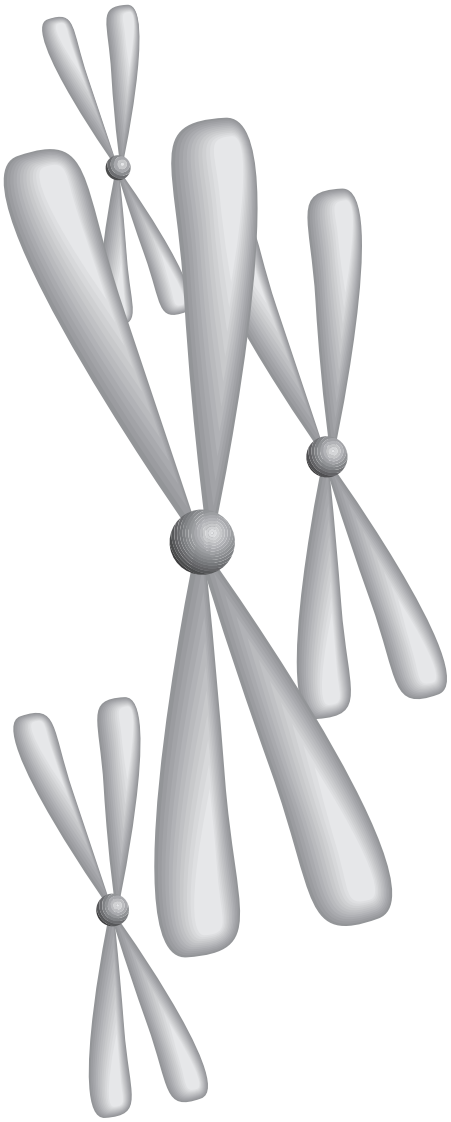


CHAPTER 3

How Genes and the Environment Influence Our Health

CHAPTER 3

How Genes and the Environment Influence Our Health

CHAPTER THREE OVERVIEW T176

SECTION A
How Stable and How Powerful Is DNA? . . . . . S161 . . . .T177
Section A Background T178
1. DNA Paradoxes . . . . . S162 . . . . . T179

SECTION B
How Do Heritable Changes in Genes Occur? . . . . . S163 . . . .T181
Section B Background T182
1. Inducing Mutations with UV Light . . . . . S164 . . . . . T184

SECTION C
Is it Nature, or Is it Nurture? . . . . . S171 . . . .T191
Section C Background T192
1. Albino Plants: A Model Gene-Environment Interaction . . . . . S172 . . . . . T193
2. Heart Disease: A Personal Gene-Environment Interaction . . . . S178 . . . . . T197

SECTION D
What Are Some of the Features of "Simple" Genetic Diseases? . . . . . S183 . . . .T205
Section D Background T206
1. Some "Simple" Heritable Defects . . . . . S184 . . . . . T207
2. Phenylketonuria (PKU) Illustrates the Complexities of Some "Simple" Genetic Diseases . . . . . S186 . . . . . T207
3. The Special Inheritance Patterns of Sex-Linked Mutations . . . S188 . . . . . T208
4. Investigating Human Genetic Diseases . . . . . S190 . . . . . T210

SECTION E
How Does a Genetic Counselor Detect Mutant Genes? . . . . . S193 . . . .T211
Section E Background T212
1. Detecting the Duchenne Muscular Dystrophy (DMD) Mutation. . . . . S194 . . . . . T216

SECTION F
How Can I Become a Genetic Counselor? . . . . . S203 . . . .T223
Section F Background T224
1. How Can I Become a Genetic Counselor? S204

Chapter Three Overview

TEACHING GENETICS IS A bit like walking a tightrope; to do it well, one has to exercise a fine sense of balance and avoid falling off to one side or the other. On the one hand, we want our students to understand that genes play a vital role in the living world. We need to help them appreciate the fact that every aspect of the life of every organism is influenced by its genetic endowment. On the other hand, it is equally important for them to understand that genes are not all powerful. We need to work equally hard to help them appreciate that the effect a gene will have – if any – is entirely dependent on the details of the complex environment inside and outside of the cell in which that gene resides.

While we were introducing our students to the basic aspects of molecular and Mendelian genetics in the first two chapters of this manual, our emphasis was necessarily on the importance of genes in the living world. But now it is time to begin striking the balance referred to above. In this chapter, we will introduce the students to some of the paradoxical features of genes and a few of the complexities of the gene-environment interactions that influence all aspects of our lives, including our health.

In the first hands-on exercise in this chapter, students will learn that something as apparently innocuous as the light shining on an organism can – depending on its wavelength – act either to cause or to prevent serious genetic changes, many of which could be fatal. In the second exercise they will discover unmistakable evidence that a favorable interaction between genes and environmental factors is required for the synthesis of even the most important molecule in the entire living world, the chlorophyll molecule, on which photosynthesis (and thus all of life) depends. The third exercise should not only reinforce their appreciation of the importance of such gene-environment interactions but should really bring the concept home. In this simulated risk-assessment exercise, students will discover that it is unlikely that they could have any combination of "good" genes that would protect them from a serious risk of heart disease if they make all the wrong lifestyle choices, and that it is equally unlikely that they could have any combination of "bad" genes that would doom them to suffer serious heart disease if they adopt a truly healthy lifestyle.

In the rest of the chapter, the students will consider additional aspects and kinds of heritable human diseases and will learn, for example, that the severity of even supposedly simple, single-gene diseases often is determined by complex interactions of multiple genes with one another and the environment. The goal of this chapter is to get the students to realize that evidence of a heritable aspect to an undesirable human trait or condition is not equivalent to evidence that nothing can be done about it. After all, appreciation of the importance of environmental changes in promoting human good – whatever the genetic endowment of the individuals concerned – is the basis for two of the world's most noble professions, education and medicine!

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION A

How Stable and How Powerful is DNA?

Chapter 3: Section A Background

WE ARE LIVING IN the middle of an amazing genetic revolution. Sequencing of the human genome is essentially complete as of early 2001. The first successful cases of human gene therapy were reported in the year 2000. In that same year a quarter of the corn seeds, more than half of the soybean seeds, and nearly three fourths of the cotton seeds planted in the United States contained foreign genes inserted by genetic engineers.

Under these circumstances, it is no great surprise that there is a strong tendency on the part of journalists and others to exaggerate the importance of genes and the DNA molecules of which they are made. Biologists and biology teachers who make careless word choices contribute to such exaggerated views of DNA potency. They might, for example, refer to DNA as a "self-replicating molecule," although they know that no DNA molecule can replicate itself and that the replication of a DNA molecule requires interactions among many different kinds of enzymes and other molecules within the living cell. The rhetoric is often as excessive and ill-considered at the whole-organism level, as research biologists call press conferences to announce that they have discovered the gene for breast cancer, or schizophrenia, or alcoholism, or obesity, or some other complex human trait. They do this despite the knowledge that every one of those conditions is a complex entity in which a wide variety of environmental factors, as well as a considerable number of genes, play determinative roles.

Surely we want to infuse our students with a sense of excitement as we introduce them to the modern age of genetics. But just as surely, we want to do it in a realistic, scientifically sound way. This section is a step in that direction.

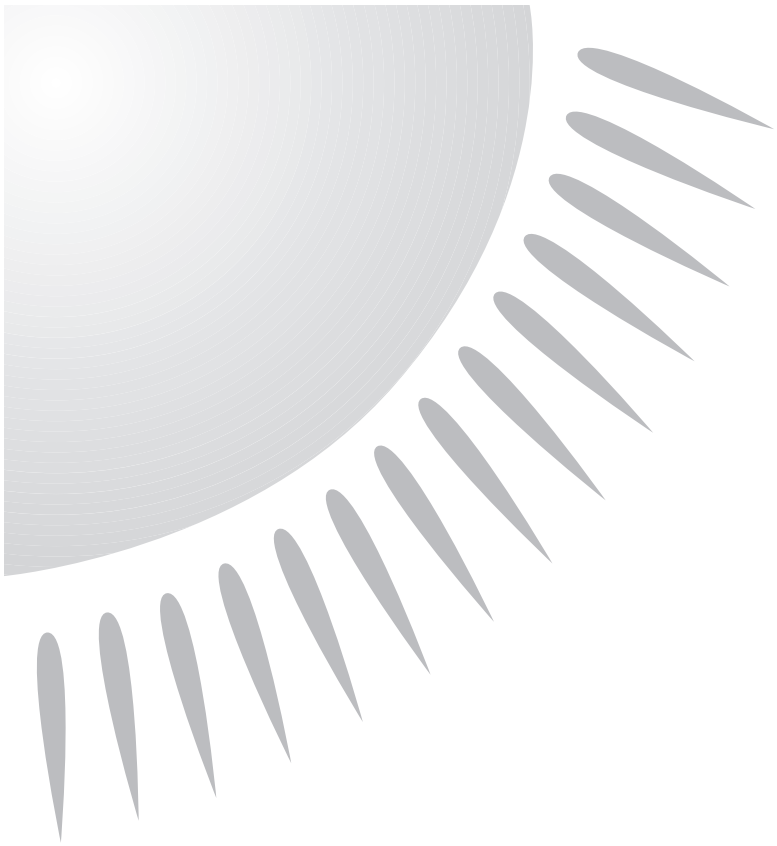
DNA Paradoxes STUDENT PAGE 162

LESSON OVERVIEW

This short reading assignment is intended to prepare your students for some of the paradoxical features of DNA and complexities of genetics - particularly the genetic aspects of human diseases - that they will encounter in this chapter.

TIMELINE

It will take an average student about 5 minutes for to read this material.



CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION B

How Do Heritable Changes in Genes Occur?

Chapter 3: Section B Background

THE MAJOR EMPHASIS OF this chapter is on genetic diseases that effect humans. However, a first step toward understanding genetic disorders is understanding the fact that genes can **mutate**, or change from one stable, heritable state to another stable, heritable state. The exercise in this section should provide your students with visible evidence of that fact. They will irradiate bacteria with ultraviolet (UV) light to induce mutations with a visible effect.

Because UV irradiation is about the easiest and surest way of inducing DNA damage experimentally, UV is one of the most extensively studied **mutagens.** It can damage the DNA in several ways, but the most common is by causing the formation of a pair of covalent bonds between adjacent thymine residues in a DNA strand, generating what is known as a **thymine dimer**. Thymine dimers cause kinks in the DNA strand that prevent both replication and transcription of that part of the DNA. Because they block DNA replication (and therefore prevent cells from reproducing), thymine dimers and other forms of UV damage cannot be inherited, and thus do not constitute mutations. Geneticists sometimes call such kinds of DNA damage **premutational lesions**, and because they prevent both transcription and replication of the genes in which they are present, these lesions are fatal if they go uncorrected. Not surprisingly, therefore, organisms (including ourselves) have a number of different DNA repair systems for eliminating such damage from DNA. But it is the very act of attempting to repair the UV-induced damage that sometimes converts it into a heritable mutation.

One of the more interesting DNA repair systems deals specifically with the thymine dimers that have been produced by UV. It is called **photo repair**, because it involves an enzyme that uses the energy of visible light to break the covalent bonds between adjacent thymines that were formed earlier by UV irradiation.

Photo repair is only one of many DNA repair systems present in cells. Other repair systems are required because UV causes more than one kind of DNA damage and UV is far from the only source of DNA damage in the living world. Many chemical compounds, both natural and man-made, can damage DNA if they get into cells. But it doesn't even take an external agent to damage DNA. It is now known, for example, that during an average day the DNA in each one of our cells loses about 5,000 of its adenine and guanine bases, due strictly to the thermal agitation that the DNA molecules experience at normal body temperature. Such **thermal depurination** is completely unavoidable at temperatures that are compatible with life, but it would be devastating to us (or any organism) if it were left uncorrected. And this is only one of several forms of spontaneous damage our DNA undergoes all of the time. It is of little surprise, then, that every living cell has several dozen different enzymes devoted to repairing DNA damage of one kind or another.

Most of these repair systems, including photo repair, are said to be **error-free**, because they restore the damaged DNA to its initial state. These systems are surprisingly efficient,

accurately repairing more than 99.99% of the DNA damage that occurs in a nucleus. Without error-free DNA repair systems neither we nor any other organism would be alive. But these repair systems are not perfect; if any damage goes undetected or unrepaired, it still can be fatal to the cell. Therefore, organisms from bacteria to humans possess a repair system of last resort, known as the **error-prone** DNA-repair system. Error-prone repair is induced by thymine dimers or other forms of DNA damage that (for whatever reason) have not been repaired by any error-free repair system.* The error-prone enzymes usually cut both strands of the DNA to remove the damaged region and then patch the molecule back together with a new piece of double-stranded DNA that they make without regard to the sequence of DNA bases that were previously present in that region. Just as the yarn used to mend a hole in a piece of clothing often does not match the original cloth, the sequence of the piece of DNA that the error-prone system uses as a patch usually bears no resemblance to the sequence that was in that region originally. As a result, the life of the cell may have been saved by the repair job, but it now has a heritable change – a mutation. Indeed, as already mentioned, it is such error-prone repair of damaged DNA that causes a mutation, not the initial damage itself. The significance of such a mutation will depend, of course, on the nature of the gene in which it occurs and the nature of the change that it causes.

* The most common form of error-prone DNA repair system in organisms from bacteria to humans is called **SOS repair**. SOS refers, of course, to the internationally recognized distress call that stands for "Save Our Ship."

Inducing Mutations with UV Light

STUDENT PAGES 164-167

LESSON OVERVIEW

In this exercise, your students will expose the bacterium *Serratia marcescens* to UV light to induce mutations with a visible effect, the loss of the ability to form the red pigment that characterizes wild-type *Serratia*.

The red pigment made by wild-type *Serratia* is called **prodigiosin**. It is a substance that is toxic to certain other microorganisms and therefore acts as an **antibiotic** that enables *Serratia* to compete more effectively with other microorganisms in its normal environment. Synthesis of prodigiosin requires the products of two separate and quite complex metabolic pathways. Because mutations that affect any of the steps in either of these pathways will interfere with pigment production, loss of pigmentation occurs with much higher frequency than a phenotype due to mutation of any single gene would.

The level of UV irradiation that your students will use in this experiment is one that (in the absence of photo repair) should induce a high level of error-prone DNA repair and therefore will lead to many mutations per cell, many of which will be lethal. Thus it will not be surprising if most of the bacteria that are grown in the dark die (particularly on the dishes that were given the longest UV exposure). Growth in the light should result in a substantial reduction in mutations and lethality because the light promotes error-free photo repair. But even when grown in the light, the bacteria should exhibit enough error-prone repair to produce some colonies that have lost the ability to synthesize prodigiosin, and are therefore colorless.

REFERENCES

Lewis, R. *Human Genetic: Concepts and Applications*, 130-133 Dubuque, Iowa: Wm. C. Brown Publishers, 1994.

Environmental Protection Agency's Stratospheric Ozone Hotline: (800) 296-1996. UV indexes, ozone layer, etc.

TIMELINE

Day 1

This part of the exercise requires 30 minutes to complete. Students spread the bacteria on nutrient agar dishes, expose them to UV, wrap them, and then set them aside for dark or light incubation. The rate limiting step will be getting the dishes irradiated, which will take 2-3 minutes per group.

Day 4 or 5

This part of the exercise should require about 20 minutes to complete. Students uncover their dishes, record their observations, and answer the questions on their work sheet.

MATERIALS

For each group of three or four students:

- 1 1000 µl micropipettor
- 1 sterile pipette tip
- 1 sterile microcentrifuge tube containing 500 µl of *Serratia marcescens* culture
- 4 nutrient agar petri dishes
- 1 marking pen
- 1 sterile inoculating loop
- disinfectant

For each class:

- 1 short-wavelength UV lamp* on a stand
- 1 watch with a second hand
- 1 waste receptacle containing 10% bleach solution
- aluminum foil
- plastic wrap
- 1 fluorescent or incandescent lamp

*It is important to use a short-wavelength UV lamp for this exercise. A long-wavelength UV lamp (sometimes know as a blacklight) will not work to induce mutations. Many UV lamps come with interchangeable filters or switches that permit them to be used in either wavelength range (for example, Carolina Biological cat. # BA-GEO9589). If you do not already have such a lamp, you may be able to borrow one from (or share the purchase price of one with) your school's earth science teacher.

ADVANCE PREPARATION

Generalized instructions for ordering and preparing microbiological media, agar dishes, etc. are given in Chapter 1, Section E.2 (pages T88 to T90).

1. Obtain a culture of *Serratia marcescens* from Carolina Biological (cat. # BA-15-5452) and use it to prepare a stock culture in liquid nutrient broth (NB) that will later be dispensed into individual tubes for student use.

   - Using sterile technique, measure appropriate volumes of sterile NB into one or more sterile, loosely capped culture tubes, allowing at least 0.5 ml of NB per lab group. (Or measure NB into non-sterile tubes, cap loosely and sterilize in an autoclave or pressure cooker.)
   - Transfer bacteria from the agar culture you received from Carolina to the NB tubes, using a sterile inoculating loop; twirl the loop in the NB to release the bacteria.
   - Incubate the tubes at room temperature under a light. Growth will be faster if you swirl the tubes carefully every hour or so during the day.
   - Incubate until the culture has developed a rich red color. Depending on the temperature of your room and the number of bacteria you transferred initially, this could take two to four days, but then the culture can be kept at room temperature for a couple of weeks with no adverse effects on the results.

2. Before class, dispense 500 µl of this *Serratia* culture to a sterile microcentrifuge tube for each lab group. Then prepare 4 nutrient agar dishes per lab group and prepare disinfectant spray bottles and waste containers (see Chapter 1, Section E.2).

3. Set up the UV lamp in an area away from student lab benches (see precautions below). The amount of UV irradiation that the bacteria receive will depend on how far above the benchtop you position the UV lamp. Because different UV lamps have different output intensities, there is no way to specify here what height will work best with your lamp. In the first year that you run this exercise, try placing the UV lamp 20-25 cm (8-10 in) above the bench top. Record the outcome so that you will be prepared to try a different height the following year if that seems advisable. The goal is to get four dark-incubated dishes on which the survival of the bacteria differs noticeably as a function of duration of UV irradiation. So if in the first year some of the dark-incubated dishes had so few surviving bacteria that they could not be easily distinguished, try placing the light higher the second year. If in the first year some had so many surviving bacteria that they could not be distinguished, try using the lamp at a lower position (or increasing the duration of the irradiation) the second year.

4. Set up a visible light source (such as a desk lamp) in the area where students will incubate their dishes.

HINTS AND TROUBLESHOOTING

1. Shortwave UV light can damage the eyes, so be sure students do not look into the UV lamp when it is lit. It is a good idea to tape cardboard blinders to the sides of the lamp to diminish the chances of accidental exposure of eyes. Brief exposure of hands to UV light won't be harmful, but students should not hold the dishes while they are being irradiated.

2. The lights used for the Wisconsin Fast Plants experiment could be used for incubation of the light-incubated petri dishes. But if these are not available, a desk lamp will be adequate. It is best to culture the dark-incubated (foil wrapped) dishes as close to the light-incubated dishes as possible. This will minimize the number of uncontrolled variables, such as temperature differences between light-incubated and dark-incubated dishes.

3. The growth rate of the bacteria will depend on the temperature of the room, particularly at night. So you may want to monitor growth on the dishes at daily intervals to determine when they will be ready for observation. Alternatively, you can schedule the observations for Day 4 or 5. If the contrast between the pigmented and nonpigmented colonies is not clear at that point, leave the dishes at room temperature for a couple of days longer and then reexamine them. (It will not be necessary to rewrap the dark-incubated dishes under these circumstances, because photo repair can only occur in the first few hours after UV irradiation.)

4. This lab lends itself well to follow-up activities. For example, students often observe that bacterial colonies on the dark-incubated, UV-irradiated dishes are larger than those on the control dishes or the light-incubated dishes, and then jump to the conclusion that this is because the bacteria have experienced beneficial mutations that cause increased growth rates. However, the size difference will usually be due to the fact that most of the bacteria on these dishes have been killed, allowing the survivors to produce larger colonies, because of the decreased competition for space and nutrients. You could use this situation to generate a discussion about developing and testing alternative hypotheses to explain an observed event. If time permits, the students could test the two hypotheses by a) growing a culture from one of the largest colonies and b) comparing the size of the colonies produced by the new culture and the original culture after they are plated at a series of different dilutions.

   Another possible follow-up is to compare the capacity of a variety of materials, such as petri lids, glass, and various plastic sheetings or bags to screen out mutagenic UV rays. This can be done by using the test material to cover about half of a petri dish during the irradiation period. Yet another possibility is to test the effectiveness of different colors of visible light in promoting photo repair. To do this, cover petri dishes with different colors of plastic filter material (borrowed from a school theater) or colored cellophane (purchased from a novelty store) after exposing the dishes to UV irradiation but before incubating the under lights. (The outcome of such experiments is difficult to predict here without knowing exactly what materials your student will test.)

Another good follow-up project is to have students use the library or the Web to investigate the chemical reactions by which chlorofluorocarbons, such as Freon, caused serious thinning of the ozone layer and created the ozone hole over the South pole. An examination of the international activities that led to bans on further production and use of such chlorofluorocarbons would be appropriate as well.

ANSWERS TO UV MUTAGENESIS WORKSHEET STUDENT PAGES 168-169

1. Draw pictures of what you observe on your dishes.
2. Describe the bacterial colonies on your dark-incubated control dish (0 seconds of UV). Then list and describe the differences among the bacterial colonies on each of the dark-incubated experimental dishes and those on the dark control dish.
   Drawings and descriptions of dishes will vary but should indicate fewer colonies and more pink or white mutants as the UV exposure time increases. Students may also observe larger colonies on irradiated dishes than on the control.

3. Based on these observations, summarize the effects that UV irradiation has on *Serratia marcescens* bacteria.
   Students should conclude that UV irradiation kills many bacteria and causes heritable changes in some of those that survive.

4. Now describe any differences among the bacterial colonies on each of the light-incubated dishes and those on the corresponding dark-incubated dish. (That is, compare the light-incubated control dish to the dark-incubated control dish, the light-incubated 30 sec. dish to the dark-incubated 30 sec. dish, etc.)
   In principle, they should not see any significant differences between the dark and light control dishes, but they should see major differences in survival and mutant frequency among the survivors on the UV-irradiated dishes that were incubated in the light vs. the dark.

5. Based on these observations, summarize the effects that cultivation in visible light has on UV-exposed *Serratia* bacteria.
   They should conclude that subsequent exposure to visible light somehow protects Serratia from the harmful effects of UV irradiation.

6. Can you formulate a hypothesis to account for such an effect of visible light?
   It will be interesting to see what they will come up with here. It should be a good test of their ability to think scientifically. The answers to this question should provide a good starting place for an interesting class discussion, particularly if students are challenged to think of ways to test their hypotheses.

7. Do all of the bacteria on a dish appear to respond to UV and visible light in the same way? Why?
   No. For example, many cells are killed, but some survive, and some of the survivors have visibly modified phenotypes, whereas others do not. This is because by sheer chance the UV will hit and damage different genes in different cells, and this will result in different phenotypes.

8. Do all of the mutations that you observe in *Serratia* after UV irradiation appear to be harmful? Explain.
   No. For example, some of the irradiated bacteria have lost the ability to make the red pigment made by wild-type bacteria, but this does not appear to have affected their ability to grow and divide enough to produce a colony of offspring.

9. Is it possible that a mutation could be beneficial? Explain.
   Some students may point out that some of the bacterial colonies on the irradiated dishes are larger than those on control dishes and then conclude that this indicates these bacteria have undergone a mutation that increases their growth rate. This can lead to an interesting discussion (see Hints and Troubleshooting).

CLOSURE

This exercise should provide your students with vivid evidence of the twin processes of environmental damage to DNA and DNA repair. Beyond that, however, it should provide a useful framework for stimulating them to think about how lifestyle decisions made by human beings today can influence their own genetic future and the genetic futures of their children, their grandchildren, and all other organisms with which we share the planet. When we and others carelessly discharge toxic substances from our homes, our automobiles, our power plants and our factories into rivers, air, and land, we may think that out of sight is out of mind. But we now have more than adequate evidence to appreciate that the mutagens and carcinogens that we release carelessly today may return tomorrow to adversely affect our genes or those of our grandchildren.

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION C

Is it Nature, or Is it Nurture?

Chapter 3: Section C Background

AS WE HAVE ALREADY mentioned, there is a potential danger in teaching genetics. The danger is that if we overemphasize the role of genes in controlling the traits of organisms, our students may adopt the mistaken concept of **genetic determinism**, which is the concept that organisms – including humans – are completely and irrevocably defined by their genes. If applied to bacteria or slime molds, genetic determinism (although scientifically unsound) probably has few serious social consequences. But when applied to human behavior, genetic determinism has had disastrous consequences, leading to the most severe forms of racism, the eugenics movement, forced sterilizations, and the ovens of Buchenwald. Even today genetic determinism rears its ugly head repeatedly in unexpected places and countless chilling ways.

If we did not all believe in the role of environment in shaping human behavior, we would not be in the education business.

This section provides a good opportunity to help students begin to strike a healthy balance in their view of genetics, by emphasizing the fact that every trait of every organism, including ourselves, is the product of some set of gene-environment interactions.

C.1

Albino Plants: A Model Gene-Environment Interaction

STUDENT PAGES 172-173

LESSON OVERVIEW

This exercise with tobacco seedlings provides a wonderful opportunity for your students to see the all-important concept given at the end of your Background section, namely, that "every trait of every organism, including ourselves, is the product of some set of gene-environment interactions."

The object of their investigation in this exercise will be one of the most important traits in the living world - the capacity to synthesize chlorophyll, the molecule upon which photosynthesis (and therefore the entire living world) depends. The seeds that the students will germinate were produced by crossing two tobacco plants that were heterozygous for a mutation at a locus encoding one of the enzymes required for chlorophyll synthesis. The wild-type allele encodes a functional enzyme and therefore is dominant with respect to the mutant allele. which does not encode a functional enzyme. However, the dominant allele, can not be expressed in the dark. Chlorophyll synthesis not only requires all of the genes encoding all of the enzymes in the pathway of chlorophyll biosynthesis, it also requires the correct environmental stimulus - light.

TIMELINE

This exercise requires little class time (particularly if the data analysis is assigned as homework), and thus it has a high benefit-to-cost ratio. Counting out the seeds and “planting” them on the agar plates requires only about five minutes the first day. Counting seedlings and recording data requires about five minutes on each of two later days.

Additional time will then be required if you ask the students to complete the optional Chi square analysis in class. However, if they performed the Chi square analyses in Chapter 2, this Chi square analysis could be assigned as homework. (On the other hand, if your students did not perform the Chi square analyses in Chapter 2 and you now wish to have them perform a Chi square analysis of their albino plant data, you will probably find it useful to introduce them to exercises E.2 and E.3 from Chapter 2 at this time.)

MATERIALS

For each group of three or four students:

- 1 petri dish with black agar
- 30 tobacco seeds
- a marking pen
- aluminum foil

ADVANCE PREPARATION

You can purchase the tobacco seeds from:
Carolina Biological
1-800-334-5551
www.carolina.com
Green:Albino Tobacco Seeds; catalog # BA-17-8400

It is a good idea to provide each lab group with a more-than-adequate sample of the seeds in a small envelope or a folded piece of paper rather than make them wait in line to count out their seeds from a central supply.

A day in advance, prepare dishes with black agar, as follows: To 500 ml of water in a 1 liter flask or beaker, add 15 grams of agar (any type of agar you have on hand will do). Heat on a burner or hot plate to bring the water to the boiling point and dissolve the agar. Allow to cool just enough that you can handle the container comfortably. Mix well and then stir in a tablespoon of powdered charcoal. Pour carefully into petri dishes and allow them to sit undisturbed until they have fully solidified. This recipe should be enough for about 25 dishes. The dishes do not need to be sterile.

Set up lights in the area where the dishes will be incubated. In order to minimize the differences in incubation conditions to which the "light" and "dark" dishes are exposed, have the students who have been assigned to the "dark" group wrap their dishes in foil and incubate them right next to the "light" dishes.

ANSWERS TO THE ALBINO PLANT WORKSHEET STUDENT PAGE 174

DATA FROM YOUR OWN PAIR OF GROUPS
Students should observe approximately a 3:1 ratio of green to white on the light dish and only white plants on the dark dish

DATA FROM THE WHOLE CLASS
Similarly, students should observe approximately a 3:1 ratio of green to white on the light dishes, and only white plants on the dark dishes

ANSWERS TO DATA ANALYSIS STUDENT PAGE 175

Use the data collected by the class as a whole to answer the following questions.

1. What ratio of green seedlings to white seedlings was present in the light-incubated seedlings on the first day of observation?
   Because students are instructed to use whole-class data, they should all have the same ratios.

2. What did you expect the ratio of green to white seedlings to be, given that this difference in color has a simple genetic basis? Explain.
   3:1 This is the ratio to be expected if the absence of green color is caused by a recessive allele at a single genetic locus.

3. Do you think that the difference between the exact ratio that you calculated for Question 1 and the expected ratio that you gave for Question 2 is significant? Explain
   Students may differ in their opinions regarding the significance of the deviation. However. if they performed exercises E.2 and E.3 in Chapter 2 they should say that one way to find out whether the difference is significant is to perform a Chi square analysis.

4. What ratio of green plants to white plants was present in the dark-incubated seedlings on the first day of observation?
   They should have observed no green seedlings following dark incubation.

5. Is this significantly different than the ratio of green plants to white plants that was present in the light-incubated seedlings on the first day of observation?
   Yes.

6. Formulate a hypothesis to explain the difference between the light-incubated and the dark-incubated seedlings.
   Students should postulate that light is required for chlorophyll synthesis.

7. How could your hypothesis be tested?
   By placing the dark-incubated plants in the light.

8. Compare the ratios of green to white seedlings that were observed in the dark-incubated seedlings on the first observation and the second observation:
   They should get a green:white ratio of approximately 3:1 on the second observation (in contrast to the absence of green seedlings that they should have seen on the first observation)

9 How do you account for any difference between these two ratios?
   Chlorophyll synthesis requires light, so you can't analyze genes involved in controlling chlorophyll synthesis in seedlings that have not been exposed to light.

10. What overall conclusion can you draw from the data collected in this experiment?
    Both genes and environment are important in determining the phenotype of an organism.

AN OPTIONAL STEP: CHI-SQUARE ANALYSIS ANSWERS STUDENT PAGE 177

1. Following the instructions for How to Perform a Chi-Square Test on Any Data Set, (Chapter 2, Section E.3) and using the data collected by the class as a whole, calculate χ^2 and p for the light-incubated seedlings on the first day of observation.
   It is hoped that the whole-class data will yield a value of p greater than 5%.

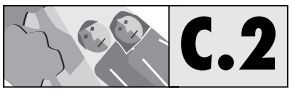
2. Based on the value of p that you obtained, do you think that the class data for the light-incubated seedlings are consistent with the hypothesis that seedling color is determined by a pair of alleles that exhibit a simple dominant-recessive relationship? Explain.
   Students should use the 5% cut-off rule to determine the significance of the deviation between their expected and observed ratios: If they get a p value greater than 5%, this indicates that such large deviations can be expected to occur more than 5% of the time by chance alone and therefore that their data are consistent with the hypothesis used to make the predictions.

3. Repeat the calculation of χ^2 and p for the light-incubated seedlings on the first day of observation, using only the data collected by your own group.
   It may be that some students will obtain p values smaller than 5%, owing to their small sample size.

4. Based on the value of p that you obtained this time, do you think that your own data are consistent with the hypothesis that seedling color is determined by a pair of alleles that exhibit a simple dominant-recessive relationship? Explain.
   See answer to Question 2.

5. Which data set gave you the higher p value, the class data or your own data? Explain.
   This could go either way because of the statistics of small numbers. Some groups may get a larger p value with their own data than with the class data, and other groups may get a smaller p value.

6. Do you think you need to perform a χ^2 test to determine whether the data that your class collected with respect to the dark-incubated plants during the first observation are consistent with the proposed hypothesis? Explain.
   No. It should be obvious by inspection that the data are inconsistent with the hypothesis.



Heart Disease: a Personal Gene-Environment Interaction

STUDENT PAGES 178-181

LESSON OVERVIEW

This exercise attempts to demonstrate the personal relevance of the principle illustrated by the previous exercise – namely that gene-environment interactions determine one's phenotype. Students will perform a simulation that allows them to see how their genes and their lifestyle will interact to determine their chance of developing heart disease.

Although heart disease is the example chosen for illustration here, most serious human diseases (such as cancer, diabetes, and schizophrenia) resemble heart disease in that they are **multifactorial**. That is to say, the probability that one of these diseases will strike any given individual – and the severity of the symptoms if it does occur – is determined by a number of interacting genetic and environmental factors.

TIMELINE

This exercise and discussion requires 50 minutes to complete.

MATERIALS

For each group of four or five students:

- 4 labeled envelopes containing Environmental Risk Cards
- 2 containers each containing 12 poker chips or other objects in three colors

ADVANCE PREPARATION

Photocopy, cut apart, and separate by category the Environmental Risk Cards, which are found on page T196. You will need a minimum of one copy of the page for every five students in your class, and lamination will make the cards much more durable. Place the cards printed side down in envelopes that are labeled *A - Body Weight*, *B - Diet*, *C - Smoking*, and *D - Exercise*.

Prepare two containers with objects of similar size and shape in three different colors. (For example, red, white and blue poker chips, although pieces of colored paper will do.) Label one container *Father's Genes* and the other container *Mother's Genes*. Place four objects of each color in each container. Make sure that the three colors are well-mixed.

PROCEDURE

Pass out envelopes containing Environmental Risk Cards to each group. Each student should take one card from each envelope and record the results on the work sheet.

Pass the containers with colored objects to each group. Each student should draw three objects from each container with eyes closed, record the colors on the work sheet, then return the objects to the bowl and mix them up before passing the bowl on. Make it clear that the intent is to simulate which allele each student inherited at each of three different loci that affect heart health. (The three different colors indicate three different alleles that can be present at each of those three loci.)

After all students have drawn and recorded three Mother's Genes and three Father's Genes on their work sheets, inform them of the genetic risk that you have assigned each color. The recommended values are 0, 2 and 4. (For example, white = a genetic risk of 0, blue = 2, and red = 4.)

HINTS AND TROUBLESHOOTING

After students have completed their work sheets, you might wish to go around the room, asking students to read out the values they have calculated for the percentage of their total heart disease risk that is due to genetic factors. (You may even wish to tabulate and average the numbers.) This exercise was designed so that the average genetic risk would be about 50% of the total risk. This ratio is arbitrary but probably not too far from reality: few individuals have such a high genetic risk that they are extremely likely to develop heart disease despite a relatively healthy lifestyle, and, similarly, few individuals can lead very unhealthy lifestyles and not develop heart disease because of a very low genetic risk.

Some of the points that you might wish to bring out during the discussion might include:

- The numbers that are used in this exercise for risk factors are for illustrative purposes only. While they are consistent qualitatively with current understanding of certain heart disease risk factors, it should not be assumed that they accurately reflect the actual numerical risks.
- Only four lifestyle factors have been considered here. Although these may be among the most important factors, they are not the only factors affecting the risk of heart disease. Others the students may be able to think of are stress, alcohol and drug abuse. One nongenetic risk factor they probably will not think of is testosterone, which is the reason heart attacks are more common in men than in women.
- Similarly only three loci, with only three alleles per locus (represented by the three different colors), have been considered in this simulation. In reality numerous loci appear to influence the development of heart disease. Moreover, these differ much more in their relative importance than the three loci considered here; some have only a modest effect, whereas others have a very pronounced effect on the probability that heart disease will strike.

- Furthermore, this simulation is based on the assumption that all genetic and lifestyle risks are additive. This is known to be a false assumption; some risks are known to interact in a multiplicative fashion. For example, dietary factors such as saturated fat and cholesterol intake assume much greater importance in individuals with genetic abnormalities in lipid metabolism than they do in the rest of the population. *
- Even with a very high risk score, it is seldom the case that one is certain to get heart disease. Nor does a very low risk score guarantee that one will never have heart disease. Chance plays a part. For example, in a pair of identical twins with virtually identical lifestyles, it is sometimes the case that one twin will suffer a heart attack and the other will not.
- Students who want more information about their personal risk of heart disease should be referred to their family doctor or other health care professional. Your local chapter of the American Heart Association can provide useful information and handouts for students.

*If time permits, you may find it useful to go into more detail about the factors affecting blood cholesterol levels, in order to make the concepts in this paragraph and the preceding one seem less abstract. The following should provide more than enough material for organizing such a discussion.

Most people now realize that diet (and particularly the amount of saturated fat eaten) is an important determinant of blood-cholesterol levels, and they know that people with high blood-cholesterol levels have a high heart attack risk. But few people realize how many different genes also contribute to determining their blood-cholesterol levels. Our blood cholesterol level is influenced not only by the amount of fat we eat, by also the alleles we happen to have inherited from our parents at each of the several dozen loci that encode proteins involved in various aspects of fat metabolism. Individually, most common alleles at most of these loci have only modest positive or negative effects on blood cholesterol levels. Nevertheless, they interact, and certain combinations of alleles at several of these loci can result in extremely large differences in blood cholesterol levels between individuals who have extremely similar diets and life styles. But in addition to these many "minor-effect" loci, there several loci at which mutant alleles can have a really major, life-threatening impact on blood cholesterol levels. The most important of these genes and its product will be discussed below, but in order to understand why it is so important, we need to understand certain aspects of the chemistry of blood cholesterol, and why elevated cholesterol levels are associated with heart disease.

Because cholesterol is a fatty substance that is not water soluble, it can only remain suspended in the blood stream if it is in the form of a complex with carrier-protein molecules known as **lipoproteins.** Cholesterol molecules that accidentally slip out of such a complex precipitate out of the blood to form waxy deposits on the walls of blood vessels. Such cholesterol deposits lead to **atherosclerosis,** or hardening of the arteries, which leads in turn to a greatly elevated risk of heart disease. The most common type of heart attacks are caused by **coronary artery disease,** in which atherosclerosis of the coronary arteries reduces the

blood flow to the wall of the heart so much that the heart becomes starved for oxygen and quits beating rhythmically.

Most of the cholesterol in the blood is carried as a complex with **low-density lipoprotein (LDL).** This LDL-cholesterol complex **(LDL-C)** is what physicians frequently call "bad cholesterol," because a consistently high level of LDL-C is what leads to cholesterol deposits in blood vessels and atherosclerosis. This is because a single LDL molecule can carry as many as 1500 cholesterol molecules, but these molecules are held rather loosely, so that they tend to fall off and form cholesterol deposits. Thus, the more LDL–C that is circulating, the more likely it is that some cholesterol molecules will fall off the complex. In contrast, the remaining 10-25% of the blood cholesterol (the **HDL-C,** which is carried by **high-density lipoprotein**) is called "good cholesterol," because HDL is capable of picking up cholesterol molecules that have been deposited on blood vessel walls, thereby counteracting the negative effects of LDL-C, and slowing the development of atherosclerosis.

If cholesterol is such nasty stuff, why do we have it in our blood at all? Why has natural selection not acted to remove cholesterol from the blood stream? The answer is simple: cholesterol is an essential component of all of our cellular membranes, and thus every one of our cells must have a supply of cholesterol. Surprising as it may seem at first, it is the "bad cholesterol" (LDL-C), not the "good cholesterol" (HDL-C), that cells tap into when they need cholesterol for making new membranes. When cells anywhere in the body are stimulated to grow or to repair their membranes, they make a plasma-membrane protein called **low-density-lipoprotein receptor (LDL-R).** LDL-R then traps circulating LDL-C complexes and causes them to be brought into the cell, whereupon the LDL is degraded while the cholesterol is used to make new membranes. When the cell has all the cholesterol it needs for membrane synthesis, it degrades its LDL–R, terminating the accumulation of cholesterol.

Whereas most body cells make LDL–R only when they are making new membranes, liver cells normally have LDL–R in their plasma-membranes at all times. This permits the liver to act as the garbage disposal for excess LDL-C in the blood. The more LDL–C in the blood, the more the liver takes up and destroys, thereby playing a critical role in regulating the amount of LDL–C in the blood.

Thus, both the uptake of LDL–C by cells that need cholesterol for growth, and the uptake of excess LDL–C by liver cells that are prepared to destroy it, requires a functional LDL–R molecule. Not surprisingly, therefore, a mutation in the gene encoding the LDL–R protein can markedly decrease the ability of the liver to take up and destroy LDL–C, which results in a marked increase in LDL–C in the blood, and the disease known as **familial hypercholesterolemia,** or **FH.** FH is one of the most common hereditary human diseases, affecting one person in 500 (which means it is much more common than cystic fibrosis or sickle cell anemia, for example). Why is it so common? The answer is: because it is inherited as an autosomal dominant condition, which means that everyone who has one mutant gene has FH symptoms. This is because it takes only one mutant gene to decrease the amount of LDL–R on liver cells by half, and this is all it takes to double the

level of LDL–C in the blood, moving it into the danger zone. Moreover, with only half as many LDL–R molecules in the liver to remove excess cholesterol from the blood, the LDL–C levels of people with FH are much more sensitive to differences in dietary fat intake than are the LDL–C levels of people with two normal LDL–R genes. Thus the occasional fatty steak that can be well tolerated by other individuals can be life threatening for someone with FH. Moreover, from the moment of birth the LDL–C levels of babies with FH average twice as high as those of babies lacking LDL–R mutations, and thus development of atherosclerotic lesions begins in early infancy, particularly if the infants have a high-fat diet.

Perhaps the statistic that indicates most clearly the importance of the LDL–R gene in human health is the fact that although the frequency of FH in the general population is only 1 in 500, the frequency of FH among those who suffer serious heart attacks is 1 in 20. Putting it differently, people with FH are 25 times as likely to have a heart attack as people without FH. Men with FH may have fatal heart attacks before age 40, and 85% of them will have heart attacks before age 60. Women with FH have nearly as great risks of serious heart attacks in the long run, but their peak risks are delayed by several years. (This difference is attributed to the effect of testosterone on cholesterol metabolism.)

If FH is so serious, why has it not been eliminated from the population by natural selection? Again the answer appears straightforward. Until blood-cholesterol assays became fairly routine, most people were unaware that they had FH until they had a heart attack, by which time their children had been born and their mutant LDL–R gene had been passed on. Even though most people now appear to be aware of the importance of controlling their blood cholesterol levels, a study performed in 2000 found that only one fourth of the people between the ages of 30 and 50 who had FH had been diagnosed before the study was performed. Natural selection is powerless to eliminate heritable traits that arise after the reproductive years are over, no matter how detrimental to later health those traits may be.

Although natural selection does not act to prevent reproduction by FH heterozygotes, it certainly does so for FH homozygotes. The unfortunate one human being in 1,000,000 who is born with two mutant LDL–R genes has LDL-C levels that are so high that heavy cholesterol deposits begin to form almost immediately – not only in blood vessels, but also in skin, in hands, feet and eyes, and various other organs throughout the body. Such children may suffer fatal heart attacks before they are two years old, and they seldom live to puberty, even with strict dietary supervision and the best medical care available.

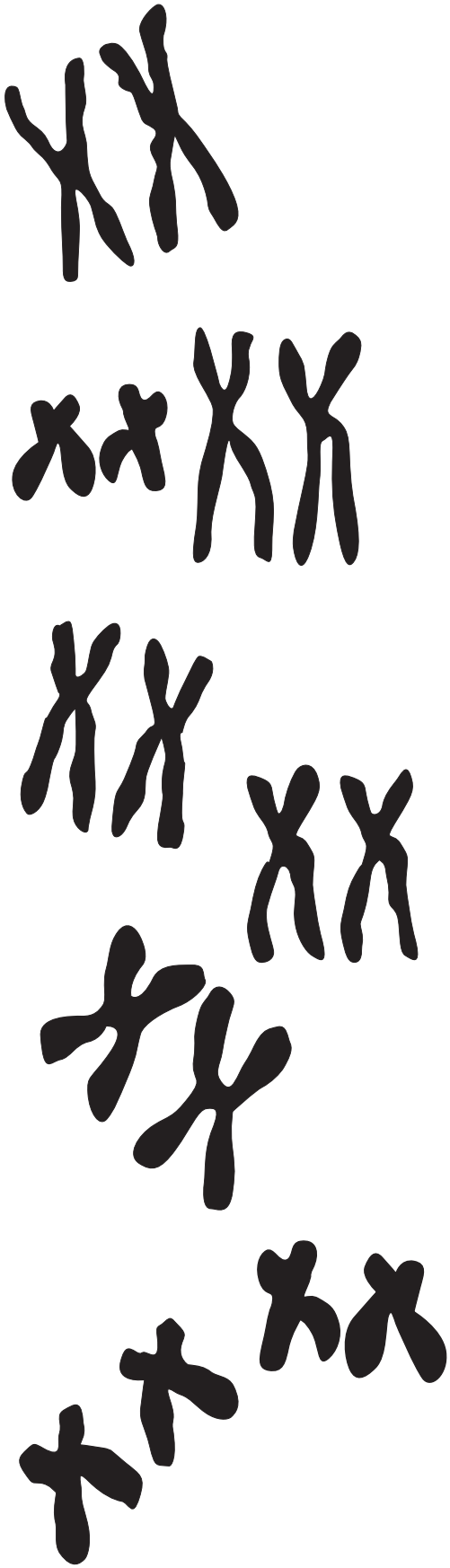
ACKNOWLEDGMENTS

This exercise was adapted from Lesson 3 in *Health in the Year 2100: What's Heredity Got to Do With It?*, a high-school genetics-curriculum unit developed at the University of South Dakota School of Education.

CLOSURE

The lessons from this exercise and the preceding one both point to the idea that genes and environment interact to determine phenotype. However, it is hoped that through this exercise your students will have come to appreciate that this is not just an abstract scientific generalization to be learned for a test but a statement about the real world that could affect their own lives in many important ways.

Maintain desirable body weight at all times SCORE = 0 **A**	Sometimes up to 20 pounds overweight SCORE = 2 **A**
Usually 10-20 pounds overweight SCORE = 3 **A**	Always 20-30 pounds overweight SCORE = 4 **A**
Always at least 50 pounds overweight SCORE = 6 **A**	Strict vegetarian SCORE = 0 **B**
Eat chicken or fish, but no beef SCORE = 2 **B**	Eat beef or pork once or twice a week SCORE = 3 **B**
Eat beef or pork most days SCORE = 4 **B**	Eat beef or pork at least once a day SCORE = 5 **B**
Never smoke SCORE = 0 **C**	Smoke less than five cigarettes a week SCORE = 2 **C**
Smoke one to four cigarettes per day SCORE = 4 **C**	Smoke about one pack of cigarettes every day SCORE = 6 **C**
Smoke more than one pack of cigarettes every day SCORE = 8 **C**	Vigorous exercise every day SCORE = 0 **D**
Vigorous exercise most days SCORE = 1 **D**	A little bit of exercise every day SCORE = 2 **D**
Exercise every other day or so SCORE = 3 **D**	Exercise once a week or less SCORE = 6 **D**



CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION D

What Are Some of the Features of "Simple" Genetic Diseases?

Chapter 3: Section D Background

THE VAST MAJORITY OF human beings are born healthy. Sadly, however, many are not. About one child in twelve in this country is born with a birth defect that will cause significant health problems by no later than age seven. Some genetic abnormality plays an important role in the development of the disease symptoms in a majority of such **congenital defects**. The term **genetic disease** is applied in all such cases, despite the fact that it is known that environmental factors usually play important roles in determining whether or when disease symptoms will appear and/or how serious those symptoms will be. More than 4,000 diseases in which a mutation of a single gene is a causative factor are now recognized. These are the so-called **single-gene diseases**. Currently, 25-30% of the patients in a typical metropolitan children's hospital will be individuals suffering from some such disease.

There may be a tendency to think of the parents of children who suffer from such a disease as "people with bad genes." Such an attitude clearly cannot be justified, however. On average, each of us is a carrier for mutations in about twenty genes, each of which would have caused us to have a serious disease had we received two mutant copies of one of those genes instead of just one. One of your twenty mutations also could cause you to be one of those unfortunate parents with a very sick child if you have a child with another person who carries one of the same mutations. The only reason that so few children suffer serious genetic disease is that each of the mutations we carry is so rare that we are extremely unlikely to meet and fall in love with someone who has mutations in the same genes.* But it could happen, of course. So serious genetic diseases are not so much the result of having bad genes as having bad luck.

This section deals primarily with single-gene diseases. Its focal point is Section D.5, in which students are challenged to learn as much as they can about one such disease and produce an informative pamphlet about that disease. (This pamphlet should be of the type that might be used by a genetic counselor.) The rest of the section provides background information that will help your students understand some general principles regarding genetic diseases and certain kinds of specific information that they may encounter as they do their research.

*The exception to this rule comes if one marries a close relative. Recognition of this fact from time immemorial is why most societies have banned marriage between cousins or more closely related individuals.

Some "Simple" Heritable Defects

STUDENT PAGES 184-185

LESSON OVERVIEW

This lesson is a student reading designed to introduce a few examples of familiar human conditions caused by a gene mutation that results in a single protein being defective.

TIMELINE

It will take an average student 5 minutes to read this material. You should discuss the table on S185 in class, as most students will have heard of many of the conditions listed, but will likely know little about the protein affected.

Phenylketonuria (PKU) Illustrates the Complexities of Some "Simple" Genetic Diseases

STUDENT PAGES 186-187

LESSON OVERVIEW

This lesson is a student reading designed to illustrate how "simple" single-gene diseases often involve complex interactions among multiple genetic and environmental factors.

TIMELINE

It will take an average student 5 minutes to read this material. It could be assigned as homework.

D.3

The Special Inheritance Patterns of Sex-Linked Mutations STUDENT PAGE 188

LESSON OVERVIEW

In this lesson, students will learn about the unusual inheritance patterns that accompany a mutation of a gene that is located on the X chromosome. Students will also deduce some of the special consequences arising from those mutations.

TIMELINE

The reading and work sheet require 50 minutes to complete.

ANSWERS TO SEX-LINKED MUTATION WORK SHEET STUDENT PAGE 189

1. If a woman is a carrier for a mutation causing a sex-linked disorder, what is the chance (in percentages) that one of her sons will have the disorder? Explain.
   50% Each of her sons will inherit one of her X chromosomes, and there will be a 50-50 chance that it will be the one carrying the mutant allele.

2. If a woman who is a carrier for a sex-linked disorder already has one son who has the disorder, what is the chance that if she has a second son he will also have the disorder? Explain.
   50% The probability that each son will inherit the mother's mutant allele is 50%, regardless of which allele any of his brothers has inherited from her.

3. If a man has a sex-linked disorder, what is the chance that he will pass it on to one of his sons? Explain.
   0 % Sex-linked mutations are carried on the X chromosome, and sons never inherit an X chromosome from their father.

4. If a man has a sex-linked disorder, what is the chance that one of his daughters will be a carrier for that disorder? Explain.
   100% Every girl inherits an X chromosome from her father, and the only X chromosome a man with a sex-linked disorder has is the one carrying the mutation.

5. If a man has a sex-linked disorder, what are the chances that one of his grandsons will inherit that disorder? Explain
   0 or 50% The sons of his sons will have a 0% chance of inheriting his disorder, but the sons of his daughters will have a 50% chance.

6. It has been postulated that a condition known as hairy ears is caused by a mutation of a gene on the Y chromosome. Assuming this is true, what is the chance that one of the sons of a man with hairy ears will inherit the hairy-ear mutation? Explain.
   100% Every boy inherits his father's Y chromosome.

7. What is the chance that one of the daughters of the man referred to above will pass the hairy-ear mutation on to one of her sons? Explain.
   0% Girls inherit their father's X chromosome, never his Y chromosome, so his daughters do not get a copy of his hairy-ears chromosome to pass on.

Investigating Human Genetic Diseases

STUDENT PAGES 190-192

LESSON OVERVIEW

This exercise is designed to help your students study particular human diseases on their own and in detail. Then they are challenged to synthesize what they have learned and produce an informative pamphlet that is of the sort a genetic counselor might give to an individual or family afflicted with the disease in question.

TIMELINE

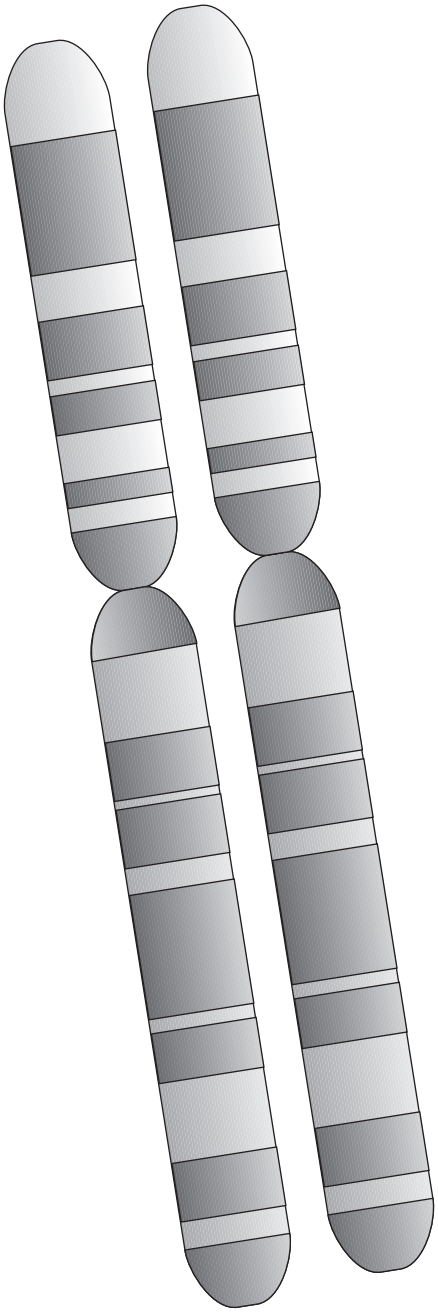
It is recommended that students be given a week to perform their research and produce a pamphlet. You will need to decide how much class time will be devoted to this and how much you will expect to be done as homework. It usually requires at least three class periods (50 minutes each) for students to do adequate research and produce the pamphlet during class time.

PROCEDURE

You need to decide whether to assign a topic to each student, allow each of them to pick a topic from the lists in this section and Section D.1, or have each of them draw the name of a disease from a hat. There are substantial advantages to the latter method: If students are allowed to choose their own topics, they often will all end up studying one of a handful of most familiar diseases, such as PKU, cystic fibrosis, or sickle-cell anemia. On the other hand, when the teacher assigns the topics, some students get disgruntled if they believe that they have been assigned a "tough" or "boring" topic, whereas a friend has been assigned an "easy" or a "cool" one.

HINTS AND TROUBLESHOOTING

Many computer word processing programs (such as Microsoft Word and Claris Works) have a multicolumn option that allows students to design an attractive trifold pamphlet.



CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION E

How Does a Genetic Counselor Detect Mutant Genes?

Chapter 3: Section E Background

DUCHENNE MUSCULAR DYSTROPHY (DMD), which is the subject of the following exercise, is a relatively common sex-linked disease. It affects about 1 boy in 3000, most of whom appear to be healthy until age 4 or 5, whereupon they begin to develop muscular weakness. Frequently, the first symptoms are problems with running and climbing stairs. Affected individuals are usually wheelchair-bound before they reach their teens and few survive into their twenties, most frequently dying from lung or heart failure. Fewer than 10% of carrier females exhibit any muscular weakness as a consequence of having one mutant allele, and female homozygotes are extremely rare, since very few affected males ever become fathers. There are currently no effective treatments for this disease.

DMD can result from any one of a variety of mutations in the gene coding for **dystrophin**, an important structural protein in the muscle, heart, and brain. (Dystrophin's essential role in the brain is pointed to by the fact that patients with DMD often show a decline in IQ scores as the disease progresses.) A milder form of the disease, called Beckers muscular dystrophy results from mutations that cause production of a partially functional dystrophin molecule.

The dystrophin gene is the largest human gene that has been studied to date and occupies almost 2% of the entire X chromosome. Perhaps because of its size, this gene has an extremely high mutation rate, and nearly one third of all cases of DMD are the result of new mutations that occurred by chance during formation of the egg from which the affected boy developed. In such cases, the mother is not a carrier for DMD and is very unlikely to have a second child with a defective DMD allele. However, because very few boys with DMD ever live long enough – or are healthy enough – to produce children, new mutant DMD alleles that show up in boys usually disappear from the population in one generation.

New DMD mutations that show up in girls persist much longer, on average, because such girls become carriers, who have a 50% chance of passing their mutant gene on to each of their offspring. Moreover, because girls receive an X chromosome from each parent, they're about twice as likely as boys to end up with a novel DMD mutation – one that occurred during formation of either the egg or the sperm from which they received their genes. It is estimated that an average genetically normal male produces a sperm cell with a new mutation in the dystrophin gene every 10-11 seconds. (As high as this mutation rate may sound, the rate of sperm production in a healthy male is so enormous that only a tiny fraction of all sperm carry a new DMD mutation.)

Most dystrophin gene defects resulting in DMD are **deletions** (the absence of some normal portion of a chromosome) of varying sizes. These deletions are the basis for many of the available diagnostic tests. Frequently, DNA samples from patients and family members are analyzed by **PCR amplification** of several different portions of the dystrophin gene where deletions are known to occur, followed by separation of the resulting DNA fragments on an agarose gel. (PCR amplification is explained in the next section.) Individuals with deletions

will either lack certain DNA bands or will exhibit smaller bands than family members without the defect.

BACKGROUND REFERENCES

Mange, A. P. and Mange, E. J. Genetics: Human Aspects. 36-40. Sunderland, Mass.: Sinauer Associates, 1990.

National Institute of Health. Understanding Gene Testing. (Bethesda, MD: NIH. 1995), Pub. 95-3905.

HOW PCR IS USED TO AMPLIFY A GENE FRAGMENT OF INTEREST

The polymerase chain reaction (**PCR**) referred to above is one of the most important, most powerful and most widely used techniques in modern biology. PCR is used routinely for a wide range of purposes by research biologists and genetic counselors (as is simulated in the following exercise). It also has become the most important method used by law enforcement agencies for personal identification. With it, a single hair or a single drop of blood found at a crime scene can be used to trap the guilty or free the innocent. Information in *Jurassic Park* notwithstanding, PCR has not yet been used to bring back the dinosaurs, nor is it likely to. However, PCR has been used to examine DNA sequences of tiny bits of plants and animals that lived long ago – including insects trapped in amber for more than 100 million years. Indeed, PCR has become so important in many areas of biology and medicine that Kary Mullis was awarded the Nobel Prize in Chemistry for inventing it.

PCR is based on one simple but important fact about **DNA polymerase**, the enzyme that replicates DNA in cells before each round of cell division. This fact is that in order for DNA polymerase to replicate any target DNA molecule (which is called its **template**), it must have a short piece of nucleic acid, called a **primer**, that is complementary in its base sequence to part of the template. The primer base-pairs to the template and acts as the starting place for DNA synthesis. DNA polymerase then functions by adding nucleotides to the primer in the sequence that is specified by the template.

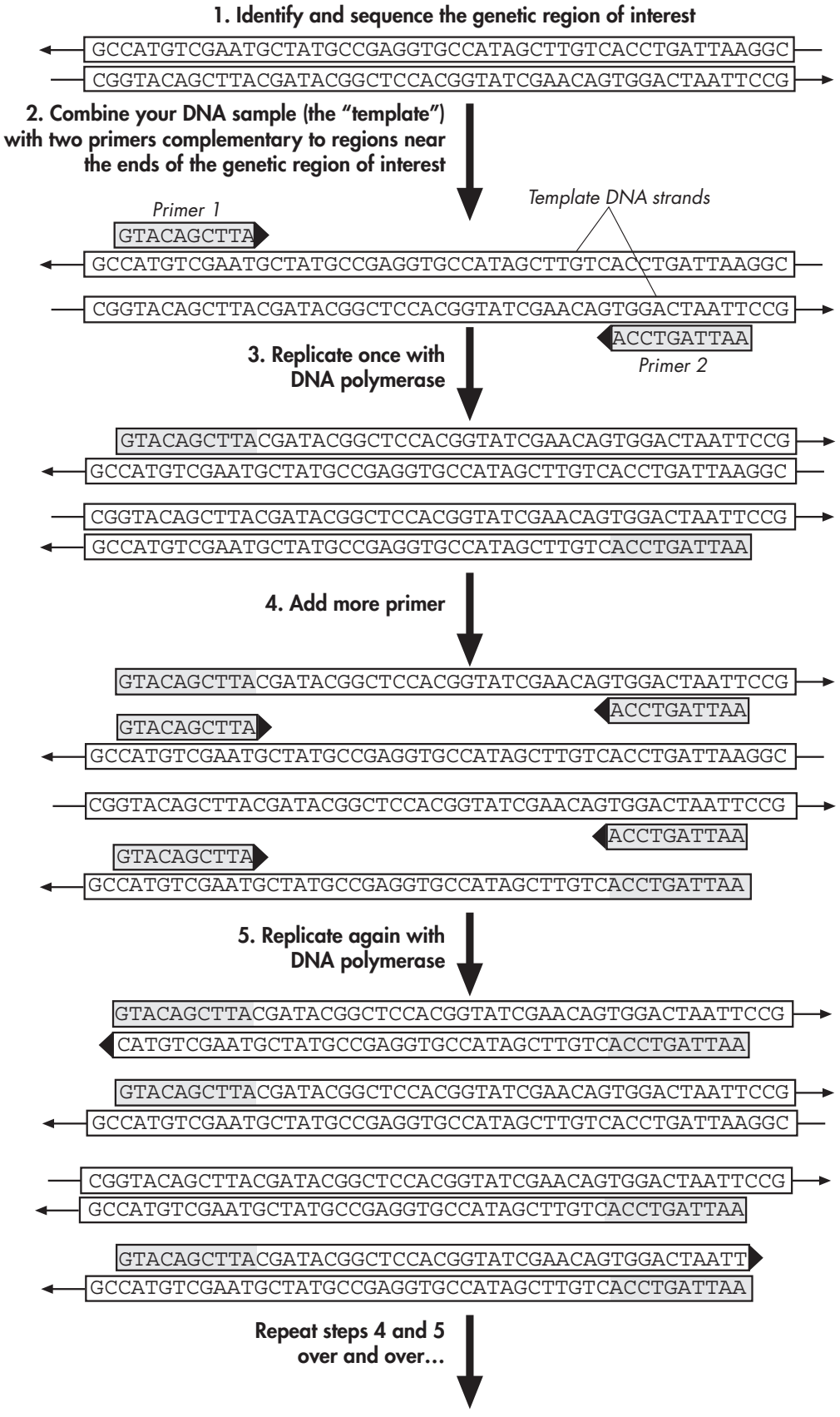
In living cells, a whole set of other proteins are required to find the location on a chromosome where replication is to begin and to make a primer that DNA polymerase can use to get started. The details of how this is accomplished need not concern us here. What is important is to recognize that if DNA polymerase has a primer and a template it can begin replicating the DNA. But if DNA polymerase has no primer, it is incapable of copying DNA. Therefore, one can get DNA polymerase to copy just exactly the part of a template DNA molecule that one is interested in by providing it with primers that define the desired starting places on both strands of the double-stranded molecule of interest. (See the diagram on the next page.)

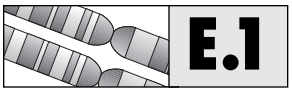
Of course, one must know the DNA sequence in the region of interest in order to design useful primers. In the diagram, the primers shown are ten nucleotides long (and with pointed ends indicating where DNA polymerase will add nucleotides). In actual practice longer primers must be used to assure that they will base pair with – and initiate DNA replication from – only the desired sites in the template DNA molecules. That is because a site comple-

mentary to a primer only 10 nucleotides long will occur by chance about once in every 4^{10} (equal to about one million) base pairs, or about three thousand times in the human genome, which is 3 x 10^9 base pairs long. In contrast, a site complementary to a primer 25 nucleotides long will occur by chance only about once in every 4^{25}, or 10^{15}, base pairs.

In the diagram, the starting DNA sample is represented by a sequence of bases in the region of interest superimposed on an arrow (which is meant to indicate that the template goes on and on). In the first round of replication, only the initial DNA serves as a template. But then in the second round the new DNA molecules can also be used as template. So in each round of synthesis the number of template molecules used, and the number of new molecules produced is doubled. This is where the chain reaction part of the term *PCR* comes from. In 30 rounds of replication, 2^{30} (more than a billion) molecules of the gene of interest are produced for every starting double-stranded molecule present in the original sample. This process produces more than enough of the DNA needed to allow for its study in a variety of ways. This includes gel electrophoresis, the process your students will simulate.

POLYMERASE CHAIN REACTION DIAGRAM





Detecting the Duchenne Muscular Dystrophy (DMD) Mutation

STUDENT PAGES 194-199

LESSON OVERVIEW

In this electrophoresis exercise, your students will pretend to be technicians in a genetic counseling lab. They will simulate the technique that would be used in such a lab to determine which family members might share the mutant dystrophin gene that has caused one of the Smith boys to develop Duchenne muscular dystrophy. To circumvent all sorts of logistic problems, in this simulation we will use two dyes with different electrophoretic mobilities to simulate two PCR fragments of different lengths - the normal allele and the DMD allele of the dystrophin gene.

Key concepts explored in this activity include a) electrophoresis as a way to separate charged molecules from one another, b) DNA analysis as a way to detect genetic abnormalities, c) X-linked genetic diseases, d) genetic counseling, and e) the personal implications of DNA analyses. It is an excellent complement to Exercise D.4, in which the students will prepare a pamphlet for use by a genetic counselor. Thus, you should schedule this activity for the period of time during which your students will gather the information for their pamphlets.

TIMELINE

There are at least three different ways you can schedule this exercise. Options 1 and 2 require that you have at least as many gel combs and at least half as many gel casting trays as you will have lab groups in all of your classes combined. If you have more than one class but only enough gel casting trays for one class, Option 3 is your only choice. Option 1 takes the least class time but requires more advance preparation on your part.

Option 1

You pour the gels in advance; the students complete the rest of the exercise, including analysis and discussion, in one class period (50 minutes).

Option 2

Day 1: One period is devoted primarily to discussion of various topics related to this exercise, such as the nature of sex-linked diseases in general and DMD in particular, the principles of electrophoresis, and genetic diagnosis and counseling. The students also pour their gels, wrap them in plastic wrap, and store them in the refrigerator overnight.
Day 2: Students load their gels (5 minutes), run the electrophoresis (15 minutes), and then record, analyze, and discuss their results (30 minutes).

Option 3

Day 1: Students perform the entire lab exercise in one class period, including pouring their gels, loading and running them as soon as the agarose has solidified, and recording the results on their work sheets. This will take most of a 50 minute period, but there will be two 10-15 minute intervals (while the gels are solidifying and while the electrophoresis is occurring) when some discussion will be possible.
Day 2: Students analyze and discuss their results.

MATERIALS

For each class:
- electrophoresis power supplies
- gel electrophoresis chambers

For each group of students (group size to be determined by equipment availability):
- 1 precast agarose gel, or 1 gel-casting tray plus masking tape, 1 or 2 gel-casting combs and 50 ml of 0.8% agarose in water
- a small container of tap water
- 1 20 µl micropipettor
- 6 dye samples labeled A-F
- 6 pipette tips

The number of electrophoresis chambers and power supplies that you have available will determine the maximum number of groups that can perform this exercise at once. A power supply that will run two gel electrophoresis chambers at once works well for this exercise. The number of groups that can run samples simultaneously with one such power supply is increased to four if extra combs are available. This will allow two sets of samples to be run in each gel. The combs and trays are inexpensive relative to the cost of the rest of the equipment, and they greatly increase flexibility. If you will be running two sets of samples per gel, you or the students who are preparing the gel will need to place two combs in each tray before pouring the agarose. One comb should be placed about 1 cm from one end and the second comb should be placed just beyond the middle of the tray.

The above mentioned materials can be ordered from:
Carolina Biological
(800) 334-5551
www.carolina.com
Electrophoresis Power Supply- catalog # BA-21-3672
Gel Electrophoresis Chamber- catalog # BA-21-3668
Extra Comb- catalog # BA-21-3666
Extra Gel Casting Tray- catalog # BA-21-3667

Agarose can be ordered from:
Carolina Biological
Agarose- catalog # BA-21-7080

Sigma Chemical Company
(800) 325-3010
www.sigma/aldrich.com/order
Agarose- catalog # A0169
Bromphenol blue and xylene cyanole can be ordered from:
Sigma Chemical Company
Bromphenol Blue- catalog # B0126
Xylene Cyanole- catalog # X4126

ADVANCE PREPARATION

1. **Prepare 0.8% agarose solution, 50 ml per group.** The following recipe is for 200 ml (4 groups):

   • To 200 ml water in a 500 ml flask add 1.6 grams agarose.

   • Cap flask with foil and heat carefully on a hot plate, or cap with plastic wrap and heat carefully in a microwave oven for 3-5 minutes.

   Safety Note: Agarose solution can superheat and either boil over during heating or erupt violently when the flask is touched. Handle hot agarose very carefully, wearing safety goggles and heavy gloves.

   • Swirl flask and make sure that all agarose has dissolved.

   • If gels are to be poured right away, cool flask to about 60°C before pouring.

   • If gels are to be poured later the same day, hold flask in a 60°C water bath until use.

   • If gels are to be poured another day, store solution covered and refrigerated. (Keeps for several weeks.) Then, well in advance of scheduled use, reheat agarose carefully on a hot plate or in a microwave oven until it is completely melted. Then bring it to about 60°C before pouring gels.

   If you will be pouring the gels yourself, follow the instructions given on the student pages for this exercise. When the gels have solidified, wrap each one in plastic wrap to prevent it from drying out.

2. **Prepare stock solutions of bromphenol blue and xylene cyanole.** In this exercise we will simulate the DNA samples of the various Smith family members with a pair of dyes, bromphenol blue and xylene cyanole. Bromphenol blue (BB) travels faster in an agarose gel, so it will be used to represent the "defective allele" that is a partially deleted version of the dystrophin (DMD) gene. Xylene cyanole (XC) travels more slowly in the gel, and so it will be used to represent the "normal" dystrophin allele. Prepare stock solutions as follows:

   • Label two small beakers or flasks BB and XC. Add 10 ml of deionized water and 1 ml of glycerol to each. Swirl to mix.

   Safety Note: Certain dyes can be dangerous to your health when they are in the dry state but become harmless once they have been dissolved. So weigh out all dyes in a fume hood, and wear a dust mask, goggles, and gloves until they are in solution.

- Weigh out 0.025 grams (25 mg) of bromphenol blue and add it to the BB container.
- Weigh out 0.025 grams (25 mg) of xylene cyanole and add it to the XC container.
- Carefully stir or swirl both containers until the dyes are thoroughly dissolved.

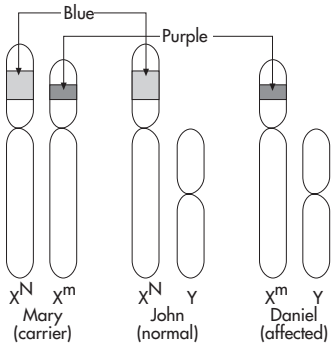
3. **Prepare simulated Smith family DNA samples from the stock dye solutions.** Set up six microcentrifuge tubes, lettered A–F, for each group. Add dyes as shown in the table below. Then cap the tubes and place each set of six in a sandwich baggie or other small container to hand out to each lab group. As you begin the experiment, inform the students which family member is represented by which letter, but do not provide the other information in the table.

Tube	Family member	Genetic condition	Dye sample used
A	Mother	Carrier	25 µl BB & 25 µl XC
B	Father	Normal	50 µl BB
C	Daniel	Affected	50 µl XC
D	Alice	Carrier	25 µl BB & 25 µl XC
E	Michael	Normal	50 µl BB
F	Fetus	Carrier	25 µl BB & 25 µl XC

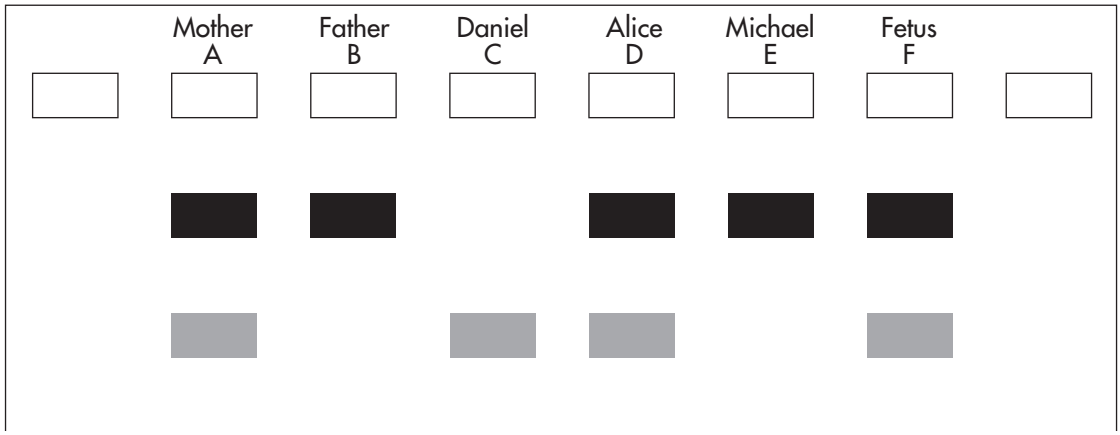
4. **Check that each group of students has established proper electrical polarity before giving them permission to turn on their power supply.** The only way that this experiment can work is if the students electrophorese their dye samples in the correct direction, which is from the black electrode (cathode) toward the red electrode (anode). Emphasize (a) that their gel should be placed in the electrophoresis chamber so that the wells will be at the end with the black electrode, and (b) that they should call you to check things over before they turn on their power supply. If you find a gel in the wrong orientation, do not try to move it, or all of the samples will be lost. Instead, switch the wires at the power supply, so that the black wire is connected to the red terminal and vice versa, thereby ensuring that current coming from the black terminal on the power supply will run to the end of the gel where the samples are located.

ANSWERS TO DMD DIAGNOSIS WORK SHEET <u>STUDENT PAGES 200-201</u>

1. On the diagram below, color the defective alleles purple and the normal alleles blue.



2. On the diagram below
   a. Above each well on this drawing of the gel, put the letter that was on the sample that was loaded into that well.
   b. Using colored pencils, draw all bands that you observed on your gel after electrophoresis.



3. Complete each of the blanks in the data table below:

Sample	Family Member	# of DMD Alleles	Genotype	Status
Tube A	Mother Mary	2	$X^N X^m$	Carrier
Tube B	Father John	1	***Y X^N***	Healthy
Tube C	Son Daniel	1	***Y X^m***	Has DMD
Tube D	Daughter Alice	***2***	***$X^N X^m$***	***Carrier***
Tube E	Son Michael	***1***	***Y X^N***	***Healthy***
Tube F	Fetus M or (F) (circle)	***2***	***$X^N X^m$***	***Carrier***

4. Which allele moves further into the gel, the normal (X^N) or mutant (X^m) allele? Why?
   The mutant (X^m) allele. It has undergone a partial deletion, so it is shorter, and shorter DNA fragments migrate faster than longer ones in gel electrophoresis.

5. Is the daughter, Alice, a carrier for DMD? How can you tell?
   Yes. Her DNA contains two fragments equal in size to the fragments representing the normal and mutant alleles present in her mother's DNA.

6. Does Michael have DMD? How can you tell?
   No. He has only the wild-type (normal) DMD allele.

7. What can you tell the Smith family about their unborn child?
   It is female, and like Mary and Alice, it will be a carrier for DMD.

8. Why are most patients with DMD male?
   A female with DMD is only produced in the extremely rare (1/10,000,000) cases when a carrier female produces an egg bearing the mutant DMD allele and that egg is fertilized by a sperm carrying a brand-new DNA mutation that arose during sperm formation.

9. Can a boy be a carrier for DMD without having the disease? Why or why not?
   No. Boys only have one X chromosome, so if they inherit a mutant DMD allele they get the disease.

10. If you were the genetic counselor in this case, what would you tell the Smiths about their test results?
    That there is some good news and some bad news. The good news is that neither Michael nor the unborn child will get DMD. The bad news is that both Alice and the unborn daughter are carriers.

Career

CHAPTER 3

How Genes and the Environment Influence Our Health

SECTION F

How Can I Become a Genetic Counselor ?

Chapter 3: Section F Background and Overview

EXPERIENCE INDICATES THAT AFTER completing this chapter (and particularly sections D and E), many students want to know what is involved in preparing for a career as a genetic counselor. This section is intended to be optional reading material for such students.