA",1,0)	=IF(D253="AA",0, IF(D253="BB",0,1))	=IF (D253="BB",1,0)
254	250	=IF(RAND()<=C$1, "A","B")	=IF(RAND()<=C$1, "A","B")	=CONCATENATE (B254,C254)	=IF (D254="AA",1,0)	=IF(D254="AA",0, IF(D254="BB",0,1))	=IF (D254="BB",1,0)
255							
256		*p* =	*q* =		*p2* =	*2pq* =	*q* 2=
257		=COUNTIF (B5:C254,"A") /COUNTA (8) (B5:C254)	=COUNTIF (B5:C254,"B") (9) /COUNTA (B5:C254)		=COUNTIF (E5:E254,1) /COUNTA (E5:E254)	=COUNTIF (F5:F254,1) /COUNTA (F5:F254)	=COUNTIF (G5:G254,1) /COUNTA (G5:G254)
258		=B257+C257			=E257+F257+G257		
259							
260				Next Generation	(10) *A*	selection	*B*
261				Allele Counts	=2*SUM(E5:E254) +SUM(F5:F254)		= 0.5* (11) 2*SUM(G5:G254) +SUM(F5:F254)
262				Allele Total		(12) =E261+G261	
263							
264				Next Generation	*p*		*q*
265				Allele Frequencies	=E261/F262 (13)	(14)	=G261/F262
266				Frequency Total		=E265+G265	

	I	J	K	L	M	N
1	*p* =	=E265 (15)		Generation		
2	*q* =	=1-K2		2		

(1) Cell C1 - Enter a value for *p* here.

(2) Cell C2 - Calculates the value for *q*.

(3) Cells B5 & C5 - Enters "A" if a random number (between 0 and 1) is less than or equal to 0.6 (*p*); if greater than 0.6, "B" is entered. ("A" is entered 60% of the time and "B" is entered 40% of the time.)

(4) Cell D5 - Combines two alleles from gametes columns to make zygote genotype.

(5) Cell E5 - Enters 1 if zygote is "AA"; if not, enters 0.

(6) Cell F5 - Enters 1 if zygote is "AB" or "BA"; if not, enters 0.

(7) Cell G5 - Enters 1 if zygote is "BB"; if not, enters 0.

(8) Cell B257 - Counts number of A gametes in columns B & C and divides by total number of gametes.

(9) Cell C257 - Counts number of B gametes in columns B & C and divides by total number of gametes.

(10) Cell E261 - Enters total number of A alleles from AA and AB individuals.

(11) Cell G261- Enters total number of B alleles from BB and AB individuals. *Also, formula allocates 50% selection against BB individuals.*

(12) Cell F262 - Enters sum of A and B alleles.

(13) Cell E265 - Enters allele frequency for *p*.

(14) Cell G265 - Enters allele frequency for *q*.

(15) Cell J1 - Enters *p* from E265 into next generation columns.

Formulas for a Mathematical Model for Evolution—50% Selection Against BB Phenotype

Figure 12-3

Investigation 3: Comparing DNA Sequences

In this investigation, you use bioinformatics to establish evolutionary relationships. **Bioinformatics** is the computer analysis of biological information, such as DNA or protein sequences, for understanding biological processes.

Part I: Using BLAST to Find Evolutionary Relationships

You begin this investigation by observing an image of a fossil obtained from an excavation in China. Using features that you've detected in the image, you decide where on a provided cladogram the fossil organism should be positioned. A **cladogram** (or phylogenetic tree) is a "tree-like" graphical representation of the relatedness of species. Each branch of the phylogenetic tree represents the divergence of a species (or group of species) from a common ancestor. Each branch may also show the **shared derived character** or the trait that species along that branch share (Figure 12-4).

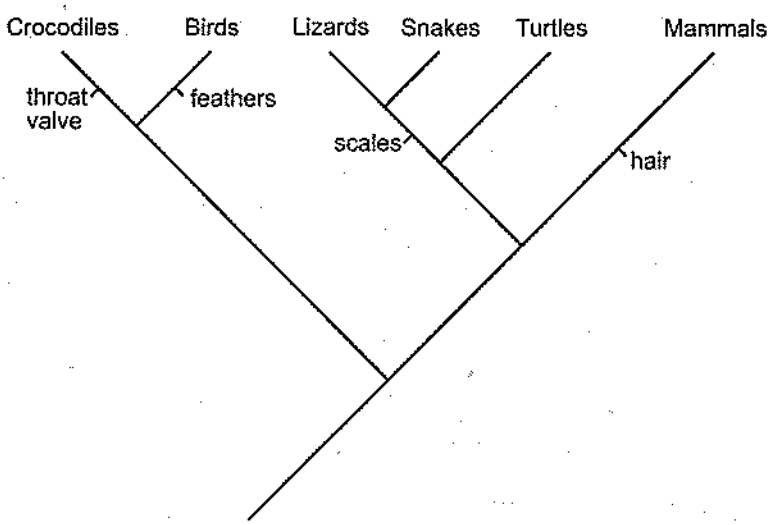
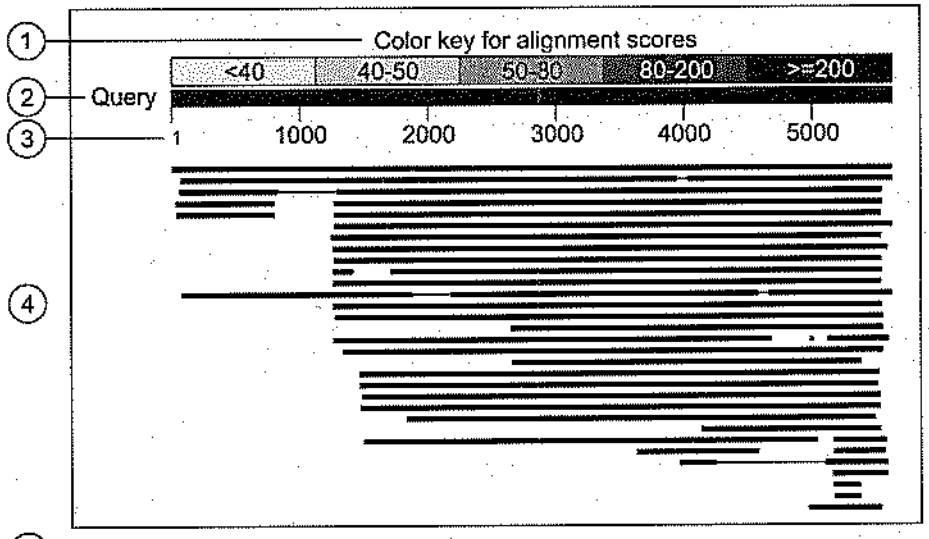


Figure 12-4

To confirm your placement of the fossil organism on the tree, you access BLAST, an online bioinformatics program, to analyze DNA obtained from tissue surviving in the fossil. You are provided with several gene sequences from the fossil (which you download from an AP Biology website) that you submit to BLAST. BLAST then compares your gene sequences with others in the database and provides a list of organisms with gene sequences that are most similar to the fossil sequence. A typical BLAST output appears in Figure 12-5.

The top section of a BLAST report provides a graphical summary of the results. Note the following information (the numbered items below correspond to the numeric callouts in Figure 12-5):

1. **Color Key.** The color key (shown in the figure as shades of gray) is a legend defining the color of each of the horizontal bars below. The bars represent alignment sequences of other organisms. The number on each color segment in the key provides an alignment score range; the higher the alignment score, the greater the similarity between the submitted sequence (from the fossil) and the sequence of another organism.
2. **Query Bar.** The bar below the color key represents the DNA sequence submitted for the fossil.
3. **Nucleotide Number.** The numbers below the query bar enumerate the nucleotides in the submitted sequence.
4. **Aligning Sequences.** Each bar in the group shown here represents a sequence in another organism that matches the submitted sequence. Match the color of the sequence to the color key to get an alignment score range for the sequence. Bars are arranged so that the best matches are at the top. The span of each bar can be compared to the submitted sequence in the query bar to determine the parts of the sequences that match and the parts that are absent (gaps).



5

Distance tree of results

Sequences producing significant alignments

			Score (Bits)	E Value	
ref |	NM 204790.1	| Gallus gallus collagen, type V, (COL...	1.029e+04	0.0	
ref |	XR 118465.1	| PREDICTED: Mmeleagris gallopavo coll...	3917	0.0	
ref |	XM 003757475.1	| PREDICTED: Sarcophilus harrisii c...	3487	0.0	
ref |	XM 001506246.2	| PREDICTED: Ornithorhynchus anatin...	3476	0.0	
ref |	XM 001372383.2	| PREDICTED: Monodelphis dommetica ...	3465	0.0	
ref |	XM 003794863.1	| PREDICTED: Otolemur garnettii col...	3227	0.0	
ref |	XR 131578.1	| PREDICTED: Equs caballus collagen al...	3221	0.0	
ref |	NM 000093.3	| Homo sapiens collagen, type V, alpha...	3177	0.0	
ref |	XM 003470915.1	| PREDICTED: Cavia porcellus collag...	3064	0.0	
ref |	XM 003222940.1	| PREDICTED: Anolis carolinensis collagen, ...	3022	0.0	
ref |	XM 537804.3	| PREDICTED: Canis lupus familiaris collage...	3020	0.0	
ref |	XR 054662.1	| PREDICTED: Taeniopygia guttata misc_RNA (...	3001	0.0	
ref |	NM 001014971.1	| Sus scrofa collagen, type V, alpha 1 (COL...	2992	0.0	
ref |	XM 002920704.1	| PREDICTED: Ailuropoda melanoleuca collage...	2976	0.0	
ref |	XM 0003312016	| PREDICTED: Meganin dadlonglegs nycusa...	2420	0.0	
ref |	XM 001118214.2	| PREDICTED: Macaca mulatta hypothetical LO...	2314	0.0	
ref |	XM 003449634.1	| PREDICTED: Oreochrois niloticus collagen ...	2279	0.0	
Ref |	XM 002935230.1	| PREDICTED: Xenopus (Silurana) tropicalis ...	2132	0.0	

6 7 8 9 10

ref |	XM 002833191.2	| PREDICTED: Pongo abelii collagen ...	717	0.0	
ref |	XR 123063.1	| PREDICTED: Nomascus leucogenys colla ...	481	4e-132	
ref |	XM 002724221.1	| PREDICTED: Oryctolagus cuniculus ...	431	4e-117	
ref |	XM 01118209.2	| PREDICTED: Macaca mulatta collagen ...	403	8e-109	
ref |	XM 002833217.1	| PREDICTED: Pongo abelii collagen ...	398	4e-107	
ref |	NM 134452.1	| Rattus norvegicus collagen, type V, ...	398	4e-107	
ref |	XR 109855.2	| PREDICTED: Homo sapiens hypothetical ...	390	6e-105	

☐ ref|NM 204790.1| Gallus gallus collagen, type V,alpha 1 (COL5A1), mRNA
gb|AF137273.1| Af137273 Gallus gallus alpha 1(V)collagen mRNA,complete cds
Length=5575

GENE ID: 395568 COL5A1 | collagen,typeV,alpha[Gallus gallus]
(10 or fewer PubMed links)

Score = 1.029e+04 bits (5571), Expect = 0.0 (11)
Identities = 5575/5575 (100%), Gaps = 0/5575 (0%)
Sttrand=Plus/Plus

```
Query 1     ATGGATACGCACACGCGATGGAAAAGGCGCAGTTGGATACGGAACTGGCAGCTGCACGTC 60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1     ATGCATACGCACACGCGATGGAAAAGGCGCAGTTGGATACGGAACTGGCAGCTGCACGTC 60

Query 61    GCGCTGGTGCTGCTGGGCGCCGCGGCCCTGGGCCGGGCAGCTGAACCTGCAGACCTCTTG 120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 61    GCGCTGGTGCTGCTGGGCGCCGCGGCCCTGGGCCGGGCAGCTGAACCTGCAGACCTCTTG 120
```

Figure 12-5

5. **Distance Tree.** You can click in this area to see a cladogram of the results (not shown here). The displayed cladogram shows the submitted sequence highlighted in yellow. Other sequences provide species names. A legend (at the far right) provides a common description for the species.

   More detailed information is provided in the second section. Here, each bar of the list of matching sequences is described in detail.

6. **References.** You can use a link in this column to get a common name for a species (e.g., *Gallus gallus* is a chicken), a description of the sequence (e.g., the sequence is associated with the mRNA that codes for the connective-tissue protein collagen), the species classification, the matching DNA sequence, the translated amino acid sequence, and published references.

7. **Species.** In this column, you are given the name of the species in which the sequence was found and the beginning of a description of the sequence. Click on the reference (1st column) to get the complete description.

8. **Alignment Score.** *The bigger the alignment score,* the greater the similarity to the submitted sequence for the fossil and the higher on the list. Clicking on this number will take you to a location (lower on this same page) that provides a comparison of the submitted and matching sequences, nucleotide by nucleotide.

9. **Expect Value (E Value).** The E value provides a numeric value for alignments that are expected by chance. If an alignment occurs by chance, then it is unlikely to indicate a biological relationship. *The smaller the E value,* the more likely the alignment is the result of common ancestry. Small E values use a notation where e represents the logarithmic base of 10. Thus, the expression "3e–08" represents 3×10^{-8}.

10. **Related Databases.** Clicking on the icons (U, E, G, M) in this column takes you to relevant information in other databases.

    In the bottom section of the BLAST report, the matching sequence from each of the other organisms is paired with the submitted sequence of the fossil, nucleotide by nucleotide.

11. **DNA Sequences.** The matching sequence and the submitted sequence are paired, nucleotide by nucleotide, for each species. A fraction and percentage are given for the nucleotides that are identical (e.g., 5575/5575, 100%) and for the gaps that occur where parts of the sequence are missing (e.g., 0/5575, 0%). The alignment score takes into account both of these values.

Once you've obtained a BLAST result for your sequence, use the alignment scores to support your placement of the fossil on your cladogram. Only consider sequences with E values smaller than 0.01% (0.0001 or 1e–04), as values larger than this suggest that the match occurs by chance and not from a biological relationship.

Part II: Additional Investigations Using BLAST

For the remainder of the lab, you explore a gene or protein of your choice using the BLAST resource.

Investigation 4: Diffusion and Osmosis

This investigation provides exercises that examine the movement of water across selectively permeable membranes. Refer to Chapter 3, "Cell Structure and Function," for a complete review of diffusion, osmosis, and plasmolysis.

In animal cells, the direction of osmosis, in or out of a cell, depends on the concentration of solutes inside and outside the plasma membrane. In plant cells, however, osmosis is also influenced by turgor pressure, the internal pressure of water exerted on the cell wall. To account for differences in both concentration and pressure, a more general term, **water potential,** is used to describe the tendency of water to move across a selectively permeable membrane. Water potential is the sum of the **pressure potential** (from any externally applied force) and the **solute potential** (osmotic potential):

$$\Psi = \Psi p + \Psi s$$

water potential = pressure potential + solute potential

Water potential has the following properties:

1. Water moves across a selectively permeable membrane from an area of *higher* water potential to an area of *lower* water potential.
2. Water potential can be positive or negative. Negative water potential is called **tension.**
3. In a living cell, water potential is always zero or negative.
4. Solute potential results from the presence of solutes and is always negative. A higher concentration of solutes generates a smaller (or more negative) solute potential.
5. Pressure potential is zero unless some force is applied, such as that applied by a cell wall.
6. Pure water at atmospheric pressure has a water potential of zero (pressure potential = 0 and solute potential = 0).
7. Water potential is measured in bars (1 bar is approximately equal to 1 atmosphere pressure) or megapascals (1MPa = 10 bars).
8. The solute potential can be calculated using the formula $\Psi_s = -iCRT$, where i is the ionization constant, C is the molar concentration, $R = 0.0831$ (pressure constant, in liter-bar/mole °K), and T is the temperature in Kelvin (273 + °C). The ionization constant is equal to the number of ions that a substance will produce in water: 2 for NaCl (two ions: Na^+ and Cl^-) and 1 for a substance that does not ionize (like sucrose).

   You do not need to memorize the solute potential formula or the value of R. They will be provided to you on the Equations and Formulas pages of the AP exam.

Think of water potential as potential energy, the ability to do work. The water at the top of a dam has a high water potential, a high potential energy, and a large capacity for doing work, such as the ability to generate electricity as it runs downhill.

Part I: Cell Surface Area

This part of the investigation examines the effect of surface area and cell size on the rate of diffusion. You cut up agar or gelatin that has been soaked in phenolphthalein into pieces of different sizes to model (represent) cells. Then you immerse the "cells" into a solution of NaOH, which turns phenolphthalein red. Cutting the "cells" in half reveals how far the NaOH has diffused.

For each size or shape "cell," the depth to which the NaOH (or red color) diffuses will be about the same for the same amount of time. But the smaller the "cell," the sooner the NaOH will reach its center. A calculation of the surface area-to-volume ratio (S/V) of the cells should reveal that "cells" with the larger S/V will have a larger portion of their volume penetrated by diffusion (turned red) of the NaOH in a fixed amount of time. Since the plasma membrane is the surface through which substances must pass (going in or out), a smaller cell, with a larger S/V, is more able to accommodate its metabolic need compared to a larger cell.

If a question on the AP exam asks you to design an experiment to investigate the effect of temperature or concentration on diffusion, you can apply the procedures of this investigation but at various temperatures or by using different concentrations of NaOH.

Part II: Diffusion and Osmosis

This part of the investigation examines the influence of solute type and concentration on diffusion and osmosis. Model cells are represented by bags made with dialysis tubing. The tubing limits the passage of solutes and models a selectively permeable membrane. It will allow small molecules to pass through, such as water and monosaccharides (glucose), but not disaccharides (sucrose), polysaccharides (starch), or proteins.

In this investigation, diffusion and osmosis are manipulated by two variables:

1. **Solute type.** Smaller molecules (water, NaCl, glucose) can diffuse across the model "plasma membrane" (dialysis tubing), while larger molecules (sucrose, proteins) cannot.
2. **Solute concentration.** The relative concentration of solutes inside and outside the bag determines the net flow of solutes or water across the membrane; the greater the solute concentration gradient, the faster the initial flow of solutes.

To investigate the influence of solute type and concentration, nine bags are prepared. Four bags are each filled with a different solute: sucrose, NaCl, glucose, and ovalbumin (a protein). These four bags are immersed in water. Another four bags are filled with water, and each is immersed in one of the four different solute solutions. A ninth bag, a control, is filled with water and immersed in water. The direction of water movement can be determined by weighing the "cells" before and after immersion.

There are several things to remember for this investigation:

1. Diffusion is *net* movement of substances from an area of higher concentration to an area of lower concentration.
2. Diffusion results from *random* movement of molecules.
3. Osmosis is the diffusion of *water* molecules across a *selectively permeable membrane.*

Also, keep in mind that the rate of diffusion (and osmosis) is influenced by these factors:

1. The initial rate of diffusion is faster when the concentration gradient is greater. But equilibrium is reached sooner when the concentration gradient is smaller. This is because the movement of a fewer number of solute molecules is necessary to establish equilibrium.
2. The rate of diffusion is faster when the temperatures of the solutions are greater. This is because molecules at higher temperatures have more kinetic energy and are moving faster.
3. The rate of diffusion is faster when solute weight is smaller. All molecules at the same temperature have the same average kinetic energy, but because kinetic energy equals $\frac{1}{2} \times \text{mass} \times \text{velocity}^2$, lighter molecules move faster.

By measuring the weights of the dialysis bags before and after immersion into the solutions, you are able to determine the direction of osmosis, the movement of *water* across a selectively permeable membrane. But how do you know if the *solutes* were able to pass across the membrane? Several qualitative tests can be used to determine the presence of the solutes inside and outside of the dialysis bags:

1. A commercial glucose testing tape can be used to test for the presence of glucose.
2. A Benedict's test can be used to test for the presence of glucose or fructose.
3. A biuret test can be used to test for the presence of proteins.
4. A flame test can be used to test for the presence of sodium.
5. Lugol's solution can be used to test for the presence of starch. Lugol's solution (iodine and potassium iodide, or IKI) is yellow-brown but turns dark blue in the presence of starch.

Part III: Observing Osmosis in a Living Cell

Cells contain water and various solutes. As a result, the cell has a negative solute potential, and pure water (water potential = 0) will enter the cell because water moves from higher to lower pressure potentials. In many plant cells, the water and solutes are stored in the central vacuole. As water enters the cell, the vacuole expands until the pressure, called **turgor pressure,** exerts a force on the cell wall. In a mature cell, the cell wall cannot stretch and expand. Thus, in a healthy cell, when the cell is fully expanded, the cell wall presses back with a pressure equal to and opposite of the turgor pressure.

In part II of this investigation, you determined the direction of osmosis across a dialysis bag, a cell *model*. This part of the investigation repeats the investigation for *real* cells.

First, you observe a plant cell, such as the aquatic plant *Elodea* or the moss *Mnium*. The most prominent features of a plant cell are the cell wall and the chloroplasts. You may also see the nucleus. The central vacuole will be apparent because of what you don't see: Much of the cell appears empty because it is occupied by the central vacuole, which contains colorless water and solutes.

Second, you use a microscope to observe the effect of osmosis on a plant cell as it responds to one of the four solutions used in part II (sucrose, NaCl, glucose, or ovalbumin). The solution you use creates one of three environmental treatments:

1. When the solution surrounding the cell is **hypertonic** (*higher* solute concentration) relative to the contents of the cell, the net movement of water is *out of the cell*, cell turgor decreases, and the plasma membrane collapses. This is called **plasmolysis** and can be identified when the cell, viewed under a microscope, appears to shrivel, leaving a gap between the cell contents and the cell wall.
2. When the solution surrounding the cell is **hypotonic** (*lower* solute concentration) relative to the contents of the cell, the net movement of water is *into the cell*. A healthy cell may not appear to change because the cell is already exerting its maximum turgor pressure. To observe a change, replace a hypertonic solution (when the cell is exhibiting plasmolysis) with a hypotonic solution to observe the expansion of the cell as turgor pressure is restored. Note, however, that the plasmolyzed cell is not equivalent to the healthy cell, as the plasmolyzed cell has a lower (more negative) solute potential as a result of having lost water (solutes are more concentrated).
3. When the solution surrounding the cell is **isotonic** (*equal* solute concentration) relative to the contents of the cell, there is *no net movement* of water into or out of the cell. Similar to the hypotonic solution above, no observable changes will occur in a healthy cell. But unlike the hypotonic solution, no change will also be observed if an isotonic solution is applied to a plasmolyzed cell. But again, be aware that the solution is isotonic with the plasmolyzed cell (not a healthy cell) and the plasmolyzed cell has a lower (more negative) solute potential (more concentrated solution in the cell) than a healthy cell.

Animal cells do not have a cell wall. As a result, immersing an animal cell in a hypotonic solution will cause the cell to expand. Unless the cell has a mechanism for removing excess water, the cell will continue to expand until it breaks apart, a process called **lysis.**

Third, you design an investigation to determine the concentration of a solution and the water potential of a plant cell. Although there are various alternative setups for this investigation, the most common one is to provide you with five sucrose solutions (0.2M, 0.4M, 0.6M, 0.8M, and 1.0M) that are labeled A, B, C, D, and E. You do not know which of the labeled solutions is which concentration. You are also provided with living cells (potatoes, sweet potatoes, or yams). One method you can use to determine the concentration of each unknown solution follows:

1. Cut, dry, and weigh five samples of living material.
2. Immerse each sample into a beaker containing one of the unknown solutions.
3. After 45 minutes, remove each sample, dry it, and weigh it.
4. Calculate the percentage change in the weight of each sample.
5. Plot your results (percent weight change vs. unknown solutions). Since a greater solute concentration in the solution will generate a greater change in potato weight, plotting the solutions on the x-axis in order of their increasing effect on the potato weight change will display the solutions in order of concentration.

From earlier parts of this investigation, you know that osmosis will occur if the water potentials on each side of the plasma membrane are unequal; the greater the difference in water potentials, the greater the net movement of water across the membrane. A cell, then, will gain (in a hypotonic solution) or lose (in a hypertonic solution) the greatest amount of water (and weight) when the difference in water potentials across the membrane is greatest. When the water potentials are equal (in an isotonic solution), there is no net movement of water, and the weight of the cell remains unchanged. Thus, you can identify the sucrose concentrations of the various solutions by the degree to which they result in a weight change. A graph of your data, with the solutions plotted such that they are arranged in increasing effect on the potato weight, will reveal the solutions in order of solute concentration.

When there is no change in the weight of a potato, the surrounding sucrose solution is isotonic relative to the potato, and the water potentials of the water and surrounding solutions are equal. The water potential (Ψ) of the sucrose solution is the sum of its solute potential (Ψ_s) and pressure potential (Ψ_p), but for a solution in an open beaker, the pressure potential = 0.

$$\Psi(\text{sucrose solution}) = \Psi_s + \Psi_p = \Psi_s(\text{isotonic solution}) + 0 = \Psi_s(\text{isotonic solution})$$

Now, the water potential of the sucrose solution and the cell can be calculated using $\Psi_s = -iCRT$.

$$\Psi(\text{potato cell}) = \Psi_s(\text{isotonic solution}) = -iCRT$$

Note that the pressure potential (Ψ_p) of the potato cell is *not* equal to zero due to the turgor pressure exerted by the contents of the water on the cell wall. Thus, the individual values of Ψ_s and Ψ_p of the potato cell remain unknown. Only the sum, Ψ, can be determined in this investigation.

In some alternative forms of this investigation, you may not be given any information about the sucrose concentrations of the four solutions. One way of dealing with this is to mix your own solutions, observe their effects, and compare them to the unknown solutions. Plotting the weight changes (vs. concentrations) for both your solutions and the unknown solutions together on the same graph will reveal approximations for the unknown concentrations.

Investigation 5: Photosynthesis

In this exercise, you investigate factors that influence the rate of photosynthesis. Photosynthesis can be summarized by the following reaction.

$$CO_2 + H_2O + \text{light} \rightarrow CH_2O + O_2$$

To determine the rate of this reaction, you can measure the disappearance of CO_2 or the accumulation of O_2. Both of these gases fill the air spaces among the spongy mesophyll of leaves. You must consider, however, that while photosynthesis is occurring in the leaf, some parts of the leaf may be generating energy through aerobic respiration. Thus, the gases that fill the air spaces are being altered by a concurrent process of respiration:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O + ATP$$

Measuring O_2 accumulation, then, is a measurement of *net* photosynthetic activity:

$$O_2 \text{ production} = \text{gross photosynthesis} - \text{respiration} = \text{net photosynthesis}$$

Part I: Determining the Rate of Photosynthesis

To measure the rate of net photosynthesis, you record the rate that submerged pieces of leaves rise in water as O_2 accumulates in their air spaces. For the treatment group, you submerge leaves in a bicarbonate solution (to supply CO_2). For the control group, you submerge leaves in a solution without bicarbonate. Here is a brief summary of the procedure:

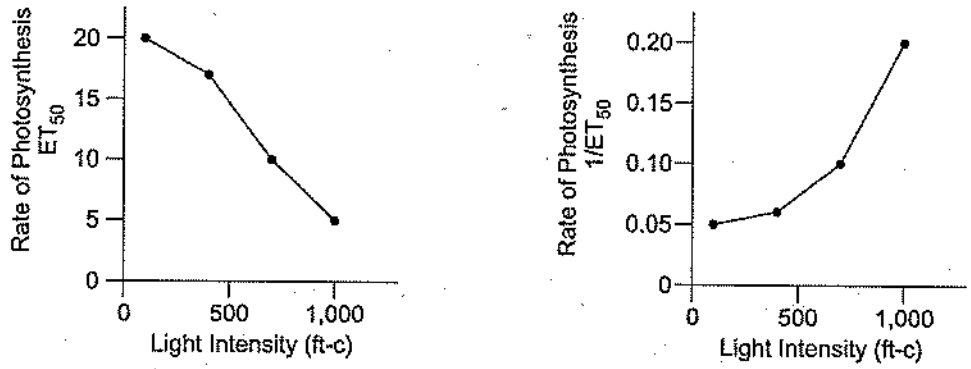
1. Cut disks of leaf tissue from leaves using a hole punch.
2. Fill one beaker with a bicarbonate solution. Fill a second beaker with a solution without bicarbonate. Add a drop of soap into each solution, as it will reduce the hydrophobic characteristic of the leaf's cuticle and will facilitate submerging the leaf disks.
3. Use a syringe to remove the gases that fill the air spaces in the spongy mesophyll. For each treatment, withdraw the plunger of a syringe, insert the cut leaf disks, and replace the plunger. For the treatment group, add bicarbonate solution (taken from the beaker) by dipping the syringe into the beaker and pulling on the syringe. Repeat for the control group, except use the solution without bicarbonate.
4. Submerge each group of disks into its respective beaker—treatment disks into a solution of bicarbonate and control disks into a solution without bicarbonate.
5. Expose both beakers to a light source and begin timing.
6. As photosynthesis progresses, oxygen will accumulate in the spongy mesophyll, increasing the buoyancy of the leaf disks. Record the time it takes each disk to reach the surface.
7. Summarize the data by calculating the median, the value at which half of the data is above and below. The median is a better statistic than the mean for this data because it reduces the impact of outliers. To obtain a *rate* for photosynthetic activity, calculate the time it takes for 50% of the disks to reach the surface $\left(\frac{\#\text{ of disks}}{\text{time for 50\%}}\right)$. This value, called the effective time (ET_{50}), has been shown to be a reliable number for photosynthetic rate for comparison across treatments.

Part II: Investigating Factors That Affect the Rate of Photosynthesis

Light intensity, light wavelength, temperature, and concentration of bicarbonate are environmental factors that affect photosynthetic rates. Biotic factors can also influence photosynthetic rates. Leaf characteristics vary among individual plants and even vary among leaves of a single plant. A leaf taken from a shady part of a plant may differ from a sun-exposed leaf by its density of stomata, density and length of leaf hairs, and thickness of cuticle. All these and other factors influence photosynthetic rates.

In this part of the investigation, you design an experiment to investigate a factor that may influence photosynthetic rates. Follow the procedure for part I, manipulating (giving different values to) the independent variable you investigate. For example, if you investigate light intensity, you manipulate the variable by assigning different values of light intensity.

After collecting the data, graph ET_{50} vs. the independent variable (the variable you are investigating). For some independent variables, such as light intensity, it makes sense to graph $1/ET_{50}$ vs. light intensity because such a graph rises as photosynthetic rate increases (Figure 12-6).



Photosynthetic Rate Expressed as ET_{50} and $1/ET_{50}$ as a Function of Light Intensity

Figure 12-6

Investigation 6: Cellular Respiration

This investigation provides a method for measuring the rate of cellular respiration. Cellular respiration is the breakdown of glucose with oxygen to produce carbon dioxide, water, and energy, as follows:

$$C_6H_{12}O_6 + O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{energy}$$

In this lab, respiratory rate is measured in seeds or other organisms by observing the changes in the volume of gas surrounding the organisms at various times. The rate of respiration can be determined by measuring the rate at which O_2 is consumed or the rate at which CO_2 is generated. In this investigation, O_2 consumption is measured, but since the volume of gas can be affected by both the consumption of O_2 and the production of CO_2, the CO_2 is removed by using potassium hydroxide (KOH). KOH reacts with CO_2 gas to produce solid K_2CO_3, as follows:

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

Since CO_2 gas is removed by its reaction with KOH, volume changes can be attributed only to the following:

1. Consumption of oxygen (due to respiration)
2. Changes in temperature
3. Changes in atmospheric pressure

Unless you are specifically investigating the effect of temperature or pressure on respiration, you must ensure that these two variables remain constant when comparing the results of experimental treatments.

Part I: Determining the Rate of Respiration

Either microrespirometers or gas pressure sensors can be used to measure O_2 generation. Here is a brief summary for using microrespirometers:

1. To assemble a microrespirometer, use a glue gun to attach a capillary tube to the needle end of a syringe. Remove the syringe plunger and insert cotton slightly moistened with a drop of KOH. Insert germinating seeds (or other living organisms) and reinsert the plunger. Attach a calibrated gauge (ruler or other graduated scale) to the capillary tube.
2. Assemble a second microrespirometer, but substitute baked seeds or glass beads for the germinating seeds. This microrespirometer is the control.
3. Place both microrespirometers (weighted down with washers) in a water bath to maintain a constant temperature.
4. Place a drop of a weak solution of soap and red food coloring to the open end of the capillary tubes. The soap reduces the adhesion of water to the glass tube, allowing it to flow freely, and the red food dye makes the water more visible. If the drop does not flow into the tube on its own, pull lightly on the plunger.
5. At regular time intervals, record changes in volume in the capillary tubes. As O_2 is consumed, the red drop should move downward toward the syringe, indicating a decrease in volume.
6. Changes in volume observed in the control microrespirometer represent responses to changes in temperature and pressure. Use these values to correct values observed in the treatment microrespirometer.
7. Graph the accumulated change in volume vs. elapsed time. The rate of respiration is the slope of the line of best fit through the plotted points.

Part II: Investigating Factors That Affect the Rate of Respiration

In this part of the investigation, you design an experiment to investigate a factor that may influence the rate of respiration. For environmental factors, consider temperature, light intensity, and light wavelength. For biotic factors, consider age or size of seed, or seeds of species that occupy different habitats. Also consider measuring respiratory rates for small insects, comparing, for example, the respiratory rates of solitary insects and pairs of same-sex and opposite-sex individuals.

Investigation 7: Mitosis and Meiosis

In this lab you explore the events that occur in cell division during mitosis and meiosis. You also investigate the influence of environmental effects on mitosis and examine the effects of mutations and cancer on cell division. As part of the review for this lab, you should read Chapter 7, "Cell Cycle." In particular, review the activities that occur during mitosis, meiosis, and cytokinesis, the cell structures involved in cell division, and the similarities of and differences between plant and animal cell division.

Part I: Modeling Mitosis

In this part of the investigation, you use clay, pipe cleaners, socks, or beads to represent chromosomes. You display the various stages of the cell cycle using these model chromosomes.

Part II: Evaluating an Environmental Effect on Mitosis

Various chemicals in the environment have the potential to influence mitotic activity. **Lectins,** a class of proteins that bind specifically to carbohydrate molecules, occur naturally throughout nature and serve various functions. In animals, they serve as cell-membrane receptors or facilitate cell adhesion or immune functions. In plants, their functions include defensive proteins that disrupt the digestion of predators that eat them. In addition, certain soil fungi secrete lectins that act as **mitogens** (substances that induce mitosis), influencing mitotic activity in the growing root tips of plants.

In this part of the investigation, you explore the effect of lectins on the roots of onions. The lectins are extracted from kidney beans and are used to simulate the effect of fungal lectins. You are provided with two kinds of onion roots—roots that have been treated with lectins and roots that have not been treated. You follow the following steps:

1. Prepare microscope slides of treated root tips (treatment group) and untreated root tips (control group).
2. Under the microscope, count the number of cells undergoing mitosis (all stages) and the number of cells not actively dividing (cells in interphase) for both treated and untreated root tips.
3. Calculate the percentages of cells undergoing mitosis and cells in interphase for both treated and untreated root tips. Use a chi-square (χ^2) statistical analysis to evaluate whether the difference between the treated root tips (the observed data) and the untreated root tips (the expected data) is statistically significant.

Part III: Cancer and the Cell Cycle

The cell cycle is tightly controlled by checkpoints and other mechanisms that evaluate cell status to ensure that each stage of the cell cycle is complete before advancing to the next stages. When DNA is damaged from replication errors or from radiation, chemicals, or other environmental effects, the mechanisms that monitor cell cycle status may fail. Cancer, characterized by uncontrolled cell division, may result.

This part of the investigation involves a discussion of two kinds of cancer:

1. **HeLa cells.** HeLa cells are cancer cells taken from Henrietta Lacks, an African-American woman, who died of cervical cancer in 1951. Although normal cells will divide only up to 50 times in culture, HeLa cells have been dividing continuously in culture in labs all over the world since they were removed from Lacks. Even for cancer cells, the tenacity of HeLa cells is exceptional. As a result, these "immortal" cells have been used for research and have contributed greatly to our understanding and treatment of disease. The cells were commercialized, and the original cells are freely distributed to researchers.
2. **Philadelphia chromosome.** Philadelphia chromosome is a chromosome translocation in which a segment of chromosome 9 has exchanged with a segment of chromosome 22. A karyotype of the condition displays a chromosome 9 that is longer and a chromosome 22 that is shorter than their respective normal chromosomes. The result of the translocation is the formulation of a gene on the chromosome 22 that codes for a protein that accelerates cell division. Various kinds of leukemia (cancers of the blood or bone marrow) result. However, a drug that limits the effect of this protein has been identified.

The AP exam may ask you to discuss the controversial issues associated with scientific research and its application to society. For example, the HeLa cells were taken from Lacks without her permission and used for research without her knowledge or compensation. In addition, medical research leading to the discovery of drugs to treat genetic disorders or illnesses requires clinical studies using human patients. In some cases, these patients may suffer from the side effects of the experimental treatment, while others, in the control group, may suffer because they do not receive an effective new treatment. Although there may not be a right or wrong answer for these issues, you should be prepared to discuss (by giving examples) the privacy, ethical, social, and legal issues that result from the application of scientific and medical research to society.

Part IV: Modeling Meiosis

Like part I of this investigation, you use clay, pipe cleaners, socks, or beads to represent chromosomes. In this part, however, you display the various stages of meiosis.

Part V: Crossing Over in Meiosis

Crossing over is the exchange of DNA between homologous chromosomes during meiosis. The process contributes to genetic recombination and genetic variation among offspring.

The results of crossing over during meiosis can be readily visualized under the microscope in the **asci** of the fungus *Sordaria*. Fungi grow as filaments called hyphae. Specialized hyphae, called asci, are reproductive filaments that contain eight haploid ascospores. The asci are embedded in other hyphae that form a fruiting body. One kind of fruiting body, a **perithecium**, surrounds the asci, except for a passageway that allows for the escape of the ascospores.

Sexual reproduction begins when hyphae from two strains fuse. Nuclei from one strain pair with nuclei from the second strain. Subsequently, these pairs of unlike nuclei fuse to produce diploid nuclei, which then undergo *meiosis*. During meiosis I, homologous chromosomes pair and separate. During meiosis II, each chromosome separates into two chromatids. At the end of meiosis, there are four daughter cells, each possessing a single chromatid originating from one of the four chromatids that made up each pair of homologous chromosomes. Each of the four daughter cells then divides by *mitosis* to produce two ascospores. The resulting order of the eight ascospores in the ascus corresponds to the alignment of chromatids during meiosis. If no crossing over occurs, then each set of four adjacent ascospores represents a single parent strain and will possess the same traits. This is illustrated in the center of Figure 12-7 for a trait that determines spore color.

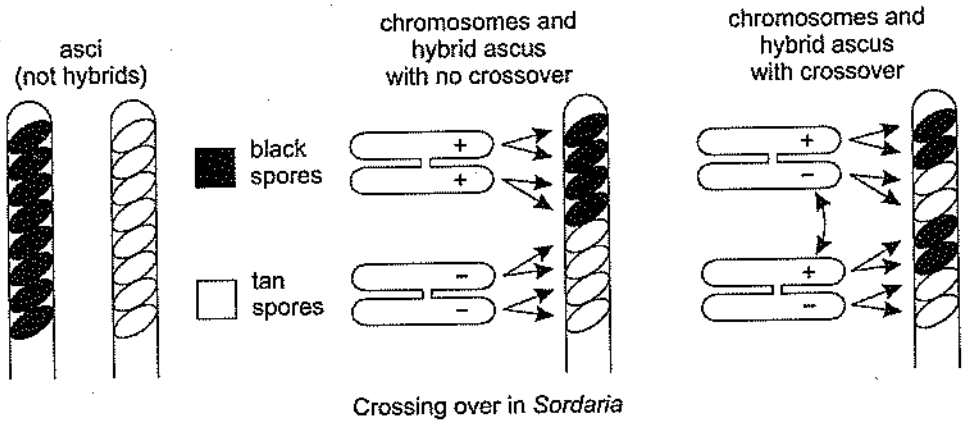


Figure 12-7

If crossing over occurs (Figure 12-7, right), then traits on two nonsister chromatids exchange, producing alternating patterns of ascospore pairs with and without the traits included in the crossover.

In this part of the investigation, you observe asci under the microscope to determine crossover frequency. Here is a summary of the steps:

1. Grow two fungal strains of the same species, each bearing ascospores of a different color, on a plate of nutrient-enriched agar. Perithecia form at the interface between the two strains and exhibit hybrid asci (spores from both strains).
2. Under the microscope, count the number of hybrid asci that contain ascospores with crossovers and the number of asci that contain ascospores without crossovers.
3. To determine crossover frequency, first divide the number of asci with crossovers by the total hybrid asci observed (asci with and without crossovers); then divide this number by 2 and convert to a percent by multiplying by 100.

Investigation 8: Bacterial Transformation

Transformation is the uptake of external DNA by cells. In this investigation, you will direct transformation by facilitating the transfer of a bacterial plasmid into *E. coli* bacteria.

Plasmids are common vectors (or carriers) for transferring DNA into bacteria. This investigation uses the plasmid pAMP, a plasmid that contains a gene that provides resistance to the antibiotic ampicillin. As a result, bacteria that have successfully absorbed the plasmid possess resistance to the antibiotic and can be separated from other bacteria by ampicillin treatment. Only those bacteria that have the pAMP plasmid survive the ampicillin treatment. In addition, you may use plasmids that also have a colored-marker gene, such as green fluorescent protein (GFP), that allows you to observe the growth of transformed cells.

Many bacteria take up extracellular DNA readily and, thus, transform naturally. Others can be induced to absorb DNA only under specific laboratory conditions. In either case, cells that are able to absorb DNA are designated **competent** cells.

Part I: Transferring a Plasmid into a Bacterium

In the first part of this lab, the bacterial plasmid, pAMP, is transferred to *E. coli* bacteria. The following steps summarize the procedure:

1. Add $CaCl_2$ to two tubes. $CaCl_2$ induces competence in the bacteria.
2. Add *E. coli* bacteria to both tubes. One tube will be transformed with pAMP plasmids. The second will be a control.
3. Transfer pAMP plasmids to one of the test tubes.
4. Facilitate the absorption of DNA (transformation) by giving the bacteria a heat shock (short pulse of heat).
5. Incubate both tubes overnight to allow for the growth of bacterial colonies. Then, transfer bacteria from one of the tubes to an agar plate with ampicillin and to an agar plate without ampicillin. Repeat for the second tube.
6. Record your results in a table (Table 12-2).
7. Interpret you results: Only bacteria transformed with pAMP plasmids can grow in the presence of ampicillin and form colonies. The control tube of bacteria confirms that without pAMP, growth cannot occur in the presence of ampicillin. It also confirms that the untransformed *E. coli* are not ampicillin resistant.

Table 12-2

	Agar Plate without Ampicillin	Agar Plate with Ampicillin
Tube 1: Bacteria with pAMP plasmids	Growth	Growth
Tube 2: Bacteria without pAMP plasmids (control)	Growth	No growth

Part II: Determine Transformation Efficiency

The transformation efficiency is a quantitative measurement of the effectiveness of the DNA transfer procedure. It is defined as follows:

$$\text{Transformation efficiency} = \frac{\text{Number of colonies}}{\text{Amount of DNA transferred } (\mu g)}$$

Each colony of bacteria on a plate is a population that began with the reproduction of a single bacterium. So the number of colonies is equivalent to the number of cells successfully transferred (transformants).

To calculate the amount of DNA transferred, you need to know each of the values listed below. Values for common protocols are provided as an example.

From protocol:	Concentration of DNA plasmid solution used = 0.005 μg/μl
From protocol:	Amount of DNA plasmid solution transferred to each tube = 10 μl
Calculate:	Amount of DNA transferred to each tube = 10 μl × 0.005 μg/μl = 0.05 μg
From protocol:	Volume of transformation suspension spread from tube to agar plate = 100 μl
From protocol:	Volume of transformation suspension in tube (before transfer to plate) = $CaCl_2$ solution + plasmid solution + nutrient broth = 250 μl + 10 μl + 250 μl = 510 μl
Calculate:	Fraction of suspension transferred from tube to plate $= \frac{100\ \mu g}{510\ \mu g} = 0.196$
Calculate:	Amount of DNA transferred from tube to plate = 0.196 × 0.05 μg = 0.01 μg

If there were 22 bacteria colonies on the agar plate, then

Calculate:	$\text{Transformation efficiency} = \frac{\#\text{ of colonies}}{\text{amount of DNA transferred}}$ $= \frac{22\ \text{transformants}}{0.01\ \mu g}$ $= 2.200\ \text{transformants}/\mu g$ $= 2.2 \times 10^3\ \text{transformants}/\mu g$

Part III: Design Your Own Investigation

Design an experiment to investigate any questions that may have occurred to you while doing parts I and II. Consider the effects of mutations from UV light or chemicals (caffeine, theobromine, food preservatives, etc.) or changes in the transformation protocol. If available, consider the use of other plasmids, such as those that produce fluorescent markers (e.g., green fluorescent protein, GFP).

Investigation 9: Restriction Enzyme Analysis of DNA

Part I: Review Restriction Enzymes

Restriction endonucleases (restriction enzymes) are used to cut up DNA. A restriction enzyme cleaves a DNA molecule at a specific sequence of nucleotides, producing cuts that are usually jagged, with one strand of the DNA molecule extending beyond the second strand. These jagged ends, or "sticky ends," allow DNA fragments with complementary jagged ends to fit back together. This technique is important for inserting genes (DNA fragments) into bacterial plasmids. When a plasmid and a foreign source of DNA (with a gene of interest) are treated with the same restriction enzyme and mixed together, the sticky ends of the foreign DNA will match the sticky ends of the plasmid DNA. The foreign DNA fragment can then be bonded to the plasmid DNA by treatment with DNA ligase. The recombinant plasmid can then be used to introduce the foreign DNA into a cell.

Part II: DNA Mapping

When DNA is collected at a crime scene, its analysis requires that it be cut up into pieces and then separated. The pieces are arranged by size to form a DNA chart or map. Because the DNA of every individual (except identical twins) is different, the map of DNA pieces from the crime scene can be compared to other DNA in an effort to determine its origin. DNA maps are "DNA fingerprints" that identify the individual from whom they came. They can help recognize mutations, identify species, produce family trees, or establish evolutionary relationships.

Part III: Separating DNA Fragments

In this part of the investigation, DNA fragments (restriction fragment length polymorphisms, or **RFLPs**) are separated using **gel electrophoresis.** The steps are summarized here:

1. To prepare a gel, a liquefied porous material (usually agarose) is poured into a tray fitted with combs, a device that allows the formation of sample wells (holes). After the gel cools and solidifies, the combs are removed to expose the wells.
2. The DNA to be analyzed is digested (cleaved) with a restriction enzyme. (This already may have been done for you.) A tracking dye is added to the sample. This allows the leading edge of DNA migration to be observed.
3. Load the DNA sample into a well in the gel in the tray of the electrophoresis apparatus. Other samples can be loaded in additional wells, including a sample of DNA of known fragment sizes to be used as a comparison standard.
4. Begin electrophoresis. The electrophoresis apparatus applies a voltage to opposite ends of the gel. DNA carries an overall negative charge due to its phosphate groups, and, as a result, fragments migrate from the negative to the positive electrode. Turn off the apparatus when the tracking dye nears the end of the gel.
5. Immerse the gel in methylene blue, a dye that allows the fragments to be observed.
6. Record the distance each fragment has migrated from the well. The migration distance for a fragment is inversely proportional to the $\log_{10}$ of its molecular weight—longer, heavier fragments move more slowly and travel shorter distances than smaller fragments. The number of base pairs (bp length) in a fragment can be substituted for its molecular weight.
7. Using semi-log graph paper, prepare a standard curve using the observed migration distances and known fragment sizes for the standard sample. The standard curve is a plot of fragment size (base pairs) against migration distance. Since migration distance is inversely proportional to fragment size, plotting migration distance against the log of the number of base pairs produces a straight line.
8. Use the standard curve to determine the size of each fragment produced by the sample under investigation.

Investigation 10: Energy Dynamics

The primary productivity of a community is a measure of the amount of biomass produced by autotrophs through photosynthesis (or chemosynthesis) per unit of time. Primary productivity can be determined by measuring the rate at which CO_2 is consumed, O_2 is produced, or biomass is stored. In this investigation, primary productivity is determined by the amount of biomass produced by plants.

Primary productivity can be examined with respect to the following factors. (Note that the term **rate** means *per unit time.*)

1. **Gross primary productivity** is the rate at which producers acquire chemical energy through photosynthesis before any of this energy is used for metabolism.
2. **Net primary productivity (NPP)** is the rate at which producers acquire chemical energy through photosynthesis less the rate at which they consume energy through respiration.
3. **Respiratory rate** is the rate at which energy is consumed through respiration.

Part I: Estimating Net Primary Productivity

Net primary productivity for Wisconsin Fast Plants is evaluated at various intervals by weighing them. This provides the mass of a live plant, which contains a considerable amount of water. Dry mass can be determined by weighing a group of live plants, then drying them in an oven, and weighing the dry matter to determine the percent of dry matter in the live plants. Chemical energy can be determined by multiplying biomass by 4.35 kcal/g. The net *rate* of chemical energy gain can be determined by calculating the change in energy per day.

Part II: Estimating Energy Flow between Producers and Consumers

Energy flow through a system is evaluated by allowing larvae of cabbage white butterflies to feed on Brussels sprouts, a close relative to Wisconsin Fast Plants (both are in the genus *Brassica*). To follow energy flow, make regular measurements of Brussels sprouts and larval masses. Dry larval masses can be approximated by multiplying live mass by 40%, but a more accurate conversion can be determined by sacrificing a group of larvae, drying them, and weighing them. Dry larval mass can be converted to kcal by multiplying the mass by 5.5 kcal/g.

To follow the energy flow, account for mass changes in the Brussels sprouts and butterfly larvae and in the mass of fecal matter (frass) produced by the larvae.

Part III: Investigating Additional Energy Flow Questions

The final exercise in this investigation is to design your own experiment. Consider the relationship between the survival and reproductive strategies of a plant and how it allocates energy to plant defenses or to various organs (root, shoot, and leaf growth and reproductive organs). Also consider how biomass varies as a function of wet mass and how that might influence energy flow to herbivores.

Investigation 11: Transpiration

Transpiration is the evaporation of water from plants. Differences in water potential move water from the soil to the leaves. From leaves (and, less so, other plant parts), water evaporates mostly through the stomata.

Review these properties of water potential:

1. Water moves across a selectively permeable membrane from an area of *higher* water potential to an area of *lower* water potential.
2. Water potential can be positive or negative. Negative water potential is called **tension.**
3. In a living cell, water potential is always zero or negative.
4. Solute potential (osmotic potential) results from the presence of solutes and is always negative. A higher concentration of solutes generates a more negative solute potential.
5. Pressure potential is zero unless some force is applied, such as that applied by a cell wall.
6. Pure water at atmospheric pressure has a water potential of zero (pressure potential = 0 and solute potential = 0).

The dominant mechanisms for the movement of water through a plant are transpiration, adhesion, cohesion, and tension (TACT). These and other contributing factors are described below.

1. **Osmosis.** Water enters root cells by osmosis because the water potential is higher outside the root in the surrounding soil than inside the root. Dissolved minerals contribute to a lower water potential inside the root by decreasing the solute potential.
2. **Root pressure.** As water enters the xylem cells (plants cells that transport water), the increase in solute potential produces root pressure. Root pressure, however, causes water to move only a short distance up the stem.
3. **Transpiration.** In leaves, water moves from mesophyll cells (the principal plant cells in a leaf) to intercellular air spaces and then out the stomata. This occurs because the water potential is highest in the mesophyll cells and lowest in the relatively dry air outside the leaf. Evaporation of water from plant surfaces is called transpiration.
4. **Adhesion-cohesion-tension.** Because water is a polar molecule, it forms weak hydrogen bonds with other water molecules and other polar molecules. As water moves up through a plant to the leaves, the adhesion (attraction of unlike molecules) of water molecules to cell walls opposes the downward pull of gravity. Also, cohesion (attraction of like molecules) between water molecules makes water act as a continuous polymer from root to leaf. As transpiration removes molecules of water from the leaves, water molecules are pulled up from the roots. The transpirational pull of water through the xylem decreases the pressure potential in the xylem, resulting in negative water potential, or tension. The cohesion-tension condition produced by transpiration is the dominant mechanism for the movement of water up a stem.

On very hot or dry days, the loss of water by transpiration may exceed the rate by which water enters the roots. Under these conditions, the stomata may close to prevent wilting.

Part I: Density of Stomata

In the first part of this lab, you investigate the relationship between the density of stomata and habitat. To calculate the density, you divide the average number of stomata by the average surface area of a leaf. To count the stomata, you prepare a stomatal peel and examine it under a microscope.

Although stomatal density varies among species, it also varies among plants of the same species and among leaves of a single plant. You should consider a variety of factors that may be responsible, many of which are described here:

1. **Temperature.** When the temperature of liquid water rises, the kinetic energy of the water molecules increases. As a result, the rate at which liquid water is converted to water vapor increases; thus, the warmer

the environment, the greater the potential for transpiration. Plants that live in hot environments must have mechanisms to conserve water, and a lower density of stomata may be a solution.

2. **Humidity.** A decrease in humidity decreases the water potential in the surrounding air. In response, the rate of transpiration increases. Dry habitats often accompany hot environments. Plants that live in dry habitats must have mechanisms that conserve water loss, and a lower density of stomata may be a solution. However, not all dry habitats experience hot or even warm temperatures. Alpine habitats and other habitats with prolonged snow coverage are dry habitats except during the short period of the year when the snow is melting. (Plants can't absorb water in the form of snow or ice.) In contrast, plants that live in moist habitats, where water is readily available, can easily remediate water loss from transpiration.
3. **Air movement.** Moving air removes recently evaporated water away from the leaf. As a result, the humidity and the water potential in the air around the leaf drop, and the rate of transpiration increases.
4. **Light intensity.** When light is absorbed by the leaf, some of the light energy is converted to heat. Transpiration rate increases with temperature. Leaves of plants growing in bright sunlight sustain higher temperatures than plants growing in shade, and such exposure may influence stomatal density. However, somewhat countering the increase in temperature is the cooling effect caused by heat removal through transpiration.
5. **Leaf size.** Because larger leaves have a greater surface area than smaller leaves, there is a greater potential for heating and transpiration. Plants with larger leaves often grow in shade where they balance surface area and the need for light against the need to minimize transpiration. Stomatal density may also be affected.
6. **Leaf orientation.** A horizontally oriented leaf maximizes surface area for the capture of sunlight but can also introduce heat stress. Some plants adapted to hot environments remediate this problem by orienting leaves vertically to minimize surface area exposure to the sun. In these leaves, you may find stomata abundant on both sides of the leaf.
7. **Leaf hairs.** By removing moisture from the leaf, air movement increases the moisture gradient across leaf cells and transpiration increases. Hairs on the surface of leaves slow air movement, reducing transpiration.
8. **Stomatal structure.** Some plant species have leaves with stomata located below the leaf surface in depressions. This reduces air movement across the stomatal opening and slows transpiration.
9. **Leaf color.** White or light-colored leaves reflect sunlight and can reduce leaf heating.
10. **Cuticle thickness.** The cuticle is a waxy coating on the surface of leaves that reduces water loss. A thick cuticle in many plants is an adaptation to hot or dry habitats. You will need to examine a cross section of the leaf to evaluate cuticle thickness, as the stomatal peel you prepare only allows you to examine the surface.

Part II: Factors That Influence Transpiration Rate

In this part of the investigation, you design an experiment to investigate the effect of an environmental factor on transpiration rate. In brief, the procedure is as follows:

1. **Choose an environmental factor to investigate.** Many of the factors above, such as temperature, humidity, light intensity, and air movement, are appropriate environmental factors for this investigation because they can easily be manipulated (varied over a range of different values).
2. **Set up apparatus.**
   - To measure transpiration rate using a single cutting of a plant, assemble a potometer by inserting a calibrated pipette into one end of a flexible plastic tube. Bend the flexible tube into a U shape so that the open ends of the pipette and tube are pointing up, and mount the tube and pipette on a ring stand. If available, you can substitute a gas pressure sensor (connected to a computer) for the pipette. Fill the tube and pipette completely with water. Cut the stem of a plant seedling under water, and quickly insert the seedling into the open end of the tube. Cutting the stem under water reduces the chance of air entering the xylem. An air bubble in the xylem (or in the plastic tube) will expand under the low pressure generated by transpiration (a process called **cavitation**) and break the continuity of the water column. Apply petroleum jelly to seal the space between the stem of the seedling and the tube.

- If equipment for a potometer is not available, you can measure the transpiration of an entire plant. Enclose the root ball in a plastic bag and remove all the flowers (as they are likely to fall off). Most weight change over a period of several days is the result of transpiration.
- Repeat the above process for each environmental condition to be investigated. Alternatively, separate groups of students can each investigate a different environmental variable.
- Prepare one additional potometer for normal conditions, a control to which the transpiration rate for the applied environmental condition can be compared.

3. **Collect data.** Record the change in water level in the pipette over several intervals of time. If you are using gas pressure sensors, the associated computer software will generate a transpiration rate. If you are using an entire plant, weigh the plant at various intervals over a period of several days while maintaining constant environmental conditions. After recording this data, you will need to remove the leaves to determine their total surface area because the number of leaves and the sizes of leaves will influence the amount of transpiration.
4. **Calculate transpiration rate.** Calculate water loss per surface area per time (e.g., ml/cm^2/min) by dividing water loss by the total surface area and by the time recorded for the intervals. If using gas pressure sensors, divide the computer-generated transpiration rate (kPa/min) by the leaf surface area to obtain kPa/cm^2/min.
5. **Analyze data.** Graph your data together with the data of other students (if available) who have investigated the effect of other environmental variables. One way to prepare the graph is to plot accumulated water loss as a function of time. Once you have a visual display of the effects of different environmental variables, explain the results, applying what you know about how plant habitat, stomatal density, and other plant adaptations influence transpiration rate.

Investigation 12: Animal Behavior

In this lab, you investigate the behavior of fruit flies (*Drosophila melanogaster*) in response to various environmental stimuli. Alternative subjects for observation include pill bugs (*Armadillidium,* terrestrial crustaceans) and brine shrimp (*Artemia,* aquatic crustaceans).

Before working with fruit flies, you should familiarize yourself with some important fruit fly characteristics:

1. **Life cycle.** Depending upon temperature, *D. melanogaster* requires 10 to 14 days to complete the stages from egg to adult.
   - **Eggs** hatch into maggot-like larvae after about 1 day.
   - **Larvae** undergo three growing stages, or instars, over a 4- to 7-day period. They molt (shed their skins) after the first two stages.
   - **Pupae** form after the third larval instar. A hardened outer case (cocoon) forms around the larva. Inside, a larva undergoes metamorphosis and emerges as an adult fly after 5 to 6 days.
   - **Adults** may live for several weeks. Females may begin mating 10 hours after they emerge from the pupa.
2. **Sex of fruit flies.** To properly identify behavior that may be sex-dependent, you must be able to distinguish the males from the females. The following *typical* (but variable) characteristics are used:
   - A female is larger than a male and has four to six solid dark stripes across the dorsal side (top) of her abdomen. The posterior end of the abdomen is somewhat pointed.
   - A male is smaller than a female and has fewer (two to three) stripes on his abdomen. The posterior end of the abdomen is rounded and heavily pigmented (as if two or three stripes have fused). A male also has a small bundle of black hairs, or sex combs, on the uppermost joint of his front legs.
3. **Virgin females.** If your class conducts genetic experiments with the fruit flies as a follow-up to this investigation, you should be aware that after mating, female flies store male sperm to fertilize their eggs. To ensure that the female does not use sperm from a mating that occurred before the investigation begins, only virgin, or unmated, females can be used. Since a female does not mate until 10 hours after emerging from the pupa, isolating the female soon after emergence will ensure a virgin fly.
4. **Fly mutations.** Your investigation of fly behavior will probably involve wild-type flies. However, you may want to investigate behavior of flies with genetic mutations. Common mutations include eye color variations, wing deformities, and antennae irregularities.

Part I: Fruit Fly Response to Light and Gravity

You begin this investigation by observing the behavior of fruit flies that you have put in a vial. Consider the following behaviors:

1. **Kinesis** is an *undirected* movement in response to a stimulus. The response is a change in the speed of an animal's movement, often movement from a stationary posture. Insects' scurrying about after lifting a rock is an example of kinesis.
2. **Taxis** is a *directed* movement toward (*positive* taxis) or away (*negative* taxis) from a stimulus.
   - **Phototaxis** is a response to light. Positive phototaxis is movement toward light, and negative phototaxis is movement away from light.
   - **Geotaxis** is a response to gravity and may be positive or negative.

Part II: Fruit Fly Response to Chemicals

In this part of the investigation, you assemble a "choice chamber" in which about 20 to 30 flies respond by moving toward or away from a chemical you put in one end of the chamber. You introduce the chemical by adding it to cotton balls that you place at opposite ends of the chamber. You can assess the strength of the response by the

number of flies that move toward the chemical after a given amount of time (e.g., 30 seconds). For each chemical test, you should rule out the influence of environmental variables such as light, gravity, and background colors or motion. You can do this by evaluating fruit fly behavior with the choice chamber positioned in different orientations. Chemicals to consider testing include the following:

1. **Water.** Use water as your first chemical to establish a control to which other chemicals can be compared. If there is a significant movement toward or away from the water, evaluate the integrity of your choice chamber or surrounding environment to determine to what, if anything, the flies are responding.
2. **Alcohol.** In alcohol fermentation, glucose is broken down anaerobically (in the absence of oxygen) by yeasts to produce ethanol (ethyl alcohol) and CO_2. ATP is produced through substrate-level phosphorylation.
3. **Vinegar.** In acetic acid fermentation, ethanol is broken down by acetic acid bacteria to produce vinegar (acetic acid).
4. **Alka-Seltzer.** Alka-Seltzer is a medication that relieves stomach pain by neutralizing excess stomach acid. When dissolved in water, it releases CO_2. Add a few drops of water to some Alka-Seltzer before adding it to the choice chamber.
5. **Various laboratory chemicals.** Diluted solutions of NaOH and HCl are usually available from your teacher.
6. **Various condiments.** Consider testing mustard, capers, or salad dressing. Check their ingredients to see if they have anything in common with other chemicals you are testing.
7. **Fruit in various stages of ripeness or decay.** Fruit flies, after all, are attracted to fruit. But why are they attracted to fruit? Try to determine if they have preferences to a fruit's ripeness and, together with your other data, determine what it is about fruit that they pursue.

Part III: Comparing Fruit Fly Preferences

In this part of the investigation, you design an experiment to determine the *degree* of preference that fruit flies have for chemicals. In other words, you determine which chemical is their favorite, which is their least favorite, and preferences of the remaining chemicals that fall in between. Pair each chemical with every other chemical in a two-pole (two-ended) choice chamber, or test multiple chemicals at once with a multi-pole chamber. Collect your data into a table (Figure 12-8).

	Substance in Corner B													
	Substance 1		Substance 2		Substance 3		Substance 4		Substance 5		Substance 6		Substance 7	
Substance in Corner A	Prefer A	Prefer B	Prefer A	Prefer B	Prefer A	Prefer B	Prefer A	Prefer B	Prefer A	Prefer B	Prefer A	Prefer B	Prefer A	Prefer B
Substance 1	15	15												
Substance 2	25	5	14	16										
Substance 3	25	5	10	20	15	15								
Substance 4	21	9	8	22	7	23	16	14						
Substance 5	6	24	0	30	3	27	6	24	15	15				
Substance 6	8	22	3	27	8	22	13	17	30	0	15	15		
Substance 7	28	2	30	0	30	0	28	2	30	0	30	0	16	14

Number of Fruit Flies Preferring Substances in Corner A or Corner B of Choice Chamber

Figure 12-8

Use a χ^2 statistical analysis to see if the combined data of your class are significant.

The AP exam may ask you to design an experiment similar to the one you completed in this lab. Begin by selecting a type of organism and observing them in the absence of applied stimuli. Then, change the environment by introducing a stimulus. Only one stimulus should be applied at a time. In the investigation that you completed

above, that one stimulus was a chemical. You manipulated the variable (gave the variable different values) by changing the kind of chemical stimulus. Be sure to include the following in your experimental design:

1. One or more experimental treatments (different values for your independent variable); some independent variables you can consider include the following:
   - Physical stimuli such as humidity, temperature, light, sound, gravity, pH, and chemicals (salt, drugs, nicotine, alcohol, caffeine, aspirin, and pesticides).
   - Biotic stimuli such as the introduction of members of the same species (males or females) or other species (predators or prey). If members of the same species are introduced, the sex of the introduced individual may influence behavior (mating or agonistic behaviors). Multiple members of the same species may elicit social behaviors.
2. A control treatment to which the experimental treatments can be compared.
3. A graph, histogram, or other graphic representation of the data.
4. An interpretation or discussion of the data.
5. A statistical analysis. If an analysis is not requested in the question, you should still suggest an appropriate analysis (e.g., χ^2).

Investigation 13: Enzyme Catalysis

In this investigation, the effect of a catalyst on the rate of a reaction is measured. The reaction investigated is the breakdown of hydrogen peroxide (H_2O_2) into H_2O and O_2. Two kinds of enzymes catalyze the decomposition of H_2O_2—catalase and peroxidase. Catalase decomposes H_2O_2 directly, whereas peroxidase decomposes H_2O_2 using an organic reducing agent. These reactions are compared in the first two equations below. The letter A represents the organic substance.

$$2\,H_2O_2\ (\text{gas}) \xrightarrow{\text{catalase}} 2\,H_2O + O_2\ (\text{gas})$$

$$4\,H_2O_2 + 4\,AH_2 \xrightarrow{\text{peroxidase}} 8\,H_2O + 2\,A_2$$

$$4\,H_2O_2 + 4\,\underset{\text{(colorless)}}{\text{guaiacol}} \xrightarrow{\text{peroxidase}} H_2O + \underset{\text{(orange-brown)}}{\text{tetraguaiacol}}$$

One option in this investigation is to use peroxidase with the organic reducing agent guaiacol. In the presence of peroxidase, H_2O_2 is broken down to H_2O as four molecules of guaiacol are converted to tetraguaiacol (third equation above). Because guaiacol is colorless and tetraguaiacol is orange-brown, the amount of product produced can be measured by the color intensity of tetraguaiacol.

At the conclusion of this lab, you should know the following:

1. The rate of a reaction is determined by measuring the accumulation of one of the products or by measuring the disappearance of the substrate (reactant).
2. The rate of a reaction is the slope of the linear (straight) part of the graph that describes the accumulation of product (or decrease in substrate) as time progresses.
3. Reaction rate may be affected by temperature, pH, substrate concentration, and enzyme concentration.

Part I: Determine a Baseline for Peroxidase Activity

The goal for this part of the investigation is to establish a **baseline** for measuring the amount of H_2O_2 that is broken down. Once a baseline is established, it can serve as a comparison to subsequent reactions that you investigate in parts II and III. You can measure the progress of the H_2O_2 reaction by measuring the disappearance of H_2O_2 or the accumulation of H_2O or O_2. If a reducing agent (like guaiacol) is used, its disappearance or the accumulation of its product (tetraguaiacol) can be measured.

In this investigation of H_2O_2 decomposition, you document the progress of the reaction by measuring the accumulation of tetraguaiacol or the accumulation of O_2. Here are three ways to do to that:

1. **Color intensity by comparing to a color palette.** A color palette can be created in which a series of test tubes with increasing color intensity are correlated with increasing concentration of tetraguaiacol product. To prepare the palette, run the H_2O_2 decomposition reaction with peroxidase and guaiacol to completion and then make a series of product dilutions (10%, 20%, etc.). You can use this palette to evaluate the progress of the reaction because each dilution bears the color of the product (tetraguaiacol) at a concentration proportional to the reactant (guaiacol). Note that *four* guaiacol molecules are converted to *one* tetraguaiacol.
2. **Color intensity by measuring absorbance.** A spectrophotometer can be used to determine the amount of tetraguaiacol product produced by measuring the absorbance of the solution. The darker the solution, the greater its absorbance, and the more product produced.
3. **O_2 generation.** An oxygen probe can be used to measure the oxygen product directly as it is being produced. In this case, an organic reducing agent (like guaiacol) is not used.

To determine the rate of a reaction in the presence of an enzyme using a color palette, follow these steps:

1. Mix the reactants (H_2O_2, guaiacol) with the enzyme (peroxidase).
2. Take pictures at 1-minute intervals.
3. Compare pictures with the color palette to determine the amount of product formed at the end of each time interval.
4. Plot product accumulation vs. time.
5. Draw a line of best fit through the plotted points. The slope of the line is the rate of the reaction.

Part II: The Effect of pH on Enzyme Activity

Now that you have a baseline for comparison, you can investigate the effect of different treatments on the rate of H_2O_2 decomposition. In this part of the investigation, you investigate the effect of pH. You prepare a series of test tubes, substituting various pH solutions for the H_2O component in the baseline solution. For each pH investigated, record the amount of accumulated product (measured by color intensity, absorbance, or O_2 generated), at various time intervals and graph that data to determine the rate of each reaction. Then plot the rates together on a graph as a function of pH.

Part III: Investigate Other Effects on Enzyme Activity

In this part of the investigation, you explore how the rate of peroxidase activity is influenced by a variable of your choice. Consider enzyme concentration, substrate concentration, or temperature. Be sure that you test only one variable at a time. For example, if you investigate the effect of enzyme or substrate concentration by adding additional enzyme or substrate, be sure to reduce the amount of H_2O by the same amount to maintain a constant volume.

Review Questions

Multiple-Choice Questions

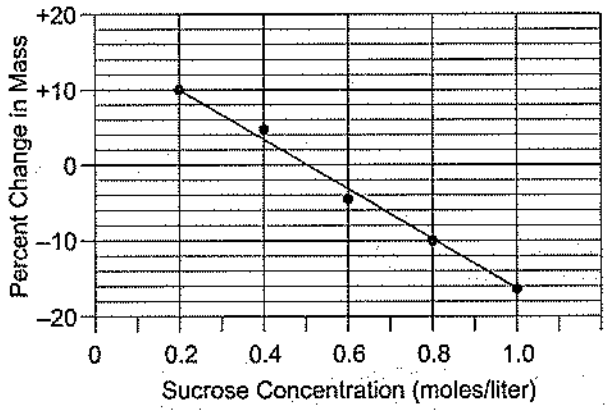
The questions that follow provide a review of the material presented in this chapter. Use them to evaluate how well you understand the terms, concepts, and processes presented. Actual AP multiple-choice questions are often more general, covering a broad range of concepts, and often more lengthy. For multiple-choice questions typical of the exam, take the two practice exams in this book.

Directions: Each of the following questions or statements is followed by four possible answers or sentence completions. Choose the one best answer or sentence completion.

1. A dialysis bag is filled with a 3% starch solution. The bag is immersed in a beaker of water containing a 1% IKI (iodine and potassium iodide) solution. IKI is yellow-brown but turns blue in the presence of starch. The dialysis bag is permeable to IKI but impermeable to starch. All of the following observations are correct EXCEPT:

- **A.** When the bag is first placed in the beaker, the water potential inside the bag is negative.
- **B.** When the bag is first placed in the beaker, the solution in the beaker is yellow-brown.
- **C.** After 15 minutes, the solution in the bag turns blue.
- **D.** After 15 minutes, the mass of the dialysis bag has decreased.

Questions 2–3 refer to five potato cores that are placed in five beakers containing different concentrations of sucrose. The following graph shows the change in mass of each of the potato cores after 24 hours in the beakers.



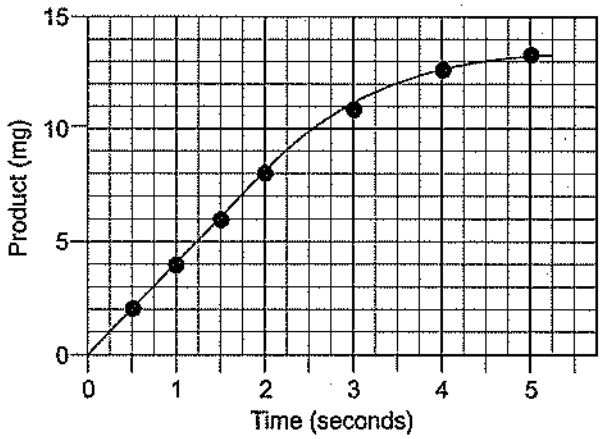
2. The water potential of the potato core can be calculated using which of the following sucrose concentrations?

- **A.** 0.2 M
- **B.** 0.4 M
- **C.** 0.5 M
- **D.** 0.10 M

3. All of the following statements are true EXCEPT:

- **A.** When first immersed in the beaker with 0.2 M sucrose, the water potential of cells in the potato core is more negative than that of the sucrose solution.
- **B.** After 24 hours in the beaker with 0.2 M sucrose, the pressure potential of cells in the potato core has increased.
- **C.** All of the sucrose solutions have a negative water potential.
- **D.** When the net movement of water into a potato core is zero, the water potential of the potato core is zero.

For questions 4–6, use the following graph of an enzyme-mediated reaction.



4. What is the initial rate of the reaction?

- **A.** 0.25 mg/sec
- **B.** 4 mg/sec
- **C.** 4.5 mg/sec
- **D.** 5 mg/sec

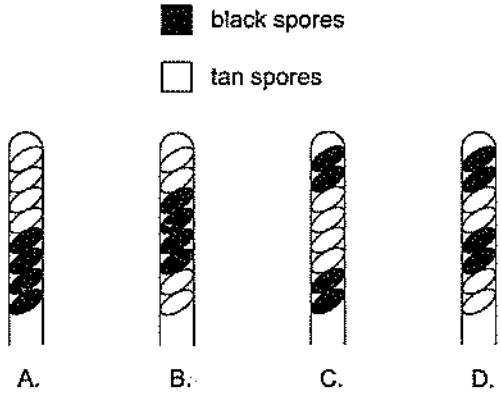
5. What will be the effect on the reaction if the enzyme is heated to 100°C before being mixed with the substrate?

- **A.** The reaction rate will increase.
- **B.** The reaction will occur at a slower rate.
- **C.** The reaction will not occur or will occur at a rate not significantly different from a reaction rate with no enzyme at all.
- **D.** The reaction rate will remain unchanged.

6. Which of the following is LEAST likely to increase the forward rate of an enzyme-mediated reaction?

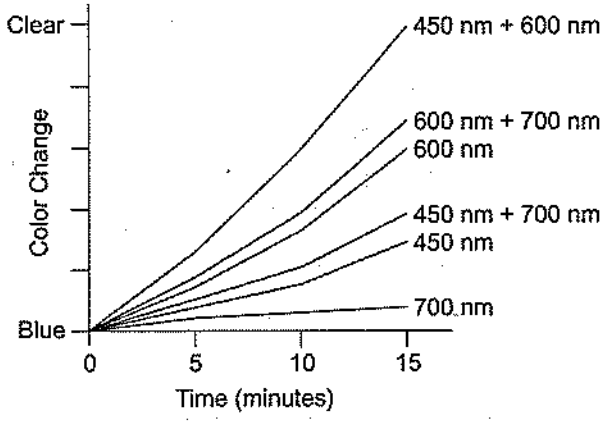
- **A.** an increase in the substrate concentration
- **B.** an increase in the enzyme concentration
- **C.** an increase in the product concentration
- **D.** an increase in pH

Question 7 refers to the following figure that illustrates four different arrangements of ascospores, all resulting from a cross between a strain homozygous for the wild type of spores (black spores) and a strain homozygous for the mutant color (tan).



7. Which of the asci in the figure contains ascospores produced during meiosis *without* crossing over?

Question 8 refers to an experiment that measures the rate of photosynthesis by using DPIP as a substitute for $NADP^+$. Oxidized DPIP (the lower energy state of DPIP) is blue, and reduced DPIP (the higher energy state of DPIP) is clear. Various wavelengths of light are used to illuminate samples that contain chloroplasts, DPIP, and an appropriate buffer. The results of the experiment are shown here.



8. Which of the following is a reasonable interpretation of the results?

- **A.** All wavelengths of light are equally absorbed and utilized during photosynthesis.
- **B.** Blue (at 450 nm) is the most efficient wavelength for photosynthesis.
- **C.** There are at least two wavelengths of light utilized during photosynthesis.
- **D.** A blue solution indicates maximum photosynthetic activity.

Questions 9–10 refer to an experiment designed to measure the respiratory rate of crickets. Three respirometers are prepared as follows.

Respirometer	Contents
1	KOH, 1 cricket weighing 2 grams
2	KOH, 1 cricket weighing 3 grams
3	KOH

All respirometers (consisting of a syringe connected to a capillary tube) are immersed in the same water bath. One end of the pipette is connected to the jar; the other end of the pipette is open to the surrounding water.

9. A pressure change registered by respirometer 1 or 2 may indicate any of the following EXCEPT:

- **A.** the cricket is alive
- **B.** a change in temperature of air inside the jar
- **C.** a change in the amount of oxygen inside the jar
- **D.** a change in the amount of CO_2 inside the jar

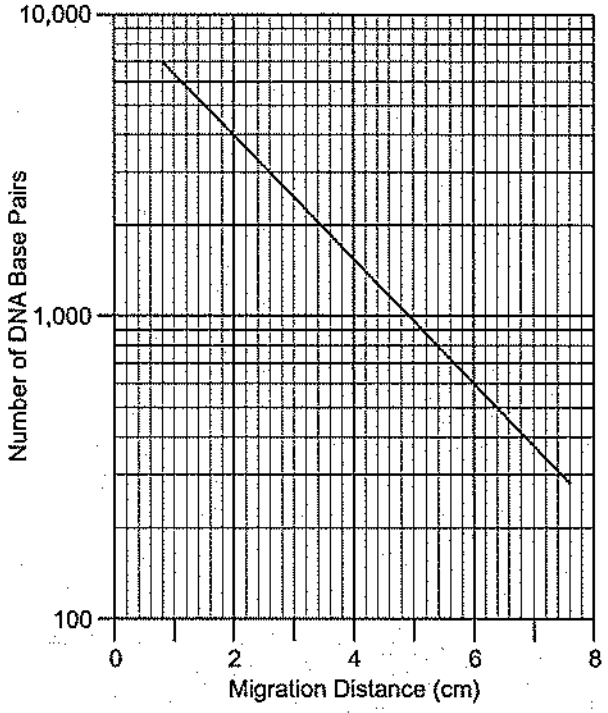
10. The purpose of respirometer 3 is to act as a control for all of the following EXCEPT:

- **A.** changes in the amount of O_2 inside the jar
- **B.** changes in water bath temperature
- **C.** changes in atmospheric pressure
- **D.** changes produced by water pressure variations in the water surrounding the respirometers

11. Competent bacteria

- **A.** are resistant to antibiotics
- **B.** can be induced to accept foreign DNA
- **C.** cannot reproduce
- **D.** cause disease

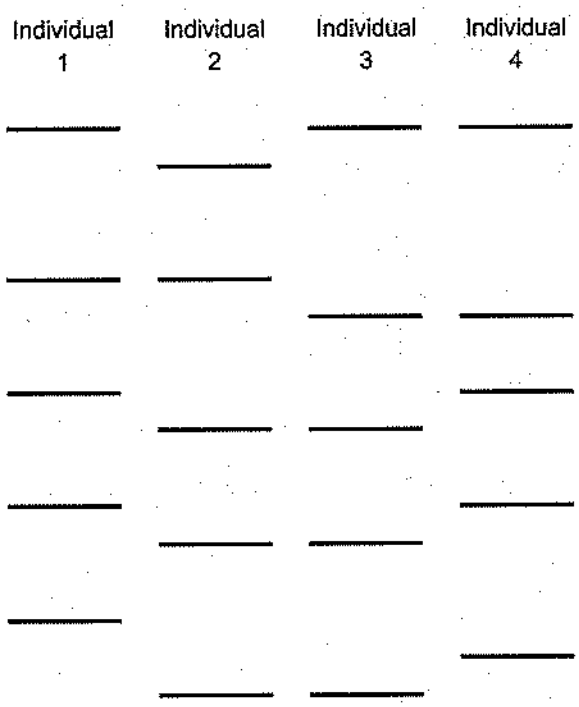
Question 12 refers to the following semi-log graph of results from the standard restriction enzyme used in a gel electrophoresis procedure. Phage lambda DNA molecules digested with Hind*III are used as the standard.*



12. A fragment of phage lambda DNA produced by *Eco*RI endonuclease migrates 6 cm. If this fragment is produced during the same electrophoresis procedure as the standard shown in the graph, how large is the fragment?

- **A.** 150 base pairs
- **B.** 600 base pairs
- **C.** 800 base pairs
- **D.** 6,000 base pairs

Question 13 refers to the following diagram, representing the bands produced by an electrophoresis procedure using DNA from four human individuals. Each DNA sample is treated with the same restriction enzyme.



13. Which of the following is a correct interpretation of the gel electrophoresis data?

- **A.** Individual 1 could be an offspring of individuals 3 and 4.
- **B.** Individual 1 could be an offspring of individuals 2 and 3.
- **C.** Individual 2 could be an offspring of individuals 1 and 3.
- **D.** Individual 3 could be an offspring of individuals 2 and 4.

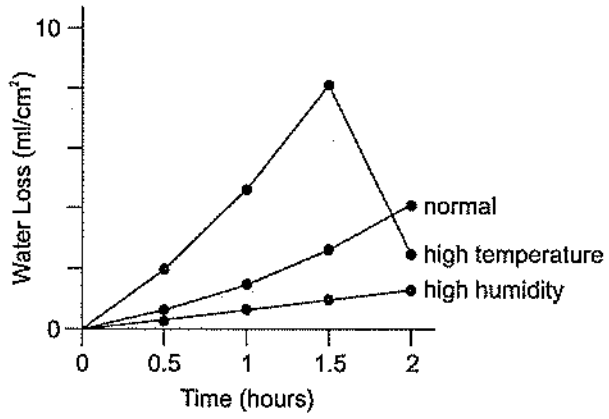
14. A population consists of 20 individuals, of which 64% are homozygous dominant for a particular trait and the remaining individuals are all heterozygous. All of the following could explain this situation EXCEPT:

- **A.** Genetic drift is occurring.
- **B.** The recessive allele is deleterious.
- **C.** All homozygous recessive individuals emigrate.
- **D.** Only heterozygous individuals mate.

15. Which of the following series of terms correctly indicates the gradient of water potential from lowest water potential to highest water potential?

- **A.** air, leaf, stem, root, soil
- **B.** soil, root, stem, leaf, air
- **C.** root, leaf, stem, air, soil
- **D.** air, soil, root, leaf, stem

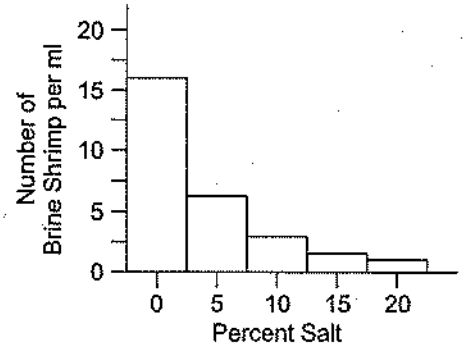
Question 16 refers to the following graph that shows the rate of water loss for three plants. One plant is exposed to normal conditions, a second plant is exposed to high temperature, and a third plant is exposed to high humidity.



16. The sudden decrease in water loss after 1.5 hours for the plant exposed to high temperatures is probably caused by

A. the burning of the leaves
B. a lack of CO_2 to maintain photosynthesis
C. a lack of O_2 to maintain photosynthesis
D. stomatal closure

Questions 17–18 refer to a laboratory experiment that allows Artemia *brine shrimp to move into water with different concentrations of salt. The results of the experiment are shown in the histogram below.*



17. According to the preceding histogram, most brine shrimp are found in a habitat in which the salt concentration is

A. 0%
B. 5%
C. 15%
D. 20%

18. Under natural conditions, *Artemia* brine shrimp are rarely found in bodies of water with salt concentrations below 5%. This is probably because

A. the brine shrimp prefer low levels of salt concentration
B. the brine shrimp prefer high levels of salt concentration
C. predators of the brine shrimp are common in fresh water and water with low salinity
D. the brine shrimp cannot survive in fresh water

19. The net primary productivity for a temperate forest was measured at 2,000 mg carbon fixed/L/day. The respiratory rate of the community was determined to be 1,000 mg carbon fixed/L/day. The gross primary productivity for this community is

A. 1,000 mg carbon fixed/L/day
B. 2,000 mg carbon fixed/L/day
C. 3,000 mg carbon fixed/L/day
D. 4,000 mg carbon fixed/L/day

Free-Response Questions

The AP exam has long and short free-response questions. The long questions have considerable descriptive information that may include tables, graphs, or figures. The short questions are brief but may also include figures. Both kinds of questions have four parts and generally require that you bring together concepts from multiple areas of biology.

The questions that follow are designed to further your understanding of the concepts presented in this chapter. Unlike the free-response questions on the exam, they are narrowly focused on the material in this chapter. For free-response questions typical of the exam, take the two practice exams in this book.

Directions: The best way to prepare for the AP exam is to write out your answers as if you were taking the exam. Use complete sentences for all your answers and do *not* use outline form or bullets. You may use diagrams to supplement your answers, but be sure to describe the importance or relevance of your diagrams.

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1. Many factors influence transpiration rate. Name three of these factors and explain how they operate.

2. In an exercise to model evolution, it was found that after 10 generations of reproduction, the frequency of one of the two alleles in the model became zero. Explain why an allele would "disappear" after such a short number of reproductive cycles.

3. In a gel electrophoresis procedure, DNA samples are placed on a gel and an electric voltage is applied. In two or three sentences, explain the purpose of applying an electric voltage.

4. An experiment was conducted to measure the effect of light on photosynthetic rate. The following three treatments were evaluated:

- Treatment I: Healthy chloroplasts exposed to light
- Treatment II: Boiled chloroplasts exposed to light
- Treatment III: Healthy chloroplasts incubated in darkness

Oxidized DPIP was added to each treatment to simulate $NADP^+$. When oxidized DPIP is reduced (energized) by photosynthesis, it turns from blue to clear. The degree to which the DPIP was reduced in each treatment was determined by using a spectrophotometer. A spectrophotometer measures the amount of light that is transmitted through a sample. The spectrophotometer in this experiment was set to measure light at a wavelength of 605 nm.

The following data were collected for the healthy chloroplasts exposed to light.

	Treatment I					
Time (minutes)	0	5	10	15	20	25
Average % transmittance (5-minute intervals)	30	45	60	70	75	78

a. Construct and label a graph for the healthy chloroplasts exposed to light. On the same set of axes, draw and label two additional lines representing your prediction of the data obtained for treatments II and III.
b. Justify your predicted data for treatments II and III.
c. Describe the process that causes the reduction (energizing) of DPIP.

Answers and Explanations

Multiple-Choice Questions

1. **D.** After 15 minutes, water will move from the beaker (higher water potential) into the bag (lower water potential), and the mass of the bag will increase. After 15 minutes, the solution inside the *bag* turns blue because the IKI that diffuses into the bag mixes with the starch. When the bag is first placed in the beaker, the water potential in the bag is negative—the sum of the negative solute potential and a zero pressure potential (flaccid bag).

2. **C.** When the net movement into and out of the potato core is zero, the water potentials inside and outside the potato core are the same, and there is no change in the mass of the potato core. A sucrose solution of 0.5 M shows a 0% change in mass on the graph.

3. **D.** When the net movement of water into a potato core is zero, the water potentials inside and outside the potato core are the same, but not zero. Because the potato core immersed in the 0.2 M sucrose solution gained weight, the water potential of its cells must have been smaller than the water potential of the sucrose solution. After 24 hours, water that enters the potato core in the 0.2 M sucrose solution causes the potato cells to expand and gain weight. Since the rigid cell walls cannot expand, pressure potential increases as the cell walls exert a restraining pressure on the cell contents.

4. **B.** The initial rate of reaction is the slope of the plotted curve at the beginning of the reaction. Since the straight-line portion of the curve from 0 to 2 seconds indicates a constant rate of reaction, the slope at any point along this portion of the line will provide the initial rate. For the entire interval from 0 to 2 seconds, the slope, determined by the change in product formed divided by the change in time, is $\frac{8\text{ mg} - 0\text{ mg}}{2\text{ sec} - 0\text{ sec}} = 4\text{ mg/sec}$.

5. **C.** An enzyme that is heated to 100°C will be structurally damaged. As a result, the reaction, no longer under the influence of the enzyme, is not likely to occur. If any activity persists, the reaction rate would be equivalent to the same reaction in the absence of the enzyme (extremely slow).

6. **C.** Since enzyme-mediated reactions are reversible (they convert product back to substrate), increasing the concentration of the product will slow the forward direction of the reaction and accelerate the reverse reaction. Conversely, an increase in the substrate concentration will increase the forward rate of the reaction. Increasing the enzyme concentration will not slow the reaction rate but may increase it if the substrate concentration is high enough to utilize additional enzymes. An increase in pH may change the rate of reaction, but the nature of the enzyme must be known in order to determine whether the rate is increased or decreased.

7. **A.** The ascus containing ascospores with two groups of four adjacent ascospores of the same color results when no crossovers occur. If no crossing over takes place, the order of ascospores corresponds to each of the four chromatids of a homologous pair of chromosomes (two ascospores from each chromatid). Thus, the first four ascospores possess traits from one parent and the second four ascospores possess traits from the second parent. If crossing over occurs, traits on two nonsister chromatids will exchange, resulting in a swap of traits between one pair of ascospores and another pair and producing an ascus that may look like any one of the images in the other answer choices.

8. **C.** Because the graph shows that 450 nm and 600 nm each produce a high photosynthetic rate, and together produce the highest rate, the graph indicates that the photosynthetic process depends on these two wavelengths. Although light of 450 nm does induce significant photosynthetic activity, light of 600 nm induces more. A blue solution indicates that little or no photosynthesis has occurred because DPIP has not been reduced.

9. **D.** An increase in CO_2 gas (from respiration) cannot be detected because it immediately reacts with KOH to produce solid K_2CO_3. A live cricket will decrease the amount of O_2 gas detected by the respirometer. Changes in temperature and atmospheric pressure also cause the respirometer to register a change in volume because temperature and atmospheric pressure affect the water pressure on the pipette inlets.

10. **A.** Since there is no insect and, thus, no O_2 consumption in respirometer 3, the purpose of this respirometer is to control all the variables that might influence the volume changes in respirometers 1 and 2, other than O_2 consumption by insects.

11. **B.** Competence refers to a stage of rapid population growth during which bacteria are most receptive to absorbing foreign DNA.

12. **B.** The vertical line at 6 cm and the horizontal line at 600 base pairs intersect on the standard curve. On the log scale for the *y*-axis, each horizontal line between 100 and 1,000 represents an increase of 100 base pairs.

13. **D.** This is an example of DNA fingerprinting using gel electrophoresis. The horizontal bands represent fragments of DNA, produced by a restriction enzyme, that have migrated across a gel, with lighter fragments moving farthest. Each lane (column) represents the DNA fragments from a different individual. Fragments in different lanes that have migrated the same distance are fragments consisting of the same DNA. Since an offspring's DNA originates from a combination of DNA from both of its parents, the DNA fragments of the offspring must match the fragments of one or the other parent. The DNA fragments from individual 3 can be found in *either* individuals 2 or 4. As a result, it is possible (but not certain) that individual 3 inherited his or her DNA from individuals 2 and 4. In an actual DNA fingerprinting analysis, many different restriction enzymes are used so that many different DNA fragments can be compared.

14. **D.** If only heterozygotes mate, 25% of the offspring, on average, should be homozygous recessive. Thus, this answer cannot explain the absence of homozygous recessive individuals in the population. Because the population is so small, genetic drift may be responsible. Alternately, natural selection against individuals with a deleterious homozygous recessive genotype may also explain why this genotype is absent from the population. Note, however, that the recessive allele remains in the population because it is masked by the dominant allele in heterozygous individuals. The absence of homozygous recessive individuals can also be explained if these individuals leave the population (emigrate) for another location.

15. **A.** Water potential is highest in the soil, decreases from root to leaf, and is lowest in the air. Water moves from the soil into the roots and through the plant and transpires from the leaf because water moves from the area of highest water potential to the area of lowest water potential.

16. **D.** When water entering roots cannot adequately replace the water loss by transpiration, the stomata close to prevent wilting.

17. **A.** The tallest vertical bar (above 0% salt concentration) indicates the preference of the greatest number of brine shrimp.

18. **C.** This question asks why brine shrimp in the wild are rarely found in water with low salt concentrations when laboratory experiments demonstrate that is where they prefer to be. The answer is that under natural conditions, shrimp predators are found in these waters and either the shrimp are eaten or the shrimp avoid these waters because of the predators. Although brine shrimp prefer low-salt water, they can still survive in water with higher salt concentrations where predators are absent.

19. **C.** The gross primary productivity is the sum of the net primary productivity (2,000 mg carbon fixed/L/day) and the respiratory rate (1,000 mg carbon fixed/L/day), for a total of 3,000 mg carbon fixed/L/day.

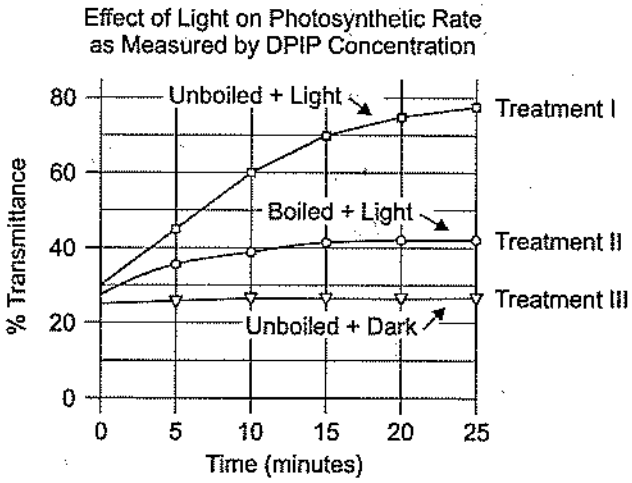
Free-Response Questions

1. A major factor influencing transpiration rate is temperature. The hotter the environment, the greater the kinetic energy of water molecules, and the faster water molecules change from a liquid state to a gas state. Wind is also a factor because it removes moisture around the leaf and increases the water gradient, which increases the rate of movement of water molecules from inside to the outside of the leaf. Leaf size is also a factor because it increases the surface area from which water transpires.

2. The population size entered into the model was probably small, less than 50 individuals. When populations are small, the effect of genetic drift (changes in allele frequencies by chance) has a strong influence on allele frequencies.

3. DNA is negatively charged due to the negatively charged phosphate groups in nucleotides. When an electric voltage is applied, DNA fragments are attracted to the positively charged end of the voltage field. As a result, the fragments move across the gel, but because larger, heavier fragments move more slowly than smaller, lighter fragments, the various fragments in the sample DNA are separated.

4. a.



b. In treatment II, the chloroplasts were boiled. Boiling damages the chloroplasts, disrupting the thylakoid membranes. Also, the enzymes and photosynthetic pigments embedded in the membranes are denatured. When these molecules lose their secondary and tertiary structures, they can no longer function properly. As a result, photosynthesis in treatment II is greatly reduced, occurring only in those few membranes that may still be intact with functioning enzymes and pigments. Only a small amount of DPIP is reduced, and the transmittance of a treatment II sample remains low (blue).
In treatment III, no DPIP is reduced because photosynthesis cannot occur in the absence of light. If photosynthesis cannot occur, electrons in the pigment systems are not reduced (energized); thus, there are no energized electrons to reduce DPIP. The DPIP remains oxidized (blue), and transmittance remains low.

c. When healthy chloroplasts are exposed to light, electrons of pigment molecules in photosystem II are energized (reduced). These electrons are eventually passed to the special chlorophyll *a* (P_{680}). Two electrons are then passed to a primary electron acceptor, the first molecule of an electron transport chain. (An electronic transport chain is a series of electron carriers, such as cytochromes.) As the two electrons are passed from one carrier to the next, energy from the electrons is used to generate 1.5 ATP molecules (on average). At the end of the chain, the two electrons are accepted by pigment molecules in photosystem I, where they are again energized by light energy, passed to a special chlorophyll *a* (P_{700}), and then passed to a primary electron acceptor. The two electrons are then used to reduce $NADP^+$. With H^+ (from the splitting of H_2O), NADPH is formed. NADPH then supplies energy for the fixation of CO_2 during the Calvin cycle. In this experiment, DPIP is added so that the process can be visualized. As oxidized DPIP is energized by electrons from photosystem I to form reduced DPIP, DPIP turns from blue to clear.