

CHAPTER 2

Passing Traits from One Generation to the Next

CHAPTER 2

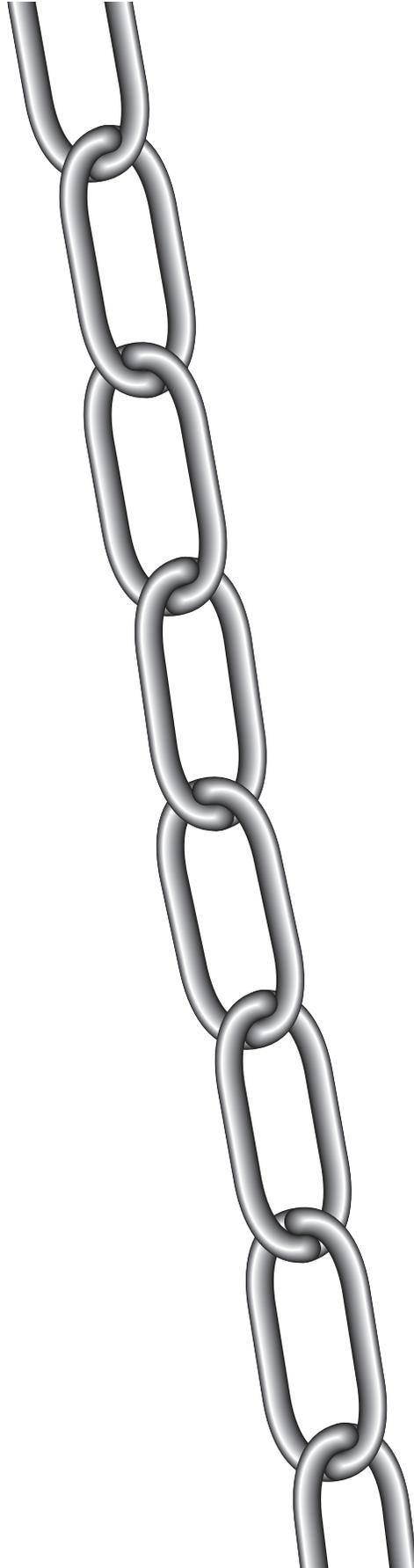
Passing Traits from One Generation to the Next

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Chapter 2 Overview

IN CHAPTER 1, YOUR students were introduced to the concept that DNA is the carrier of hereditary information. This should have raised more questions in their minds than it answered. For example, how does DNA get passed on from parents to children? Or if each child inherits DNA molecules from both parents, doesn't this mean that each person must have twice as much DNA as his or her parents? Or if my sister, my brother, and I all got our DNA from our Mom and Dad, and if the DNA we inherited determines all of our heritable traits, why don't we look just like one another? And why don't we look like some sort of average of Mom and Dad?

The purpose of this chapter is to help students answer such questions. To do this, we must move the discussion of heredity to a slightly more complex level. We have to consider how DNA actually gets passed on between the generations. And we have to think about how the information that this DNA contains is used to generate a whole new individual, not just a bunch of new protein molecules.



CHAPTER 2

**Passing Traits from
One Generation
to the Next**

SECTION A

**What is
Inheritance?**

An Introduction to Inheritance

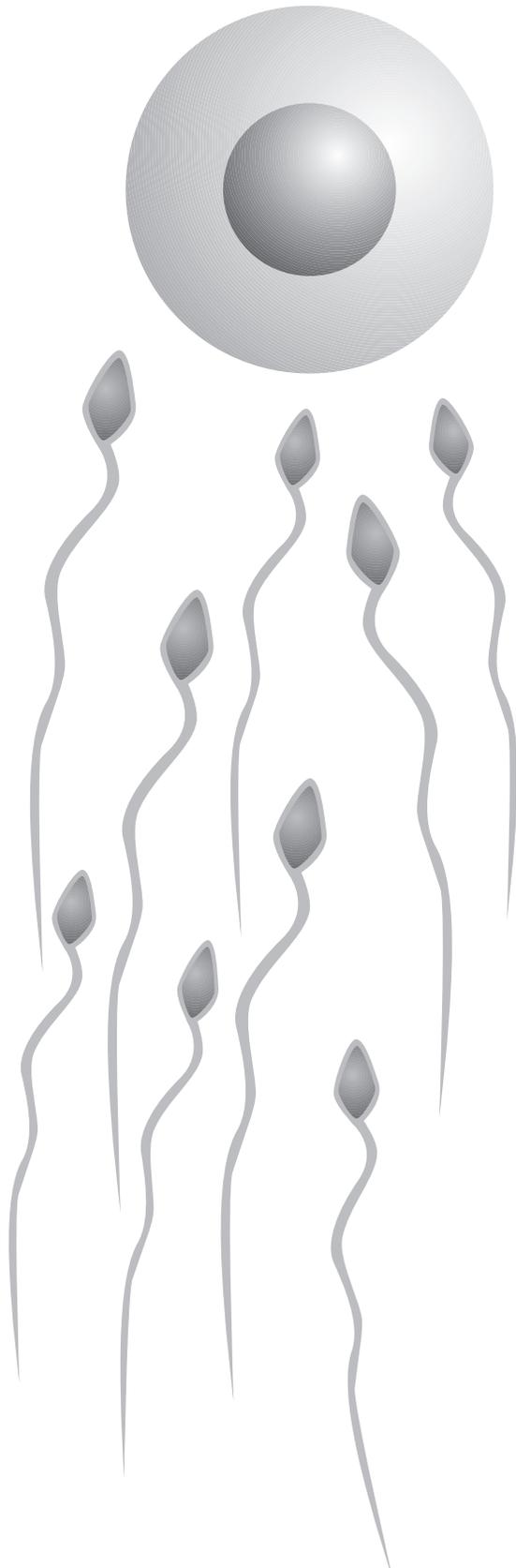
STUDENT PAGES 76-77

LESSON OVERVIEW

This section consists of only one part: a reading assignment designed to familiarize your students with the general concepts underlying the study of inheritance.

TIMELINE

It will take an average student 15 to 20 minutes to read this material. It could be assigned as homework.



CHAPTER 2

**Passing Traits from
One Generation
to the Next**

SECTION B

**How Does
a New
Generation
Get Started?**

Chapter 2: Section B Background

IT MIGHT SEEM THAT studies of embryos are out of place in a discussion of genetics. But nothing could be further from the truth.

It is only through the processes of embryonic development that the potential present in the DNA in an egg and a sperm can be converted into a physical reality – a new individual. Or, to say the same thing in technical terms that your students will learn to use in this chapter, it is during embryonic development that a **genotype** (an individual's complete set of genes) becomes transformed into its **phenotype** (its set of visible features).

This section begins with a discussion of what **model systems** are, and how they are used both to study heredity and development, and to yield insight about humans that could never be obtained by studying human beings directly.

In the second part of this section, students will work with one such model system, sea urchins, which are the premier model for studying the first few stages of the beautiful and mysterious process by which genotype is transformed into phenotype. Here they will watch the fusion of egg and sperm and the initial stages of embryonic development.

In the third and final part of this section, they will encounter an even more captivating example of genotype-to-phenotype transformation. In the *Nova* video, *The Miracle of Life*, they will benefit from extraordinary, award-winning cinemagraphic techniques that record the development of a human being from the time when egg and sperm are released, through the instant of their fusion, and the subsequent nine months of development, to the moment of birth.

Model Systems for Studying Heredity & Development

STUDENT PAGES 80-81

LESSON OVERVIEW

Progress in genetics has always been most rapid when geneticists have found an appropriate **model system**, an organism whose biological features make it very well suited for the experimental analysis of a specific set of questions.

Garden peas were the ideal model system for Gregor Mendel, a monk and botanist who was interested in asking the basic questions about heredity 150 years ago. But garden peas could never have been used to determine the chemical nature of the hereditary material or the way it works. For addressing that sort of genetic question, bacteria turned out to be the ideal model. But bacteria are of little value in addressing questions about the way particular genes control the development of specific body parts in complex organisms like ourselves. Such questions must be addressed with animals. The animals that have been most useful as model systems for asking this kind of developmental-genetic question include fruit flies, round worms, zebra fish, frogs, and mice.

Even the most ardent advocates of such developmental-genetic models have regularly been quite astonished, however, to discover the extent to which studies of the genetic control of embryonic development in one of these model systems turns out to apply to others – including ourselves. An expanded version of the example that is mentioned in the student pages may help to underscore this very important concept.

A few years ago, biologists studying fruit fly development discovered that an eyeless fly was produced whenever both copies of one particular gene were defective. For obvious reasons, they named this the “eyeless” (*eye*) gene. The wild-type *eye* gene is normally expressed (transcribed and translated) only in the region of the fruit fly head where the eyes will form. But when cells on a leg, or the back, or a wing of the fly were tricked into expressing the wild-type *eye* gene and making the corresponding protein, extra eyes developed in those regions of the leg, or back, or wing! This clearly suggested that the *eye* gene is a “master control gene” that is responsible for triggering the entire series of events that is involved in making an eye. Soon it was found that all animals with eyes, including squid, sea scallops, flatworms, fish, birds, mice, and humans, have a gene of extremely similar DNA sequence that plays a similar role in eye development in every case. In fact, the mouse version of the *eye* gene is so similar to the fly gene, that when the mouse gene was introduced into fruit flies and expressed anywhere on the fly body, an extra eye was formed in that location. (Notably, the eyes that formed under these conditions were fly eyes, not mouse eyes.)

Human babies who have the misfortune to be born with one defective copy of the human version of the *eye* gene have small eyes that lack an iris, which results in serious vision problems. Babies that are born with two defective copies of this gene have no eyes at all. Thus, it is clear that the *eye* gene plays the same role in human eye development as its counterpart does in that of the fly.

Similarly detailed genetic parallels between flies, birds, mammals and ourselves have been discovered with respect to the **master control genes** that control development of the heart (a gene called *tinman*), legs, nervous system, and other body parts. If most biologists had not already been thoroughly convinced that (as a famous biologist said 50 years ago), “nothing in biology makes sense except in the light of evolution,” such modern observations would undoubtedly drive them to such a conclusion.

TIMELINE

It will take an average student 10 minutes to read this material; it could be assigned as homework.

REFERENCE

The Genes We Share with Yeast, Flies, Worms and Mice: New Clues to Human Health and Disease. (2001). Available by writing to the Howard Hughes Medical Institute, Office of Communications, 4000 Jones Bridge Road, Chevy Chase, MD 20815-6789. This colorful and well-written booklet is the eighth in a series of reports about biomedical research that has been prepared by the Howard Hughes Medical Institute specifically to provide teachers and students with up-to-date information about current research developments in biology and biomedical sciences. It is an unusually rich source of information about the powerful new methods that are now being used to study the genes of model organisms as well as the novel insights that such studies have already provided us about our own genetic makeup.

Starting a New Generation: Sea Urchin Fertilization

STUDENT PAGES 82-85

LESSON OVERVIEW

This activity is meant to bring the processes of early development vividly to life for your students. The sea urchin sperm and eggs that they will see under their microscope serve as wonderful models to help them understand their own biological and genetic origins. Students enjoy working with live animals, particularly ones that are unfamiliar, yet non-threatening. So this is a wonderful time to talk about the unity and diversity of life: It is the fundamental unity of all life forms that allows a model organism such as the sea urchin to give us insights into our own biological nature. This exercise is also, of course, a wonderful time to introduce or review the concepts of mitosis and meiosis, and the roles that each plays in the processes of heredity and development.

TIMELINE

The sea urchins need to be ordered at least two weeks before you plan to use them in your class. They are only shipped on Monday or Tuesday and will usually arrive sometime during the next day. Be sure you are prepared to take care of the urchins properly when they arrive (see below). It is best to do the fertilization exercise as soon as possible after delivery. So plan accordingly.

MATERIALS

For the entire exercise:

- 3 or 4 aquariums or holding tanks (see Advance Preparation for details)
- 1 package of a dry artificial sea water mixture (such as Instant Ocean™)*
- 1 hydrometer*
- 1 aquarium pump*
- 3 or 4 air stones*
- tubing and adapters to connect air pump to air stones*
- sea urchins
- 2-5 ml syringe with a 20-30 gauge needle
- 30 ml of 0.5 M KCl (1.13 g of KCl in 30 ml water)
- disposable petri dishes
- dropping pipettes
- microscope slides and cover glasses for checking gametes
- 1 or 2 microcentrifuge tubes for storing sperm
- 3 or 4 250 ml beakers to hold female sea urchins during spawning
- 3 or 4 16 x 100 mm test tubes for making sperm suspensions
- 3 or 4 50 or 100 ml beakers for fertilizing eggs
- 1 500 ml bottle for cultivating embryos

*available at most pet stores, or from lab supply catalogs

For each group of four students:

- 1 or 2 depression slides
- 2 or 3 dropping pipettes
- a compound microscope

Sea urchins can be ordered from:

Carolina Biological Supply

(800) 334-5551

www.carolina.com

Sea Urchin Embryological Kit, catalog no. BA-16-2505 or

Sea Urchins, catalog no. BA-216-2949

Pacific Bio-Marine Laboratories

(310) 677-1056

Gulf Specimen Corp.

(904) 984-5297

Urchins are sold by Carolina Biological Supply Co. in sets of 14, which is usually adequate to supply enough gametes for all of the classes taught by one (or possibly even two) teachers. Because different species are available from different suppliers and/or at different times of the year, and because this will affect the way you will need to handle the urchins when they arrive (as explained below), it is a good idea to ask what species will be shipped when you place your order.

ADVANCE PREPARATION

- Order sea urchins at least three weeks before the activity.
- Visit the sea urchin website (<http://www.stanford.edu/group/Urchin/fert.htm>) well before the urchins arrive. As discussed in the reference section below, this site is full of useful and interesting information.
- Have 3-4 aquaria or holding tanks filled with sea water, aerated, and equilibrated for a few days by the time the sea urchins are scheduled to arrive. See discussion of Sea Urchin Care below to determine temperature at which your aquaria or holding tanks should be equilibrated.
- If you have aquaria already on hand, use them. However, genuine aquaria are not essential. Large, clear plastic boxes (such as 3-gallon Rubber Maid, #2220) purchased at a discount store will work equally well as holding tanks. You should soak them for a few days with several changes of tap water (to leach out any plasticizers that might be toxic to sea urchins) before filling with sea water.
- Air pumps, air stones, a hydrometer, and sea salt are available in many pet stores. These supplies can be obtained from most any biological supply company as well.
- Prepare the sea water according to the instructions on the package, using dechlorinated tap water. Check the specific gravity with the hydrometer. Adjust as necessary to a spe-

cific gravity of 1.020 and 1.023 (at 75°C), adding more sea salt to increase or more dechlorinated water to decrease the specific gravity.

- Collect and organize other supplies. Most of the additional supplies can be purchased from any scientific supply company. For example, from Fisher Scientific [(800) 766-7000, www.fishersci.com] you might order:

Potassium chloride, 500 g, cat. no. P217-500

Plastic dropping pipettes, box of 400, cat. no. 13-711-37

Hanging drop depression slides, package of 12, cat. no. 12-560A

3 ml disposable syringes with 22 Ga needles, box of 100, cat. no. 14-826-85

- It is important to use glassware and other supplies that are clean and free of detergent residues or other potentially toxic substances. Glassware that has been used previously should be rinsed extensively with clear tap water before being used for this exercise.
- When you finish this exercise, rinse all of your equipment thoroughly with clear water. Do not use any soap or detergent, or you may leave a residue on them that will kill next year's sea urchins.

SEA URCHIN CARE

Identify which species of urchins you received. Different species have different fertile seasons, so the month in which you order will often determine which species you will get. The four species most commonly shipped from the sources listed above are:

Strongylocentrotus drohbachiensis, a cold-water North Atlantic species;

Strongylocentrotus purpuratus, a purple, cold-water Pacific species;

Lytechinus variegatus, a light-colored, warm-water species from Florida;

Arbacia punctulata, a dark purple, warm-water species from Florida.

If you have received either of the warm-water species, they should be kept in tanks or holding trays in a cool room (60-68° F) but not a refrigerator. Your prospects of having healthy, cooperative sea urchins for your class will be improved if you can allow the urchins time to become acclimated to their new environment gradually. If they were shipped in bags of sea water, float the bags in the aquarium about half an hour. Meanwhile, assess each urchin. Examine its spines and the water it has been shipped in. If its spines are falling off, or it smells foul when the bag is opened, it is dead or dying; discard it. If the urchin looks healthy, and its water smells all right but is cloudy, it is likely that the urchin has spawned. If you put an urchin that has spawned in a tank with ones that have not, chemicals diffusing from the eggs or sperm will usually cause the others to spawn immediately, ruining them for your purposes. So place any questionable urchins in individual beakers or jars of sea water, not in an aquarium with other urchins.

Once the bags with healthy urchins have equilibrated in temperature with the aquarium, open the bags and use the hydrometer to check for salinity differences between the aquarium and the sea water in the bag. If there is a difference, add portions of the aquarium water to the bag. When you have doubled the volume of the water in the shipping bags, remove the sea

urchins from the bags and place them in the tank. One sea urchin per half gallon of sea water is a good ratio.

Sometimes sea urchins are shipped in sea-water-soaked, shredded newspaper rather than in bags of water. If this is how your urchins have arrived, unwrap each one carefully and examine it. If its spines are falling off, or it smells foul, it is dead or dying and should be discarded. If you see a white or yellow-orange exudate on the **test**, or shell, between the spines, this will indicate that the urchin has spawned; isolate it from the rest of the urchins, and rinse your hands thoroughly before handling other urchins. Place healthy looking urchins in individual beakers or bags of cool (not cold) sea water, float the bags in the aquarium, and monitor carefully for the next half hour or so. If the water in a bag turns cloudy during this period, this also will indicate that the urchin has spawned and should not be housed with unspawned ones. Place healthy, unspawned urchins in a tank together, as above.

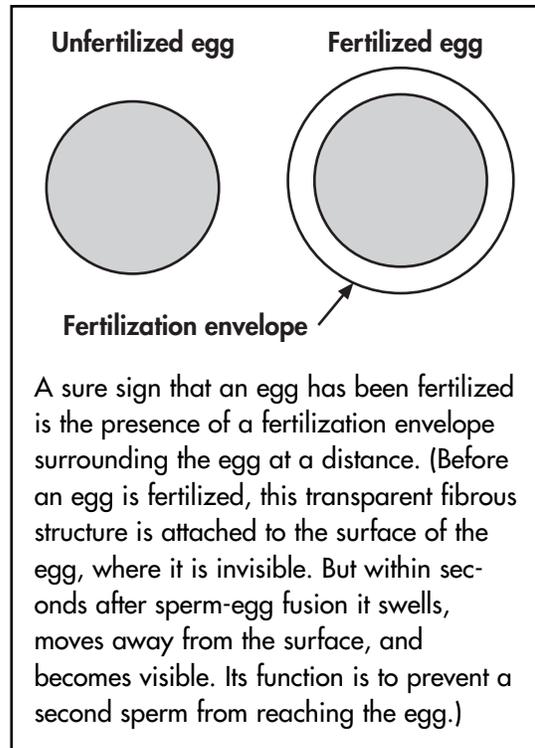
If you have received either of the cold water sea urchins, either use them immediately or keep them in refrigerated sea water. Assess each urchin as above. Place the healthiest looking ones together in a shallow plastic tray with enough sea water to just cover them. Place them in a refrigerator at 45-50° F. Urchins held in this manner should remain healthy for 1-2 days.

PROCEDURE FOR COLLECTING AND USING SEA URCHIN GAMETES

Experience suggests that the chances of success are substantially increased when the teacher injects the urchins and collects the gametes rather than charging the students to do it. However, it is a good idea to allow students who wish to do so to hold and examine one of the urchins. (For many of them it will be a novel and broadening experience.)

1. Fill a syringe with 2.0 ml of the 0.5M KCl solution.
2. Hold a sea urchin mouth side up. Insert the needle through the soft membrane surrounding the mouth, while pointing the needle away from the mouth. There may be a little initial resistance, but then the needle should slide in easily. *Slowly* inject the KCl solution.
3. Following injection, place the urchin, mouth down, on a clean, dry Petri dish. Check periodically for appearance of an exudate (the gametes) between the spines on the upper part of the test. It may take 5-10 minutes for the gametes to appear, and some urchins may not be mature and will not release gametes in response to the KCl.
4. If you see a smooth, milky white fluid being released, you have a male that is releasing sperm. But if you see a somewhat grainy suspension being released that is colored yellow to red (depending on the species), you have a female releasing eggs. If in doubt as to which kind of gametes are appearing, use a pipette to put a small drop on a microscope slide, dilute it with a drop of sea water, and examine it under the microscope.
5. Immediately after you have determined the sex of your sea urchin, do one of the following:
Female urchin: Place her, mouth up, over a 250 ml beaker that contains enough sea water to make contact with the test. Allow the eggs to flow to the bottom of the beaker.
Male urchin: Leave him, mouth down, on a dry Petri dish. Use a dropping pipette to pick up the sperm from the surface of the test and transfer them to a dry microcentrifuge tube. As long as the sperm have not come in contact with sea water, they will remain alive in such a tube for at least 24 hours in a refrigerator (45° F).

6. As soon as you have a reasonable number of eggs in the bottom of a beaker, check the appearance of a drop of egg suspension with a microscope. If there is a lot of debris visible around the eggs, wash them. To wash the eggs, transfer the female to a second beaker of sea water to continue spawning. After the eggs in the first beaker have settled completely, pour off the water and add fresh sea water. Repeat the process. A clean suspension of healthy eggs will survive in a refrigerator for several hours.
7. As soon as you have a clean suspension of eggs, have each group of students transfer a small sample to a depression slide, examine it in the microscope, and draw what they see. Emphasize that their best chances of seeing fertilization take place will occur if they have only 10-20 eggs in the depression slide, and that if they have more, they should remove some of them and replace them with more sea water.
8. After all students have eggs on their slides, the sperm can be prepared. Dilute one or two drops of the “dry” sperm in 10 ml of sea water. Use a microscope to view the sperm with a 40X objective to make sure that they are moving. The sperm are only viable for about 15 minutes after they have been exposed to sea water.
9. As soon as you have an active sperm suspension, transfer two or three pipettefuls of an egg suspension to each of two clean small beakers and add a pipetteful of sperm suspension to each. Label each beaker with the time that the sperm and eggs were mixed.
10. Now have each group of students add a drop of the sperm suspension to the eggs in their depression slides. Emphasize that they should only need a little of the sperm suspension, enough so that they will see 10-100 sperm around each egg.
11. Check samples of the sperm-egg mixtures you have in the beakers to be sure that the eggs have been fertilized (see drawing). If they have, have each group of students examine a drop of the suspension every 15 minutes or so to monitor development. The first cleavage division should occur about an hour after fertilization.
12. Place the remaining unfertilized eggs in the refrigerator for use later in the day. Save samples of fertilized eggs from one class to the next, so students can see the later stages of development.
13. Toward the end of the day, transfer a suspension of developing embryos to a clean, dry, screw-cap 500 ml bottle and dilute it to about 100 ml with sea water. Screw the cap on tightly and float the bottle on its side in the aquarium. Samples of this preparation can then be examined over the next day or two to monitor development and behavior of the sea urchin larvae or **plutei** (singular **pluteus**).



HINTS AND TROUBLESHOOTING

1. If your schedule permits, it would be a good idea to fertilize a batch of eggs one to two hours before your first class, so that students in that class will be able to observe some cleaving embryos. Alternatively – or in addition – you may want to make arrangements so that students can drop in later in the day to observe more advanced stages of development.
2. If you have a video microscope, have it available for this exercise, so that the whole class can observe any particularly nice specimens at the same time. Also, try to use a VCR to record any particularly good views of fertilization and cell division for later viewing.
3. One never knows for sure what is going to happen when working with live animals. It is best to be prepared for the unexpected. It is possible that in the first hour you could inject five urchins and they would all be males (or all females). The thing to remember is that once you get one female and one male, you should have enough gametes to last for all your classes that day. Many teachers have backup activities planned for down time and in case the urchins are uncooperative. Some ideas include: a videodisc or tape with sections on mitosis, meiosis, and fertilization, a video from other classes where the activity was successful or prepared microscope slides of sea urchin development. If computers with Internet access are available, students can view the sea urchin website referenced above.
4. If you get eggs and sperm, but the eggs do not seem to be getting fertilized, check the temperature of the sea water and make certain that they are not getting overheated. The next thing to try is to dilute a new batch of sperm and check to be sure it is active. If neither of these things seem to identify the problem, chances are that the eggs are immature. This can sometimes be diagnosed by seeing a very large nucleus, since an egg that has not yet undergone meiosis will still have a diploid number of chromosomes and a large nucleus.
5. If the eggs are getting fertilized but do not divide, or they divide abnormally, the cause is probably **polyspermy** (fertilization of each egg by more than one sperm). Dilute the sperm, make sure it's still active, and start over.
6. It may be interesting to your students to discuss how different the tempo of sea urchin development is from that of human development. With sea urchins the first division of a fertilized egg occurs in about an hour, and then subsequent divisions occur about every half hour. With humans, however, the first division does not occur until more than a day after fertilization, and at the end of two days, while the sea urchin is already swimming around as a pluteus larva and feeding itself, the human embryo is just getting around to dividing the second time. Moreover, by the time the sea urchin has become a mature adult and is having “babies” of its own (about 18 months), the human infant is nine months old, still wholly dependent on its parents for food and everything else required for survival. The magnitude of these differences in timing makes it seem all the more astonishing how similar in quality many of the chemical changes are that occur in the two kinds of eggs following fertilization.
7. Keeping sea urchins for a prolonged period of time in an inland location is difficult. Even teachers who maintain a salt-water aquarium have often found that adding the

left-over urchins to such an aquarium is a mistake; they can foul the aquarium and cause severe difficulties. The most humane way of killing urchins after you are through using them is to put them in a freezer. After they are dead, you may preserve the tests (shells) by washing them in diluted bleach and setting them out to dry. Students who are squeamish about holding a live urchin may be willing to hold and examine a lifeless test.

REFERENCES

Dr. David Epel of Stanford University has prepared an excellent resource regarding sea urchin development. It can be found at <http://www.stanford.edu/group/Urchin/fert.htm>. This site takes students through gamete collection, fertilization, and development. It has good animated sequences, lots of useful information, and great ideas for further studies.

Womb with a View (1995), Kendall/Hunt Publishing Company. This reference book for teachers contains several activities related to meiosis and mitosis and is an excellent resource for sea urchin background information. It discusses the historical uses of the sea urchin as well as its anatomy and taxonomy. The book has more detailed ordering information and includes several activities related to meiosis, mitosis, sea urchin fertilization, and sea urchin behavior.

From Egg to Adult: A Report From the Howard Hughes Medical Institute (1992). Available by writing Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, MD 20815-6789. This colorful brochure illustrates beautifully the way in which fruit flies have served as a model system to identify the genes that regulate formation of the human body from head to toe.

ANSWERS TO POSTLAB QUESTIONS STUDENT PAGE 86

1. If an adult sea urchin of one particular species has 14 chromosomes, how many chromosomes would an egg or sperm of that species have?
It would have seven chromosomes.
2. What do you think would happen if one of the gametes (either the egg or the sperm) had the wrong number of chromosomes? Why?
The embryo derived from the fertilized egg would not develop normally. This is because an embryo needs to receive an equal number of chromosomes from each parent in order to develop normally.
3. What are some differences between a fertilized and unfertilized egg?
A fertilized egg has twice as many chromosomes as an unfertilized egg. It also has a fertilization membrane that protects it from being penetrated by a second sperm. It also is capable of dividing and forming a sea urchin larva, something an unfertilized egg cannot do.

4. What is the function of the fertilization membrane? Why would that be important?
It is to prevent a second sperm from fusing with the egg. That is important because an egg that fuses with more than one sperm never develops normally.
5. What happens to the fertilized egg about an hour after fertilization?
It undergoes its first division.
6. Whenever a cell of an embryo divides, how are each of the newly formed cells similar to one another and to the original fertilized egg but different from the unfertilized egg?
They are diploid, whereas the unfertilized egg was haploid.
7. Mitosis and meiosis are essential aspects of the cycle of life and development. Complete the adjacent diagram by writing mitosis or meiosis on the correct lines.
 - a. *meiosis*
 - b-e. *mitosis*



The Miracle of Life

STUDENT PAGES 87-88

LESSON OVERVIEW

If all went well, the preceding exercise provided your students a chance to watch gamete release, fertilization, and the early development of a sea urchin. Now, thanks to the spectacular cinematography included in this video, which was first shown on *Nova*, they will get to see the equivalent processes taking place in human beings.

TIMELINE

Viewing this video and answering the questions will take an entire 50 minute period.

MATERIALS

The Miracle of Life can be ordered from:

Carolina Biological Supply Company

(800) 334-5551

www.carolina.com

The Miracle of Life, catalog no. BA-49-3555V

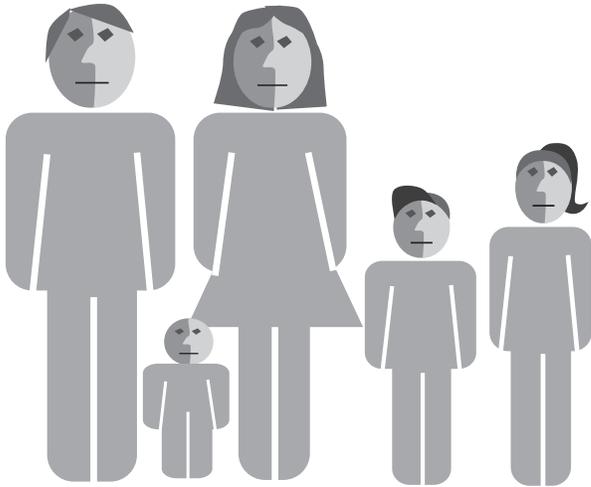
ANSWERS TO THE MIRACLE OF LIFE QUESTIONS STUDENT PAGES 87-88

1. Describe the journey of the egg as it becomes mature and travels toward the sperm.
The egg matures in a follicle in the ovary, is released when the follicle ruptures, and is then swept into the fallopian tube, where it is pushed in the direction of the uterus by cilia on the surface of the cells lining the fallopian tubes.
2. Describe the journey of the sperm as they leave their site of origin and travel toward the exterior.
Sperm are formed in the seminiferous tubules in the testes and stored in the epididymis. During ejaculation they travel through the vas deferens, are mixed with secretions released by the seminal vesicles and prostate gland, and then travel through the urethra and are expelled into the vagina.
3. About how many sperm does a man produce in his lifetime?
A typical male produces about 400 billion sperm in his lifetime.
4. About how many sperm are released in a single ejaculation?
About 200-300 million sperm are released in a typical ejaculation.
5. After sperm are released into the vagina, how long are they viable?
Sperm remain viable in the female reproductive tract for 24-48 hours.

6. Describe the barriers that the sperm face as they travel up the female reproductive tract toward the egg.
The acidic condition of the vagina kills many sperm, as do protective cells that attack them. Then once they reach the fallopian tubes, the sperm must swim upstream against the current generated by the cilia.
7. Where is the egg when the sperm reach it?
It is in one of the fallopian tubes.
8. About how many sperm reach the egg?
Only about 50 sperm ever make it to the vicinity of the egg, and only one enters it.
9. What happens to the sperm after it enters the egg?
The sperm loses its tail and midpiece, and its head swells and then ruptures, releasing its genetic material into the egg.
10. When does the fertilized egg begin dividing?
The egg divides for the first time about a day after fertilization.
11. What is the fertilized egg called after it divides?
When the egg is fertilized, it becomes a zygote; then when it begins to divide, it becomes an embryo.
12. How long after fertilization does the embryo implant itself in the uterine wall?
Implantation in the uterine wall begins about 10 days after fertilization.
13. Describe the human embryo at the following stages:
 - 4 weeks: It has arm buds and the beginnings of eyes.
 - 5 weeks: *It has a nose and leg buds.*
 - 6 weeks: *It is about 1/2 inch long.*
 - 7 weeks: *It is about 3/4 inch long and has fingers*
 - 8 weeks: *Bones and joints are visible in its fingers and toes.*
 - 10 weeks: *It is now about 2 inches long.*
 - 14 weeks: *It is able to suck its thumb.*
 - 18 weeks: *Its eyes are still closed, but it can detect light.*

CHAPTER 2

**Passing Traits from
One Generation
to the Next**



SECTION C

**If All the Kids
Have Mom and
Dad's Genes,
Why Don't They
All Look Alike?**

Chapter 2: Section C Background

IN SECTION B, YOUR students saw how the fusion of sperm and egg initiates the beautiful and mysterious process by which a genotype gives rise to a phenotype. Now it is time to get the students to contemplate the rules of heredity that relate the phenotype of the offspring to the genotypes of the parents that produced the egg and sperm. Our goal in this section is to help our students understand the answer to the question, If all the kids have Mom and Dad's genes, why don't they all look alike?

The field of inquiry to which your students will be introduced in this section is called Mendelian genetics, because it involves using methods of investigation very similar to the ones Gregor Mendel used more than 150 years ago.

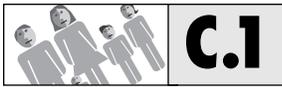
In the first exercise, your students will have the fun of crossing two members of a very charming species of imaginary animals – the Reebops – and producing a young Reebop of distinctive phenotype. Although all the progeny produced by the class will be children of the same pair of parents (Mom and Dad Reebop), it is extremely unlikely that any two of the young Reebops will look exactly alike. In the course of this exercise, your students will have a painless introduction to many of the most important terms and concepts of Mendelian genetics.

Then in the second exercise, they will learn how to use a Punnett square, as well as a very simple mathematical method, to make genetic predictions. In this exercise, they will perform the equivalent of one of the most important tasks a genetic counselor must perform: predicting the probability that two parents of known genotype will have a baby with some particular phenotype.

In the third exercise, genetics will strike much closer to home. The students will pair up to produce an imaginary human baby together, and will discover which of them the baby will resemble with respect to 22 different phenotypic characters.

In the final exercise they will take information from Exercise 3 and use a more complicated Punnett square to make a more complicated genetic prediction.

All in all, this section should not only introduce your students to many important Mendelian-genetic concepts, it should reinforce the concept that genetics (and science in general) can be fun!



Really Relating to Reebops STUDENT PAGES 90-94

LESSON OVERVIEW

Reebops (invented by Patti Soderberg at the University of Wisconsin*), are model “organisms” that students can use for a variety of genetic studies. In the initial exercise described here, students receive a set of diploid “chromosomes” that represent the genotypes of a pair of parental Reebops. The students then simulate meiosis, combine the resulting two haploid sets of chromosomes, and then determine phenotypes for individual offspring, which they construct from inexpensive materials. The class then compiles data for this F1 generation and forms hypotheses about the inheritance patterns. This exercise can be carried through a number of generations, if desired, to test various hypotheses.

TIMELINE

Two 50 minute periods.

MATERIALS

For each pair of students provide at least:

4 standard white marshmallows	2 thumbtacks
1 red miniature marshmallow	4 red pushpins
1 orange miniature marshmallow	4 blue pushpins
1 yellow miniature marshmallow	1 short and straight pipecleaner
3 green miniature marshmallows	1 long and curled pipecleaner
8 toothpicks	1 set of red and 1 set of green chromosomes in an envelope
2 small nails	

ADVANCE PREPARATION

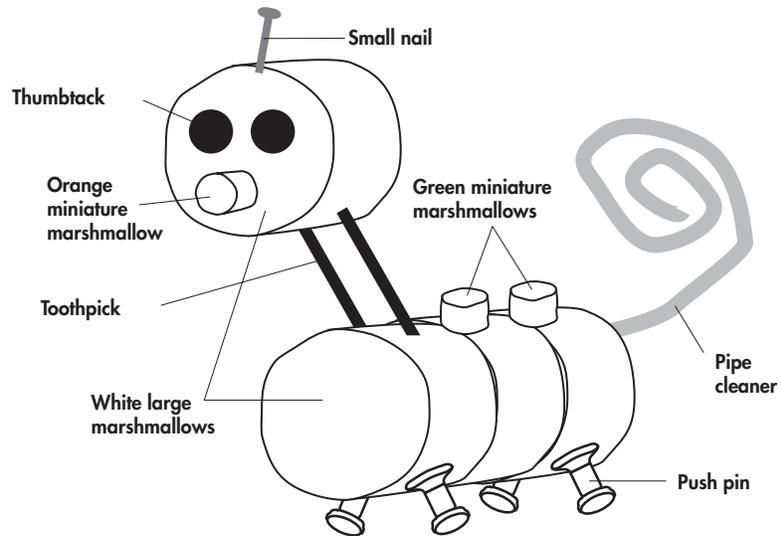
- Prepare two diploid chromosome sets per pair of students, using the templates provided. For convenience, we have put two sets of chromosomes on one page, but you will need one Mom set on red paper and one Dad set on green paper for each student pair. These chromosomes will be much more durable if they are laminated before they are cut out.
- Assemble Mom and Dad Reebop. The chromosomes indicate that both Mom and Dad Reebop are heterozygous at each locus (or gene pair). So the phenotype of each of them should be as follows: three body segments, one antenna, orange nose, two eyes, two green humps, curly tail, and blue legs (see drawing).

**The lesson included here has been adapted from Patti Soderberg's version with her permission.*

PROCEDURE

Day 1

- Place body parts in boxes at the front of the room.
- Give each pair of students an envelope with their chromosomes and introduce the class to Mom and Dad Reebop, which you should leave in a conspicuous place during the rest of the exercise. Make sure that the students sort the



Toothpicks function as the unseen ligaments and tendons that hold the Reebop together.

- parental chromosomes in the kit and perform “meiosis” with code letters face down, gathering one haploid set of chromosomes from each parent at random. (They may then put the extra chromosomes back in the envelope without looking at them.) Now they “recombine” the haploid chromosome sets produced by Mom and Dad Reebop to generate the genotype of baby Reebop. After turning their baby’s chromosomes letter-side up, they should record this genotype on the worksheet. Then they should use the Genotype-Phenotype Conversion Table to determine (and record) what the baby’s phenotype will be. Finally, they should assemble a baby with this phenotype.
- If there is enough time left after the babies have been “born,” you may wish to have the students work on the Reebop Review. Alternatively, you may wish to assign it as homework. (Note that it includes an introduction to predicting probabilities with a Punnett square.)

Day 2

- This session is used to compile and discuss the data from the population of Reebop babies in the class.
- Try to work the key concepts of Mendelian genetics into the discussion at appropriate places. See the Genetic Glossary on S93.
- Some teachers may have a tendency to downplay the concept of a locus, because students may initially have a bit of trouble understanding it. But it is very difficult to discuss any but the very simplest of genetic concepts without using the concept of a **locus**. So please work on getting students to understand it. A locus is the chromosomal site where alleles affecting one particular heritable trait are located. A clear example may help: The ABO locus is the place on a particular chromosome where the proteins responsible for the A, B and O blood-group antigens are encoded. The A allele at this locus encodes an enzyme that puts one kind of sugar on a red blood cell (RBC), mak-

ing it type A. The B allele encodes a different form of this enzyme, which puts a different kind of sugar on the RBC, making it type B. The O allele at this locus encodes an inactive form of the enzyme. (RBCs in AB heterozygotes have both kinds of sugar on their surface and type AB blood, whereas OO homozygotes have neither sugar on their cells and thus have type O blood.)

See “Suggested topics for additional discussion” in the next exercise.

ANSWERS TO REEBOP REVIEW STUDENT PAGE 95

1. Define the following terms and give an example of each from this activity. (You may refer to the Genetic Glossary.)
allele: *one of two or more forms of a gene that can exist at a single locus*
genotype: *the specific combination of alleles that an individual possesses at one or more loci*
phenotype: *the outward appearance of an individual with respect to one or more traits that is associated with some particular genotype*
homozygous: *having two identical alleles at a particular locus*
heterozygous: *having two different alleles at a particular locus*
2. If a Reebop female with a red nose and a male with a yellow nose marry and have children, what genotype and phenotype for nose color will their children have? (You may refer back to the Reebop Genotype-Phenotype Conversion Table.)
genotype *Nn*
phenotype *orange nose*
3. If a Reebop female with one antenna and a male with no antennae marry and have children, what genotypes and phenotypes might their children have with respect to number of antennae?
genotypes *Aa or aa*
phenotypes *one antenna or no antennae*
4. If a Reebop female with one antenna and a male with one antenna marry and have children, what is the probability that they will have a baby with no antennae? (If you have difficulty answering this question, check out section C.2.)
The probability is 1 in 4, or 25%.
5. If a Reebop female with two green humps and a male with two green humps marry and have children, what is the probability that their first baby will have two green humps?
The probability is 1 in 2, or 50%.
6. If a Reebop female with three green humps and a male with three green humps marry and have children, what is the probability that they will have a baby with two green humps?
The probability is zero.

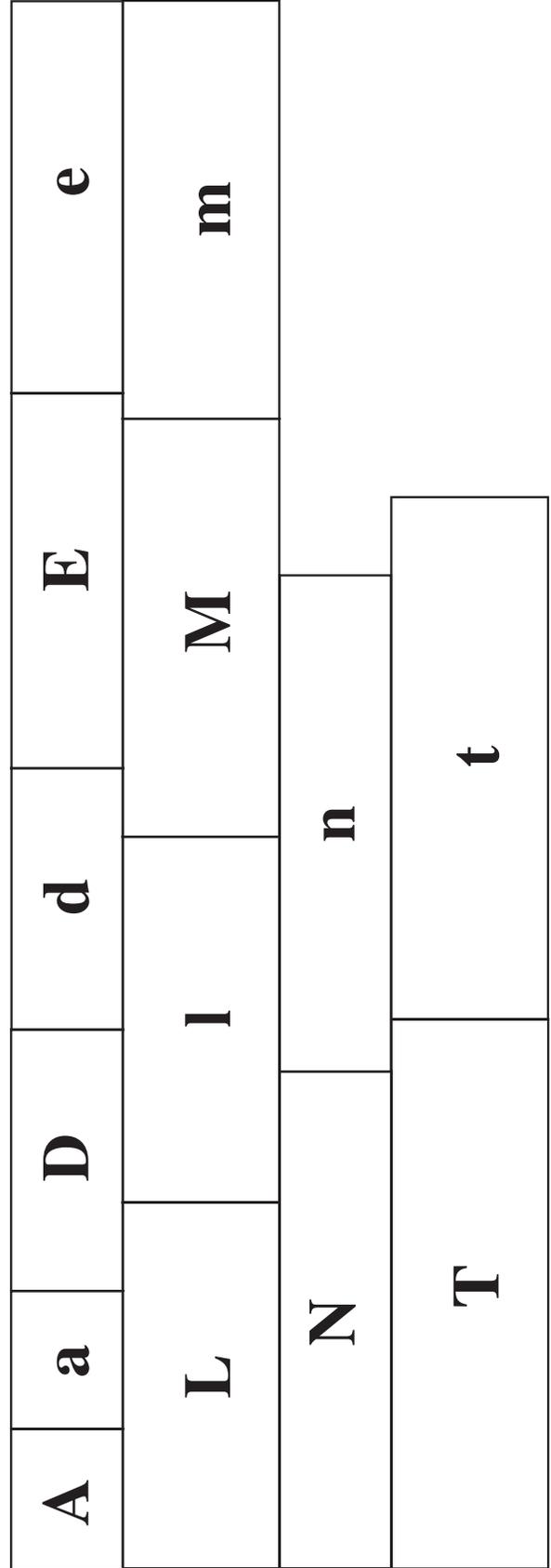
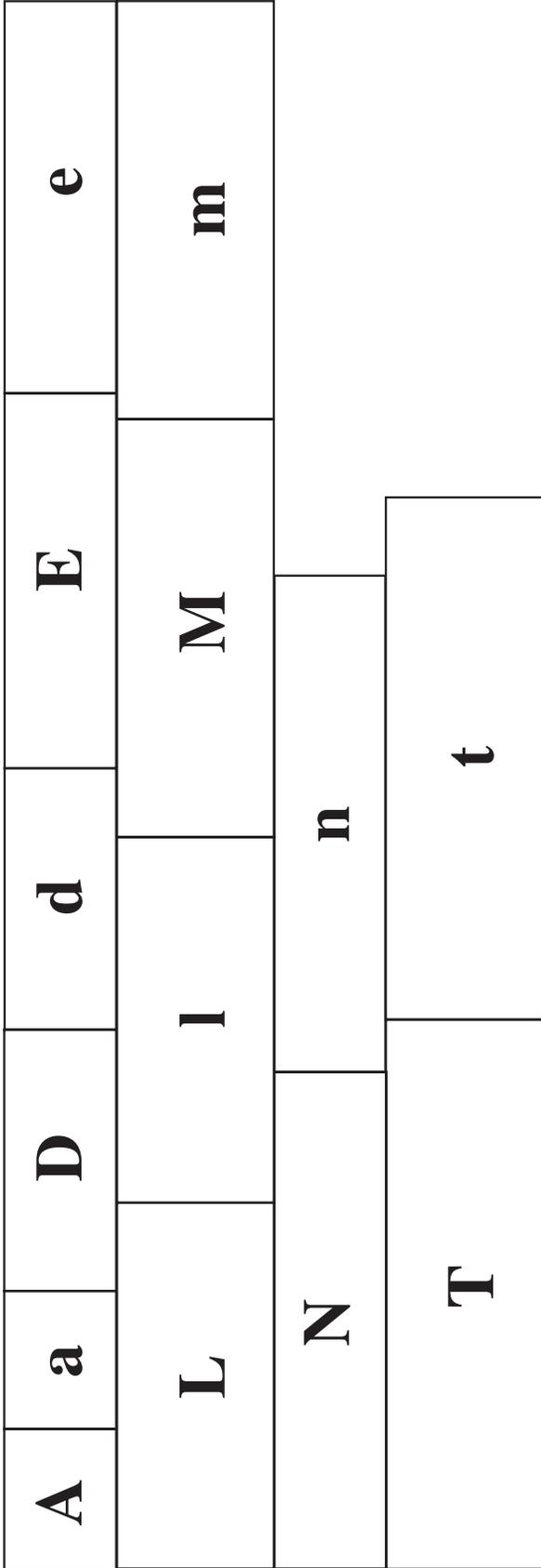
7. If a Reebop baby has a straight tail, but both of his parents have curly tails, what are genotypes of the two parents?
With respect to this trait, both parents have the genotype Tt. (Because the baby has the genotype tt, it must have inherited a t allele from each parent. Therefore both parents must be heterozygous.)

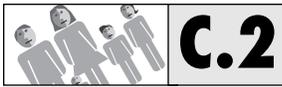
ANSWERS TO ANALYSIS OF REEBOP FINDINGS STUDENT PAGES 96-97

1. Describe the phenotypes of Mom and Dad Reebop.
three body segments, one antenna, orange nose, two eyes, two green humps, curly tail, and blue legs.
2. Using the information in the Reebop Genotype-Phenotype Conversion Table, list all of the genotypes that would produce the phenotypes exhibited by Mom and Dad.
DD or Dd, Aa, Nn, EE or Ee, Mm, TT or Tt, LL or Ll.
3. How many of the Reebop babies in your class have the same phenotypes as Mom or Dad?
None
4. Do any two babies in your class have exactly the same phenotypes?
(Probably) no
5. Why do some Reebop babies have traits that are not seen in either Mom or Dad?
These are all traits for which the parents are heterozygous but the babies are homozygous recessive.
6. Which Reebop traits are dominant?
three body segments, two eyes, curly tail, and blue legs
7. Which Reebop traits exhibit codominance?
number of antennae, nose color, and number of humps
8. Use the information you have about the phenotypes of all of the Reebop babies in your class to figure out what the genotypes of Mom and Dad Reebop must be. Write the answer below.
Dd, Aa, Nn, Ee, Mm, Tt, Ll
9. If you know the genotype of the parents, is it possible to predict all of the possible genotypes of babies that they might produce?
yes
10. If you know the genotype of the parents, is it possible to predict the genotype of any particular baby, such as their first one?
Not unless both parents are homozygous at all loci. (If they were both homozygous at all loci, all of their offspring would have the same genotype, but such a situation would be extremely rare in the real world.)

11. The Reebops appear to have only one gene on each chromosome. Do you think this is true of real, living organisms?

Probably not. More than likely they all have many more genes than they have chromosomes.





Determining Genetic Probabilities with a Punnett Square

STUDENT PAGES 98-99

LESSON OVERVIEW

One of the most important uses of genetic information outside of a classroom or laboratory is to predict the probable genotypes and phenotypes of unborn or potential offspring. For example, if a man and a woman both have siblings who suffer from cystic fibrosis (a heritable disease), they undoubtedly would wonder what their chances would be of having a child with cystic fibrosis if they were to marry and raise a family. We will get into this sort of prediction with respect to human diseases in a later chapter. The Reebops activity provided a great opportunity to introduce the concepts of genetic predictability and probability in a less somber context.

Students saw how many different Reebop phenotypes were generated with only seven allele pairs; from that exercise they should have gained some insight into the enormous phenotypic diversity that can be generated by the twin processes of meiosis and recombination in natural populations of real organisms. It is such phenotypic diversity that serves as the raw material on which natural selection acts to bring about evolutionary change.

The **Punnett square** provides a way for students to visualize all of the genotypic and phenotypic possibilities in a cross between parents of known genotype and to determine the relative probabilities of each of those possibilities. It is a good idea, however, to make the point that a Punnett square is just a way of visually confirming a basic rule of probability that applies to many events, not just sperm and egg fusion. The rule highlighted by the Punnett squares is that **the probability of two events occurring together is equal to the product of their independent probabilities.**

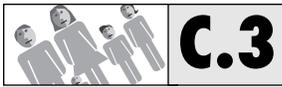
To apply this mathematical rule to the problem being addressed here, one would proceed as follows: The probability that an egg produced by an **Aa** female will contain the **a** allele is 1 in 2, and the probability that a sperm produced by an **Aa** male will contain the **a** allele is 1 in 2; therefore, the probability that a baby produced by this couple will have the **aa** genotype is $1/2 \times 1/2$, or $1/4$.

An advantage of using the Punnett square is that it gives students a way to visualize all of the possible outcomes as sperm and eggs combine in a particular genetic cross. But the mathematical approach of multiplying individual probabilities is applicable to many more situations in science and life.

This lesson will use Punnett squares and mathematical calculations in a guided practice activity. You could easily pick other Reebop traits and have the students work through the same process on their own.

TIMELINE

This exercise will only take about 5 to 10 minutes of class time. But you could assign it as homework, together with a few other problems in Reebop heredity for the students to work out using Punnett squares and/or product-of-probability methods.



Exploring Human Traits: Create-a-Baby

STUDENT PAGES 100-105

LESSON OVERVIEW

Now that your students have encountered the basic concepts of Mendelian genetics by working with the Reebops, they get to apply them to beings with which they should have even less trouble identifying – their own imaginary offspring! Working in pairs, the students will analyze their own phenotypes and genotypes with respect to 22 visible features of the head and face. These will include dominant/recessive traits, codominant traits, and simple multigenic traits. Having established their own genotypes, they will use a coin toss to determine which alleles they will pass on to their offspring at each locus where they are heterozygous. When alleles from two partners have been recombined in the imaginary baby, they will work out the complete phenotype of the baby and name it. Then each partner will draw a picture of what they think the baby would look like.

TIMELINE

The reading, the phenotype and genotype diagnoses, and the Create-a-Baby Table require 50 minutes to complete. If you chose to have the students do their drawings in class, a second period will be required. However, the drawings and the Create-A-Baby Review could be assigned as homework.

MATERIALS

- a coin for each student
- extra paper
- colored pencils

HINTS AND TROUBLESHOOTING

Many teachers compile all the baby pictures produced that year into a baby book. Students often seem to take more pride in their work when they know it is going to be displayed to the following year's students.

ANSWERS TO CREATE-A-BABY REVIEW STUDENT PAGES 106-107

1. Define each of the following terms:

chromosome, codominant, diploid, haploid, meiosis, multigenic, recombination

chromosome: a structure in the nucleus of a eukaryotic cell that contains a linear array of many genes. A chromosome is composed of a single DNA double helix molecule wound around many protein molecules that stabilize it and regulate its function.

codominant: refers to a pair of alleles, both of which exert an effect on the phenotype when they are present together. In codominance, the heterozygote has a phenotype different from that of either homozygote and sometimes (but not always) is intermediate in phenotype.

diploid: having two complete sets of chromosomes, one set derived from the mother and one from the father.

haploid: having only one set of chromosomes (as in a sperm or egg nucleus).

meiosis: the “reduction division” in which a diploid cell divides to produce haploid cells that will function as gametes (eggs or sperm).

recombination: the process in which two haploid sets of chromosomes are brought together in a pair of gametes to produce a new diploid offspring. Usually this new diploid will be different in genotype from both of its parents.

2. What was the probability that you and your partner would produce a boy? A girl? Explain.

There was a 50% probability it would be a boy and a 50% probability it would be a girl, because the gender was determined by the toss of a coin, and the coin had a 50-50 chance of coming down heads versus tails.

3. Explain how it is possible for your baby to have a visible trait that neither you nor your partner have.

If both partners are heterozygous at a particular locus, the baby could inherit one recessive allele from each parent and exhibit the recessive version of that trait.

4. If you and your partner repeated this exercise and produced another imaginary baby, do you think it would look just the same as the one you produced already? Explain.

It would probably look quite similar, but not identical (as siblings usually do). This is because in every case where a coin toss was used to decide which allele the baby would get, there is a 50-50 chance that the outcome would be different the second time.

5. A woman who is heterozygous for the chin-dimple trait marries a man without a chin dimple. What are the possible genotypes and phenotypes of their children?
The woman could contribute either a P or a p allele, but the man could contribute only the p allele. So the children could be either Pp (dimpled chin) or pp (no dimple).
6. What is the probability that the man and woman discussed in the preceding question will have a baby with a chin dimple?
50%
7. A man and a woman who are both heterozygous for two traits, the cheek-dimple and the chin-dimple traits, get married. What is the probability that they will have a baby that has cheek dimples but not a chin dimple? (If you have trouble answering this question, check out section C.4.)
The probability that one of their babies will have cheek dimples but not chin dimples is 3 in 16, or 3/16. (One way of obtaining this answer is by performing a 4-by-4 Punnett square. A second way is by multiplying the probability that one of their children will have cheek dimples [3/4] by the probability that one of their children will not have a chin dimple [1/4]; $3/4 \times 1/4 = 3/16$.)
8. What is the probability that a man with dark blonde hair and a woman with red hair will have a baby with brown hair?
100%! (According to the information used in this exercise, the genotype corresponding to dark blonde hair is rr SS and the genotype corresponding to red hair is RR ss. All of the children of parents with these genotypes will be double heterozygotes: Rr Ss. The phenotype associated with the doubly heterozygous genotype is brown hair.)

Using a More Complicated Punnett Square

STUDENT PAGE 108

LESSON OVERVIEW

Question 7 on the previous lesson leads students into the use of a 4 x 4 Punnett square to deal with alleles at two loci. Once again, after the students have used the Punnett square to answer a question, you should discuss how the answer could also be obtained by multiplying independent probabilities.

For example, the question asked in the Punnett square discussion read, “When a man with an oval face and wavy hair marries a woman with an oval face and wavy hair, what is the probability that they will have a baby with a square face and curly hair?”

The parents both have oval face and wavy hair, so they are both heterozygous at both loci of interest: they both have the genotype **Aa Hh**. Rephrasing the question, what we want to know is, What is the probability that two parents with the genotype **Aa Hh** will have a baby with the genotype **aa HH** (the genotype for square face and curly hair)?

- We can break this problem into two parts. First we ask, What is the probability that the baby will be homozygous recessive (**aa**) at the “**a**” locus? There are two ways we can answer this part of the question.
- We may simply remember that the probability of any two heterozygotes producing a homozygous-recessive offspring is always 1/4. Or we may get the same answer by the process of multiplying the independent probabilities. The probability that the baby will inherit the **a** allele from the mother is 1/2, and the probability that it will inherit the **a** allele from the father is 1/2; therefore, the probability that it will inherit two **a** alleles is $1/2 \times 1/2 = 1/4$.
- Next we ask, What is the probability that the baby will be homozygous dominant (**HH**) at the “**h**” locus? By one of the two processes that we used above, we establish that the probability that the baby will inherit two dominant alleles at the **h** locus is also 1/4.
- So now, to determine the probability that the baby will have the **aa HH** genotype, we simply multiply the probability that it will be **aa** (1/4) by the probability that it will be **HH** (1/4). $1/4 \times 1/4 = 1/16$.

In the C.4 exercise, students were guided very carefully through the use of the 4 x 4 Punnett square and given the answers. It would be a good idea to choose a pair of different Create-a-Baby traits and have students perform a Punnett square analysis of those traits (perhaps as a homework assignment).

An advantage of the Punnett square method is that once the diagram has been filled in, one has information regarding the probabilities for all possible genotypes and phenotypes that could result from that particular cross. On the other hand, the advantage of the “product-

of-independent-probabilities” method is that it can be applied to many situations in which the Punnett square is not useful, such as many non-genetic probability problems and genetic problems that are much too complex to be solved with a Punnett square.

For example, suppose we wanted to know what the probability is that one of the babies produced by Mom and Dad Reebop would have the homozygous-recessive genotype at all seven loci at which Mom and Dad are heterozygous. Because there are seven loci, we would need a large square containing 2^7 , or 128, squares on each side if we were to use the Punnett square method. Completing and analyzing such a huge Punnett square without any errors would be tedious, to say the least. On the other hand, to solve the same question by the product-of-independent-probabilities method is quite simple. We know (or can quickly calculate) that if both parents are heterozygous at any one locus, the probability of the baby being homozygous recessive at that locus is $1/4$. Therefore, to calculate what the probability is that the baby will be homozygous recessive at all seven loci at which the Mom and Dad Reebop are heterozygous, all we have to do is calculate the value of $(1/4)^7$. This reveals that the probability of a Reebop baby being homozygous recessive at all seven loci is 1 in 16,384, or 0.006%.

TIMELINE

It will take an average student about 10-15 minutes to read through this exercise carefully enough to really understand it.

SUGGESTED TOPICS FOR ADDITIONAL DISCUSSION

1. Why don't all the babies in either the Reebop or the Create-a-Baby exercise look just like their parents?

They have different combinations of alleles than their parents have.

2. Does it make any difference whether you get a particular allele from your father or from your mother?

In nearly all cases it does not. In fact, until a few years ago the answer to this question was thought to be a simple “no.” But in recent years it has been discovered that for a few genes (particularly ones that control aspects of very early embryonic development) it does make a difference whether a particular allele was inherited from the father or the mother. However, this is an exception to the general rule, which is that any particular allele has the same effect in the offspring whether it came from the mother or the father.

3. Are dominant traits always more common than recessive traits?

*No. For example, in humans the allele for six fingers and six toes is dominant over the allele for five fingers and five toes, but the **polydactyly** (extra digits) allele is quite rare.*

4. If you know the genotype of the parents, can you predict the possible genotypes of the babies?

Yes. But when the parents are heterozygous for several different genes (as Mom and Dad Reebop were in the previous exercise, for example), it becomes complicated. When Mom and Dad are both heterozygous at only one locus (Aa), three different genotypes of babies are possible: AA, Aa, or aa. If they are heterozygous at two loci (Aa and Bb), there would be nine possibilities: AABB, AABb, AAbb, AaBB, AaBb, Aabb, aaBB, aaBb, and aabb. So we can quickly see that the relationship is...what? (Answer: There are 3^n different genotypes possible, where n is the number of loci at which Mom and Dad are heterozygous.) So, since Mom and Dad Reebop were heterozygous at seven loci, how many different genotypes of babies could they have produced? (Answer: 3^7 , or 2,187! That is why there will very rarely be two Reebop babies of identical genotype produced by a class.)

*If two members of this class had been heterozygous with respect to 20 of the traits that were included in the Create-a-Baby Table, there would have been 3^{20} , or **more than three billion**, possible genotypes for the offspring they produced. Most humans are probably heterozygous at thousands of different loci, so the number of possible genotypes of their offspring is astronomical. That is why siblings never look exactly alike unless they are identical twins (derived from a single fertilized egg).*

5. Why are there so many different skin and hair colors in humans?

Skin and hair color, like many other human traits, are determined by the effects of more than one gene, so there are many possible combinations. For example, it is thought that alleles at seven different loci have major effects on skin color in humans. So, as we just calculated, if there are only two different alleles at each of these seven loci, there are 2,187 possible genotypes. So at least 2,187 different skin color genotypes are possible in humans. This is without even making any allowance for the effect of sunlight on skin color.

6. Are there only two possible alleles at each locus?

No, there can be any number. As a simple example, there are three common alleles at the ABO blood-group locus. Alleles A and B encode different forms of an enzyme, thereby causing two different kinds of sugars to be put on the surface of red blood cells (RBCs). Alleles A and B are codominant, so a person who is heterozygous for these two alleles has both types of sugar on the RBCs and thus has type AB blood. The O allele (which encodes an inactive form of the enzyme and thus is unable to put any sugar on the surface of the RBCs) is recessive to both the A and B alleles.

As a more complex example, in humans there are more than 100 alleles at most of the HLA histocompatibility loci, which encode proteins on the surfaces of cells in most human tissues. It is differences in these proteins between donor and recipient cells that cause the immune system to recognize grafted tissues as foreign and attack them. With 100 alleles at 11 loci, there would be 100^{11} , or 10^{22} , possible combinations of different histocompatibility alleles, which is far greater than the number of human beings that have ever been born. That is why it is impossible (except in the case of identical twins) to find an organ donor who is a perfect match for someone who needs an organ graft.

7. Mom and Dad Reebop have only one gene on each chromosome. How many different genes do humans have on a chromosome?

As a result of the sequencing of the human genome, which was completed in 2001, we now know that humans have about 35,000 genes per haploid chromosome set. These are distributed on 23 kinds of chromosomes that differ quite a bit in size, suggesting that the number of genes per chromosome is probably also quite variable. So a good guess would be that on average a human chromosome probably contains around 1,500 genes, but that the range might be from about 750 to about 3,000.

HINTS AND TROUBLESHOOTING

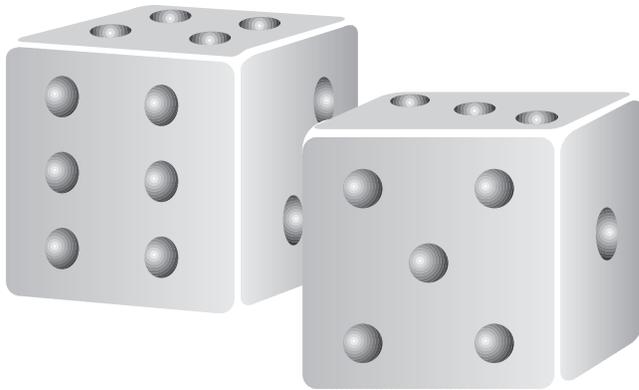
Create-a-Baby is another fun exercise and a good complement to the Reebops. It reinforces students' newly gained knowledge of genetic concepts and terminology by referring to organisms with which they have no trouble identifying: themselves, their friends, and their future progeny.

You can contribute to the mutually reinforcing nature of these two exercises by tying the two discussions together as much as possible. Among the major take-home messages should be the following: (a) as a result of meiosis and recombination, a pair of sexually reproducing organisms have the potential to produce progeny with many different genotypes and phenotypes, but (b) if we know the genotype of the parents, we can predict how probable it is that one of their offspring will have any particular genotype of interest.

This would be a good time to provide your students with copies of Appendix C (“Some Presumably Simple Heritable Human Traits”). If you do, be sure to call their attention to the introduction, which indicates some of the reasons that the information in this table should be considered tentative.

CHAPTER 2

**Passing Traits from
One Generation
to the Next**



SECTION D

**How Are
Genetic
Experiments
Actually
Performed?**

Chapter 2: Section D Background

IMAGINARY ORGANISMS ARE FUN. They can also be instructive, as we saw in Section C. But Mendelian genetics is not about imaginary organisms any more than chemistry is about imaginary elements. So it's time to introduce your students to some real Mendelian-genetic experiments, with some real, live organisms.

Although all sexually reproducing eukaryotic organisms that have been studied adhere to the principles of Mendelian genetics, there are a very limited number of them that are suitable for performing Mendelian genetic experiments in a secondary-school classroom. In order to be useful in such a context, organisms must be quite small (so a large number of them can be produced and maintained in a small space). They must be relatively easy to raise in captivity (so teachers and students with little prior experience can work with them effectively). They must have a relatively rapid sexual reproductive cycle (so progeny from a sexual cross can be produced and analyzed within the confines of a standard school term). And they must be available in strains exhibiting reproducible visible differences that students can readily distinguish (so students can easily collect the data that will enable them to deduce the genetic basis for the phenotypic traits(s) in question).

Fruit flies meet all of the above criteria and therefore probably have been used for classroom Mendelian genetic experiments more than all other organisms combined. Nevertheless, they do not always meet one other criterion not listed above: to be actually adopted for classroom genetic studies, organisms must be aesthetically acceptable to the teacher. Our experience indicates that many teachers say they prefer not to have rogue fruit flies buzzing around their classrooms all semester or refuse to use fruit flies for some other reason. So the exercises developed here are based on two very different kinds of organisms that meet the above criteria: a fungus (baker's yeast) and a plant that was developed for studying genetics in the classroom (Wisconsin Fast Plants).

As you will read in the Overview to Section D.1, baker's yeast has become one of the most intensively studied organisms in the world. Here your students will use it to generate a Punnett square that illustrates the results of a monohybrid cross, not with letters on paper, but with live organisms that are growing and exhibiting two readily distinguishable diploid phenotypes.

As you should already have read by now (on the second page of this manual), *fast* is a relative term when it comes to plants. If you have made the recommended preparations, we believe your students will have a truly rewarding experience, as they see the results of a dihybrid cross unfold before their eyes. However, we do provide an alternative, less time-consuming option that will permit your students to gain some of the benefits of the Fast Plants, even if you are unable to devote as much time to this topic as the two-generation Fast Plant exercise requires.

A Colorful Experiment in Yeast Genetics

STUDENT PAGES 112-119

LESSON OVERVIEW

In this exercise, students perform a genetic cross that provides information about a single gene trait. Haploid strains of baker’s yeast (*Saccharomyces cerevisiae*) come in two mating types, **A** and **α** (alpha), each of which is available in red and white varieties. Therefore, students can use yeast matings to produce a visible Punnett square.

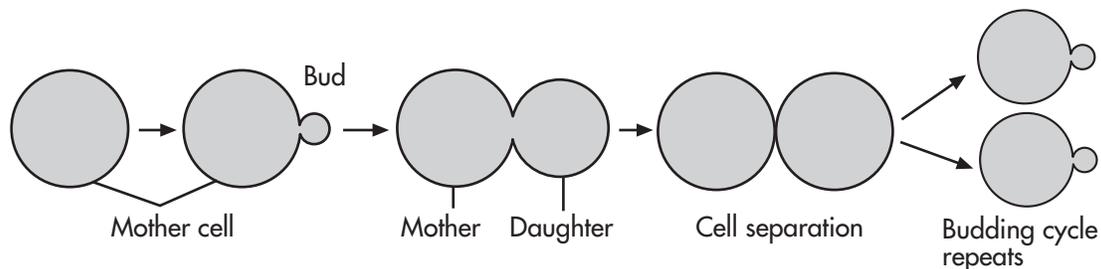
BACKGROUND

Baker’s yeast is a unicellular organism that can be grown on culture plates like bacteria. Nevertheless, it is a genuine eukaryotic organism with a nucleus, mitochondria, and many other features shared by all eukaryotic cells. This, combined with its interesting life cycle, which involves an alternation of haploid and diploid phases, makes it a good model organism for studying basic cell biology and genetics.

Yeast was the first eukaryotic organism to have its DNA completely sequenced as part of the Human Genome Project. The reason that yeast was included in the Human Genome Project is because it is known to have thousands of genes that are extremely similar to those of humans, but it is much easier to study the function of many of those genes and their products in yeast cells than it is in human beings. Yeast is the most intensively studied eukaryotic organism. Nevertheless, thousands of yeast genes of unknown function were discovered as a result of the DNA sequencing. Now that the sequence of each of those genes is known, work has begun to understand all of their functions.

Cell Growth and Division

Baker’s yeast is known as a **budding yeast**. This refers to its unusual form of cell division. It does not just grow larger and then divide in the middle, as many animal and plant cells do. Instead, it forms a small bud at some point on the surface of each **mother cell**. This bud, or **daughter cell**, grows until it is as large as the mother cell, and then it pinches off



Cell Division in Budding Yeast

as a separate cell. (By then, the chromosomes will have been replicated, the nucleus will have undergone mitosis, and the bud will have received a nucleus identical to that in the mother cell.) Under good growth conditions, both the mother cell and the daughter cell (now about to become a mother cell itself) begin to bud again as soon as they have separated. Both haploid and diploid cells of baker's yeast divide this way.

Sexual Reproduction and Beginning of the Diploid Phase

Saccharomyces cerevisiae has a well-characterized sexual cycle, which is illustrated on S113. A haploid cell population consists of cells of one of two mating types, usually referred to as **a** (little a) and **α** (alpha). But because of the difficulty students may have in distinguishing **a** and **α** in writing, we will refer to the two mating types as **A** (capital a) and **α** (alpha) throughout the rest of this exercise. Although haploid cells can reproduce asexually (by budding) indefinitely, they also can act as gametes and fuse with cells of opposite mating type to form a diploid.

Each mating type releases a chemical substance that prepares cells of the other mating type for sexual fusion. Thus, as soon as cells of opposite mating type encounter one another, they initiate a series of cellular events that will lead to **conjugation** in which an **A** cell fuses with an **α** cell.

Sporulation and Beginning of the Haploid Phase

Given suitable conditions and adequate nutrients, diploid yeast cells can grow and divide mitotically for an indefinite period. However, if a diploid culture becomes nutritionally deprived, growth and mitotic division ceases, and the cells prepare to undergo meiosis and **sporulation** or spore formation. As a result of meiosis, four haploid cells are produced. These four haploid cells develop as four **ascospores**, dormant, resistant cells, within the wall of the original diploid cell. Sporulation takes at least 15 hours. If ascospores are simply returned to a nutritionally adequate environment, they will germinate and begin to reproduce asexually. But because both mating types will be present under these conditions, they will quickly mate and reform diploids.

On the other hand, if the ascospores are separated from one another while they are still dormant, each will form a stable haploid culture of a single mating type when it is returned to rich medium. This is how the haploid strains that your students will be using were produced.

The Basis for the Red Phenotype in Yeast

In most organisms, presence of color is a dominant trait and colorless (white or albino) is recessive. This is because the colorless trait is usually caused by a mutant gene that encodes an inactive form of an enzyme required for producing a normal pigment. Even when the enzyme encoded by the mutant allele is totally inactive, the heterozygote usually is normal in color, because the enzyme acts as a catalyst, and therefore half the usual amount of enzyme is enough to make the usual amount of pigment. In some cases that is not true, so the traits exhibit codominance, and the heterozygote is intermediate in color between the two homozygotes. (For example, it has pink flowers instead of red or white.)

Yeast normally are not colored. The red strain that will be used in this exercise is unusual in that regard. It has a mutation of a gene that encodes an enzyme required for one particular step in a series of reactions that normally produces the nitrogenous base adenine. When this mutant gene is present in a haploid strain or in the homozygous condition in a diploid strain, this step in adenine synthesis cannot take place.* As a consequence, the compound that is produced in the preceding reaction accumulates in the cell. And as luck would have it, this accumulating intermediate product causes the cell to turn red. However, in a diploid cell that has one copy of the wild-type gene, enough functional enzyme is produced to prevent accumulation of the intermediate. Thus, the cell remains colorless. Or, putting it differently, white is dominant.

Experience indicates that, by one means or another, most students have developed a sense of the usual dominance relationship between color and colorless alleles and therefore will predict that the heterozygous diploid yeast will be either red or pink. The contrast between what most students usually predict and what they actually observe often provides a good opening for discussing the scientific approach as a way of testing assumptions and hypotheses.

*Adenine is essential for life. It is required for making DNA, RNA, ATP and several other very important cellular components. Thus, the red yeast strain could not grow if you did not provide adenine. However, the yeast extract that you included in the medium provides all of the adenine that the mutant strain needs for normal growth.

REFERENCE

Handbook for Using Yeast to Teach Genetics, T.R. Manney and M.L. Manney. 1991. Manhattan, KS, Kansas State University Department of Physics.

This handbook and the accompanying video have several interesting experiments for further study.

The Genes We Share with Yeast, Flies, Worms and Mice: New Clues to Human Health and Disease. (2001). This booklet contains an up-to-date discussion of the way in which baker's yeast is being used to obtain important new insights into human genetics. A more complete description and ordering information are given on page T106.

TIMELINE

The terms *Day One*, *Day Two*, and *Day Three* in this exercise are based on the assumption that you will be able to incubate the culture plates at 30° C. If you do not have a 30° incubator, incubate the dishes at room temperature, but allow the yeast to grow for two days between steps, instead of one.

Day One About 15 minutes. Students label their Petri dishes and transfer haploid yeast of four types from stock plates to their own test plates.

Day Two 15-30 minutes. After the haploid cultures have grown enough on the Petri dishes to show their color clearly, students will set up their crosses and then complete the Day Two Worksheet.

Day Three About 15 minutes. Students examine the results of their crosses, record observations and complete the Day Three Worksheet.

(If most of the lab groups find that their cultures have not grown enough to make the color of each diploid entirely clear, all groups should allow their cultures to grow another day before continuing with the analysis.)

MATERIALS

For each group of four students:

1 petri dish containing yeast growth medium (YED)	culture of Red, Mating type α yeast
marking pen	culture of White, Mating type A yeast
a packet of sterile toothpicks	culture of White, Mating type α yeast
culture of Red, Mating type A yeast	waste container
	disinfectant

The four strains of yeast can be ordered from:

Carolina Biological
(800) 334-5551
www.carolina.com

Mating type	Color	Strain Designation	Catalog number
A	red (R)	HA2	ER-17-3624
A	white (W)	HAT	ER-17-3630
α	red (R)	HB2	ER-17-3626
α	white (W)	HBT	ER-17-3631

At the same time that you order the above yeast strains, order the premixed powder that you will use to prepare the medium on which the yeast will be grown: Carolina cat. no. ER-17-3651 Yeast-Extract Dextrose (enough to make 2 liters of YED medium). You should also order 10 ml of 10 mg/ml Gentamycin solution (Cat. # G1272) from Sigma, P.O. Box 14508, St. Louis, MO 63178, (800) 325-3010, www.sigma-aldrich.com.

ADVANCE PREPARATION

NOTE CAREFULLY: Preparations for this exercise will not occupy a lot of your time, but at several points culture plates will need to sit for several days to a week at a time. Therefore, you need to begin preparations for this experiment at least two weeks in advance, and if you do not have an incubator in which you can incubate the plates at 30°C, you should start preparations at least three weeks in advance. If plates and cultures are ready before they are needed, they will keep. But if they are not ready when you want to use them, there will be nothing you can do to rush them along!

1. Preparation of yeast extract/dextrose plates (YED plates) for culturing the yeast. You will need four YED plates for your own use, plus five times as many plates as there will be student groups in your largest class, plus one plate for each group of students in each of your other classes. For example, assuming that you have seven groups of students in your largest class and six groups of students in each of your other three classes, you will need to prepare $4 + (5 \times 7) + (1 \times 3 \times 6) = 57$ YED plates. But because YED plates are very easily contaminated, we recommend making a few extras. The general procedure for pouring YED plates is similar to the one that you used to prepare nutrient agar (NA) plates for *E. coli* (Chapter 1, Section E). The major difference is that because YED is richer in nutrients than NA medium is, more of the bacteria and fungi that are present in room air can grow on YED plates than can grow on NA plates.

The gentamycin that we recommend adding will greatly reduce the chance of bacterial contamination, but the plates will nevertheless be likely to become contaminated with airborne fungal spores unless they are poured and used with great care.

It is possible to buy the individual ingredients for YED and weigh them all out for each batch of medium. But because you will probably need to make several batches of medium, that could become tedious. We strongly recommend that you buy premixed YED powder from Carolina Biological Supply Company as noted earlier.

Below are the materials and steps required to make a batch of 12 dishes:

15 g of YED powder	Gentamycin solution (10 mg/ml)
300 ml deionized water	1000 μ l micropipettor with sterile tip
500 ml flask	12 petri dishes
piece of aluminum foil	disinfectant
autoclave or pressure cooker	1 Bunsen burner or alcohol burner
hot water	

- a. Weigh out 15 g of YED powder.
- b. Add 300 ml of deionized water to a 500 ml flask. Swirl the flask while adding the powder slowly.
- c. Cover the flask with aluminum foil. Sterilize 30 min at 15 psi in an autoclave or pressure cooker
- d. While medium is being sterilized, wipe down a flat surface with disinfectant and spread out 12 petri dishes on it.
- e. Set up a Bunsen burner or alcohol burner near the petri dishes.
- f. Allow flask containing sterile YED to cool just enough that you can handle it comfortably. Use a micropipettor with a sterile tip to add 450 μ l of gentamycin solution (10 mg/ml) to the medium. Swirl the flask to ensure that the contents are thoroughly mixed.
- g. Light the Bunsen or alcohol burner. Remove the foil lid from the flask. Pass the mouth of the flask through the flame.
- h. Lift the lid of a petri dish, fill it about half way with YED medium, and cover it back up quickly.
- i. Repeat the preceding two steps until all twelve plates have been poured. Fill the flask with hot water to simplify cleaning later.

Repeat the above procedure as many times as necessary, until you have enough plates for all of your classes, plus a few to spare. If necessary, carefully stack one set of plates four-high and move them aside, in order to make space for pouring the next set of 12 plates.

After all plates have solidified, they should be spread out in a place where they can be left undisturbed for a day or two to dry out. (YED plates have a tendency to accumulate a great deal of moisture on their lids initially. If this moisture is not allowed to evaporate before the plates are stored or used, contamination with unwanted organisms is almost inevitable.) When all, or nearly all, of the condensation has disappeared from

their lids, the YED plates can be turned upside down, wrapped (either in the plastic sleeves from which the petri dishes came or in plastic wrap) to prevent further drying. They can then be stored upside down in the refrigerator until they are needed.

2. Preparation of stock culture plates. The yeast cultures from Carolina Biological will arrive in small vials. Use them to establish petri dish subcultures, using techniques similar to those you used to subculture *E. coli* for “Shine On!” (Chapter 1, Section E.2.c). The difference here is that you will grow your yeast subcultures on the YED plates that you have prepared in step 1. You will greatly decrease the chances that you will contaminate your stock subcultures (and thereby possibly blow the whole exercise!) if you keep your YED plates upside down while streaking the yeast out on the surface of the agar. This will seem a bit difficult to do at first. However, it is the technique that you will be asking your students to use later. So some practice now might come in handy later.

Be sure to label and date the bottom of each subculture plate as you prepare it. Rather than using the catalog numbers or the strain codes that are used by Carolina Biological to identify the cultures, label your plates with the clear, simple designations that you will have your students use, as shown in the following table:

Catalog number	Carolina strain code	Your label
BA-17-3624	HA2	Red, Mating type A
BA-17-3630	HAT	White, Mating type A
BA-17-3626	HB2	Red, Mating type α
BA-17-3631	HBT	White, Mating type α

If you have a thermostatically adjustable incubator, set it for 30°C for growing yeast cultures. Otherwise, grow them in whatever place you can find that is closest to 30°C. At 30°C, it should take only two to three days for the white yeast colonies to grow to the extent that they have a rich, creamy consistency and an ivory-white color. At this point they will be ready to use in the next step. The red colonies, however, probably will not look as lush and creamy in consistency as the white colonies and will not be ready for use. If that is the case, put the white yeast in the refrigerator and allow the red ones to grow another day or two, until they also have a rich, creamy appearance and are very dark pink or red in color. If you grow the yeast at room temperature, it might take twice as long to get cultures that are ready to use. The cooler the spot where you grow them, the longer it will be before you are ready for the next step. On the other hand, you should not assume that this means that the higher the temperature, the better. Yeast grow better in an incubator set at 30°C than one set at 37°C, for example.

Once the red and the white strains on your stock plates have all produced rich, creamy-looking colonies, use them to make one subculture of each strain for each student group in your largest class. (All of your classes will be able to work from the same set of stock plates.) Be sure that you label each plate carefully as you set it up. After establishing the second generation of subcultures for your classes, wrap the first generation plates in plastic film and store them in the refrigerator. It will take just about as long

for your second generation plates to reach a useable condition as it did your first generation plates. Once again, permit the red strains to grow longer than the white strains if necessary. If these subcultures are ready for use before your class is ready for them, wrap them in plastic film and refrigerate them; they will hold for weeks with no apparent loss of viability. (We do not recommend trying to hold them over from one year to the next, however.)

3. Sterile toothpicks. Provide each lab group with an unopened box of toothpicks that has just had one corner cut off with a razor blade, so that toothpicks can be shaken out one at a time. (Toothpicks in an unopened package are essentially sterile, so they do not require any additional sterilization.) As long as no contaminated toothpicks have been put back in the package, the package can be passed on from class to class with no problem.
4. Disinfectant. Prepare disinfectant spray bottles as for Chapter 1, Section E.2.

HINTS AND TROUBLESHOOTING

1. The YED plates get contaminated easily, so always make a few extra and emphasize the importance of working with them carefully to avoid contamination. Experience indicates that they will have much lower contamination levels if students keep the plates upside down while working with them. As mentioned above, you should practice this technique in advance and then demonstrate it to the class.
2. Unless students are cautioned against doing so, they will tend to ignore the instructions and use as much yeast as they can possibly load on the end of a toothpick to set up each cross. Emphasize that “more is better” does not apply to this case. Indeed, if there are too many haploid cells present, they will interfere with the growth of the diploid cells and will obscure the outcome of the cross. Emphasize that each cross should be set up with just enough yeast of each mating type to be barely visible. Then also emphasize the importance of mixing the two kinds of cells together thoroughly with the second toothpick.
3. After students have set up their crosses and completed the Day 2 Worksheet, have them discuss it as a class. Most students will think either that red will be the dominant color, so that the diploids will be red, or that red and white will be codominant, so that the diploids will be pink. Actually white is dominant, as explained above, but don’t reveal this to the class yet.
4. If students have a colony that is mostly white, but with a red center, have them incubate the plate another day, by which time the white diploids should overgrow the red haploids, clarifying the result.
5. At the end of the exercise, have the students open their plates, spray them with bleach, reclose them, tape them shut, and dispose of them in a standard trash container.

DAY 2 WORK SHEET STUDENT PAGE 116

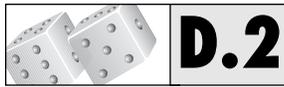
After each group of students has predicted the outcomes of the crosses, have them discuss their predictions as a group. Make a tally of the class predictions.

ANSWERS TO DAY 3 WORKSHEET STUDENT PAGE 118

1. Use the results you observed for your yeast crosses to fill in the blanks on the diagram below:

		Mating type A	
		R	W
Mating type α	R	Genotype <u>RR</u> Phenotype <u>Red</u>	Genotype <u>WR</u> Phenotype <u>White</u>
	W	Genotype <u>RW</u> Phenotype <u>White</u>	Genotype <u>WW</u> Phenotype <u>White</u>

2. What ratio of phenotypes did you observe as a result of the four crosses you performed?
1 red to 3 white
3. What does this indicate about which allele is dominant and which is recessive?
White is dominant, and red is recessive.
4. Is this what you predicted on your Day 2 Worksheet?
(You might want to discuss the class tally here and use the disparity between predictions and observations to discuss the strength of the scientific approach as a way to test – and falsify – hypotheses.)
5. In the table below, list what you predicted and what you observed for each of the four crosses.
Did anyone in the class predict the outcome correctly?
6. If your predicted and observed phenotypes do not agree, how can you account for that, and can you propose a good hypothesis to account for the results you actually observed?
See what students come up with, but then tell them what biologists have discovered about the basis for the color of the red strain. (See the Background Information for an explanation of why red is recessive to white.)
7. If you have come up with a new hypothesis, can you think of a way to test it?
It should be interesting to see what your students come up with here.



Experimenting with Wisconsin Fast Plants

STUDENT PAGES 120-156

LESSON OVERVIEW

Paul Williams, a plant biologist at the University of Wisconsin, performed an exceptional service for biology teachers and students everywhere when he devoted 15 years to developing the Wisconsin Fast Plants. These plants have made it possible for students to carry out meaningful experiments in plant genetics in less than a semester.

Most plants take at least one full growing season — six months to a year — to go through a full life cycle. (That is, to go from a seed to an adult plant with mature seeds.) Furthermore, most plants get so large by the time that they produce mature seeds that you could not grow more than a few of them in a standard classroom. In contrast, Fast Plants go from seed to seed in about six weeks, and they are so small when they are full-grown that hundreds can be kept in just a few square feet.

Another name for Wisconsin Fast Plants is **rapid-cycling brassicas**. *Brassica* is the plant genus that includes such familiar vegetables as broccoli, cabbage, cauliflower, collards, kale, kohlrabi, and mustard greens (in addition to the rape seed plant, from which the increasingly popular canola oil is obtained). The species of *Brassica* from which the Fast Plants were derived is *B. rapa*, a species from which bok choy, Chinese cabbage and turnips also were derived. Professor Williams planted seeds that had been collected from thousands of brassica plants around the globe, and observed their offspring carefully. The plants of each species that flowered and set seed most rapidly were crossed with one another. This selection process, continued for many generations, resulted in *B. rapa* plants that germinated within a day or two, flowered within two weeks, and had mature seed in less than six weeks. These became the Wisconsin Fast Plants used for teaching, but Fast Plants belonging to five other species of *Brassica* were also produced for use in professional plant research.

Seeds of a number of Fast Plant mutants are now commercially available. Among the mutants with homozygous-recessive phenotypes that can be easily recognized are the following: The *rosette* mutant is much shorter than a wild-type plant because its **internodes** (the regions of stem between successive leaves) fail to elongate. The *elongated internode* mutant has the opposite abnormality and thus is much taller than a wild-type plant. The *petite* mutant is about half as tall as a wild-type plant as a result of a reduction in size of nearly all plant parts, not just an internode abnormality. The *anthocyaninless* mutant lacks the purple-red pigment, **anthocyanin**, that is normally present to a varying degree in the stems, leaves, and various other plant parts; thus these plants have a much brighter, clearer green color than wild-type plants do. On the other hand, the *yellow-green* mutant, as its name suggests, has a less intense green color than wild-type plants do.

The Mendelian-genetics experiment that is outlined in this unit involves a **dihybrid cross**: a cross involving alleles at two different loci (in this case, the *anthocyaninless* and the *yellow-green* loci). It will provide your students with an opportunity to rediscover Mendel's law of independent assortment of alleles at two loci.

The term *law* is put in quotation marks because independent assortment of alleles at two loci is not a basic law of heredity as Gregor Mendel believed it was. The phenomenon of independent assortment (which Mendel observed in every dihybrid cross that he analyzed) is only observed when the two loci being studied are located either on separate chromosomes or far apart on a single chromosome. When the two loci being studied are located near one another on a single chromosome they do not exhibit independent assortment; they exhibit linkage, as will be explained in section 2.E.

Symbols Used to Identify Genotypes and Phenotypes of Fast Plants (and Many Other Organisms)

Wherever you look for information about Fast Plants — whether it is in the Carolina Biological catalog, in information distributed by the Wisconsin Fast Plants Project, or even in the ordering information provided in the MATERIALS section of this unit — you will find symbols used to identify the various Fast Plant genes that look a bit more complicated than the symbols that we've been using to identify genes and their alleles earlier in this chapter (in the Reebop and the Create-a-Baby exercises, for example).

Be assured that these unfamiliar symbols are not being introduced just to complicate your life as a teacher! They are the kind of symbols that geneticists around the world have agreed to use to symbolize the genotypes and phenotypes of organisms involved in genetic studies. After explaining why such symbols are used, and how they are used, we will suggest how you can avoid introducing such apparent complications into your classroom, if you prefer.

When students are introduced to genetics, they and their teachers are usually given a very simple and easily understood set of symbols to use for representing dominant and recessive alleles. This is what we usually call the **big-A-little-a convention**; a single capital letter represents the dominant allele associated with some particular trait, and the corresponding lower case letter represents the corresponding recessive allele. For example, *T* was used for the dominant Reebop curly tail allele, whereas *t* was used for the recessive straight tail allele (see S94). In contrast, the symbols used for the various Fast Plant genes all use three letters.

Why can't geneticists leave well enough alone and stick with the simple, easily understood big-A-little-a convention for naming genes? One reason is that such a convention only provides a way of naming 26 genes per organism, and even the simplest real organisms have thousands of genes. A second reason is that in most cases a single letter conveys no information about the function of the gene for which it stands. For example, who would guess that *D* stands for a gene that determines the number of segments in the body of a Reebop — without looking it up in the Reebop genotype-phenotype table?

In contrast, in the **three-letter convention** used to identify genes of Fast Plants (and many other organisms), the symbols used for genes are usually abbreviations of the phenotypic traits that they influence. As a result, the gene symbols convey useful information. For example, one of the genes your students will study in this exercise controls the synthesis of the purple-red pigment, anthocyanin. In plants that are homozygous for the recessive allele at this locus, no anthocyanin can be made. Therefore, the symbol for this recessive allele is *anl*, which is short for *anthocyaninless*. Similarly, the second trait that will be studied gives the leaves of homozygous-recessive plants a yellow-green color, instead of the usual dark green color. So the symbol for the recessive allele in this case is *ygr*, which is short for *yellow-green*.

Like the big-A-little-a convention, the three-letter convention distinguishes the dominant and recessive alleles by use of capital and lower case letters, respectively. Thus, the dominant and recessive alleles at the *anthocyaninless* locus are symbolized *ANL* and *anl*, respectively. And the dominant and recessive alleles at the *yellow-green* locus are *YGR* and *ygr*, respectively.

Notice that in the three-letter convention the allele symbols, as well as the names of the genes themselves, are always italicized (or underlined when handwritten). This is because there is a special use for the corresponding nonitalicized symbols: to provide a shorthand identification of phenotypes. Here is how it works:

- The recessive (mutant) phenotype is identified by three non-italicized letters, of which only the first one is capitalized. For example, **Anl** stands for the anthocyaninless (no-purple color) phenotype of the homozygous recessive (*anl/anl*) plants.
- The dominant (wild-type) phenotype is identified by the same three-letter symbol, followed by a superscript-plus sign. For example, **Anl⁺** stands for the anthocyanin-containing (purple stems, etc.) phenotype exhibited by both the homozygous dominant (*ANL/ANL*) and the heterozygous (*ANL/anl*) plants.

The table below may help you visualize all of these relationships:

Trait being considered	Dominant allele	Recessive allele	Dominant phenotype	Recessive phenotype
Presence versus absence of purple anthocyanin	<i>ANL</i>	<i>anl</i>	(purple stems, etc.) Anl⁺	(anthocyaninless) Anl
Yellow-green versus dark green	<i>YGR</i>	<i>ygr</i>	(dark green leaves) Ygr⁺	(yellow-green leaves) Ygr

You need to understand the three-letter convention to interpret the symbols for genotypes and phenotypes in the Fast Plant section of the Carolina Biological catalog or in literature distributed by the Wisconsin Fast Plant Project. However, you are free to decide whether you will introduce these symbols to your students or whether you will continue to use the big-A-little-a convention in your classroom. If you prefer, you may have your students use A and a (instead of *ANL* and *anl*) for the dominant and recessive alleles at the anthocyaninless locus and B and b (instead of *YGR* and *ygr*) for the dominant and recessive alleles at the yellow-green locus. The choice is yours.

TIMELINE

Some Important Scheduling Considerations

Fast Plants provide a virtually unrivaled opportunity for students to perform a genuine study in Mendelian genetics in a standard classroom setting. However, fast is a relative term when applied to plants. Although Wisconsin Fast Plants have a generation time of less than one-fourth that of most annual plants, it still takes at least seven weeks to complete a two-generation Mendelian-genetics experiment with them. Some teachers decide that they cannot fit an experiment of such length into their curriculum — even though the plants will require little attention during most of the seven weeks. Recognizing this, we provide two options for conducting the Fast Plant experiment. Details of each option will be given later, but here we will briefly outline both options, so that you can consider which of them better suits your schedule and your teaching philosophy.

Outline of Option 1: A Seven-Week, Two-Generation Experiment

1. About seven weeks before you expect to finish the rest of Chapter 2 have your students plant their F₁ Fast Plant seeds. Do not discuss the genetic aspects of the experiment; only tell them that they are starting an exercise in plant growth and development that will take on additional meaning later.
2. Day 2: The seeds have germinated, and the students watch the plants develop.
3. Week 2: The plants are flowering, so the students pollinate them like busy little bees.
4. Week 5: The F₂ seeds are mature, and the plants are dried out.
5. Week 6: The F₂ seeds are dry and the students plant the seeds.
6. Week 7: The F₂ plants germinate, and the students quickly see that (in distinction to the F₁ plants) the F₂ plants are not all alike. While many will appear wild-type (as their F₁ parents did), some will be *anthocyaninless*, others will be *yellow-green*, and yet others will be both *anthocyaninless* and *yellow-green*. They record the number of plants in each of these categories and then analyze the data to see how closely they correspond to the ratios predicted by Mendel's law of independent assortment.

Total elapsed time: about seven weeks.

Outline of Option 2: A One-Week Analysis of the F₂ Generation

1. Students begin with Step 5 of the above outline, when they plant F₂ seeds that you have purchased and provided to them. As controls, they will also plant seeds from the parental and F₁ generations.
2. Within the first week the students see the same set of phenotypes as the students performing Option 1 do, record the same kind of data, and perform the same kind of data analysis.

Total elapsed time: about one week. If you are already teaching from this manual when you read this page, there is little doubt: Option 2 is for you. (But consider Option 1 for next year!)

PROS AND CONS OF OPTION 1

The major advantage of Option 1 is that the students can follow the F₁ plants through an entire life cycle, pollinate the F₁ flowers, watch them produce seeds, and then harvest and plant those seeds to determine what kinds of plants will be produced in the F₂ generation. This will probably be the first opportunity for most of your students to follow a plant through its entire life cycle. In addition, Option 1 will allow your students to observe one of the most fundamental facts of life: namely, that certain heritable abnormalities can be carried in a latent, invisible form by one generation of individuals, only to appear in a very visible — and sometimes devastating — form in their progeny.

The disadvantages of Option 1 are (a) that it must be started about seven weeks before the students obtain genetic data for analysis, and (b) during the intervening weeks the students will need to water and observe their plants from time to time, even though they will be engaged in other kinds of learning activities in your classroom.

PROS AND CONS OF OPTION 2

The advantage of Option 2 is that your students can collect and analyze the very same kind of genetic data as with Option 1, but in one week instead of seven.

The disadvantage of Option 2 is that it is much more of a cookbook experiment than Option 1. They do not have the opportunity of seeing any phase of the plant life cycle other than the first few days of seedling growth. More importantly, they do not have the opportunity of harvesting their own F₂ seeds from the F₁ plants they raised. Instead, they are handed several sets of seeds and told what the relationship among them is.

MATERIALS OPTION 1

Seeds required per 12 student groups (groups of four are recommended):

Catalog number	No. of seeds	Phenotype	Genotype	Genetic role in exp.
BA-15-8812	50	anthocyaninless (Anl, Ygr+)	<i>anl/anl, YGR/YGR</i>	PA
BA-15-8818	50	yellow-green (Anl+, Ygr)	<i>ANL/ANL, ygr/ygr</i>	PB
Two packs of BA-15-8891	2 x 200	wild type (Anl+, Ygr+)	<i>anl/ANL, YGR/ygr</i>	F1
BA-15-8888	250	segregating all four phenotypes	all of the above and below	F2 (optional backup)
BA-15-8842	50	anthocyaninless, yellow-green (Anl, Ygr)	<i>anl/anl, ygr/ygr</i>	double-mutant

Carolina Biological Supply Company 1-800-334-5551 or Fax 1-800-222-7112

For all classes combined:

- 6 liters of potting mix (1 part Peatlite, RediEarth, or JiffyMix to 1 part medium vermiculite)
- Peter s Professional Fertilizer 20-20-20, diluted to 1X and 1/8X (instructions below)
- 1 1-liter and 1 2-liter soda bottle, with labels removed
- masking tape
- felt-tip marking pens
- 1 pack of dried bees (Cat. # BA-15-895)
- 1 box of toothpicks
- 1 tube of fast-drying Duco cement

The components for the potting mix and the Peter s Professional Fertilizer are available at most garden centers.

For each set of four groups of four students:

- 1 plant lighthouse (instructions below)
- 6 8-inch x 10-inch pieces of 1 inch thick rigid insulating foam

Sheets of insulating material can be purchased inexpensively from building supply stores. Your shop teacher may be able to cut up such a sheet for you, or you can cut it with a utility knife. Your students will use these six pieces of foam underneath their growth systems initially, so that when the seedlings emerge, they will be close enough to the light to grow vigorously. Then as their plants grow, students will remove the foam pieces one at a time, in order to keep the growing tips of the plants at the recommended distance from the light.

For each group of four students:

- 1 film-can growth system (instructions below)
- 30 F₁ seeds in a small envelope
- 1 water bottle (instructions below)
- 14 25-cm bamboo skewers (available in most supermarkets)
- 14 split-ring ties made from 9 or 10 mm O.D. Tygon or aquarium tubing (instructions below)
- 1 brown paper lunch bag
- 1 small envelope

For each group of four students in Phase 2:

- 1 envelope with 6 PA seeds
- 1 envelope with 6 PB seeds
- 1 envelope with 6 double-mutant seeds
- 1 envelope with the students own F₂ seeds

MATERIALS OPTION 2

Seeds required per 12 student groups (groups of four are recommended):

Catalog number	No. of seeds	Phenotype	Genotype	Genetic role in exp.
BA-15-8812	50	anthocyaninless (Anl, Ygr+)	<i>anl/anl, YGR/YGR</i>	PA
BA-15-8818	50	yellow-green (Anl+, Ygr)	<i>ANL/ANL, ygr/ygr</i>	PB
BA-15-8890	50	wild type (Anl+, Ygr+)	<i>anl/ANL, YGR/ygr</i>	F ₁
BA-15-8888	250	segregating all four phenotypes	all of the above and below	F ₂
BA-15-8842	50	anthocyaninless, yellow-green (Anl, Ygr)	<i>anl/anl, ygr/ygr</i>	double-mutant

For all classes combined:

- 3 liters of potting mix (1 part Peatlite, RediEarth, or JiffyMix and 1 part medium vermiculite)
- Peter s Professional Fertilizer 20-20-20 diluted to 1X and 1/8X (instructions below)
- masking tape
- felt-tip marking pens
- 1 1-liter and 1 2-liter soda bottle, with labels removed

The components for the potting mix and the Peter s Professional Fertilizer are available at most garden centers.

For each set of four groups of four students:

- 1 plant lighthouse (instructions below)
- 6 8 inch x 10 inch pieces of 1 inch thick rigid insulating foam

Sheets of insulating material can be purchased inexpensively from building supply stores. Your shop teacher may be able to cut up such a sheet for you, or you can cut it with a utility knife. Your students will use these six pieces of foam underneath their growth systems initially, so that when the seedlings emerge, they will be close enough to the light to grow vigorously. Then as their plants grow, students will remove the foam pieces one at a time, in order to keep the growing tips of the plants at the recommended distance from the light.

For each group of four students:

- 1 film-can growth system (instructions below)
- 1 envelope with 6 P_A seeds
- 1 envelope with 6 P_B seeds
- 1 envelope with 6 F₁ seeds
- 1 envelope with 6 double-mutant seeds
- 1 envelope with 18 F₂ seeds
- 1 water bottle (instructions below)

ADVANCE PREPARATIONS

Whether you use Option 1 or Option 2, the educational potential of the Fast Plants will be realized only if you provide the plants all of the resources they need to grow vigorously and fully express their genetic potentials. The most important resource is light. Fast Plants have been developed to grow rapidly and express their full potential under inexpensive fluorescent lights of the sort used in the home if — but only if — such light is provided at relatively high intensity and for 24 hours per day.

The lighting system that the Wisconsin Fast Plants Program currently recommends is a plant lighthouse (see below). The plant lighthouse has several significant advantages over the older light-bank system that was recommended previously*: It provides a more nearly ideal light intensity; it is both easier and cheaper to construct; and it takes up far less classroom space per plant grown. The plans given below are for making lighthouses that can be folded up and stored flat when they are not in use. They should remain useable for many years.

The other resources that your Fast Plants require are water, soil, and fertilizer. Information regarding simple, inexpensive ways of providing all these resources are also given below.

*Until rather recently, the lighting system that most teachers used was a set of 6 4-foot fluorescent tubes spaced 10 cm apart (as in the Carolina Biological Plant Light Bank kit, Cat. # BA-15-8998). If your school already has such a light bank, it may be used. Similarly, if your school has a collection of the watering systems and quad growing units purchased from Carolina for growing Fast Plants, they may be used. But in either case, please see the *Hints and Troubleshooting* section for ways to improve the performance of these components.

A. CONSTRUCTING A PLANT LIGHTHOUSE (FIG. 1)

Whether you are implementing Option 1 or Option 2, you will need one plant lighthouse for each four student groups.

Materials

For the entire project:

- 1 felt-tip marking pen
- 1 quarter
- 1 utility knife or other sharp, pointed cutting tool
- 1 or more glue sticks
- 1 roll of 12 inch-wide aluminum foil
- 1 pair of scissors
- 1 roll of clear, glossy tape

For each lighthouse:

- 1 empty copy-paper box (approximately 23 x 30 x 45 cm)
- 1 lid from a plastic container, 10-15 cm in diameter
- 1 foot of self-adhesive Velcro tape (hook and loop)
- 1 30 to 39 watt energy saver fluorescent circle-light with a socket adapter**
- 1 electric cord with a standard light socket on one end
- 1 wooden paint stirrer or other thin wooden strip approximately 30 cm long
- 1 20 x 26 cm piece of cardboard

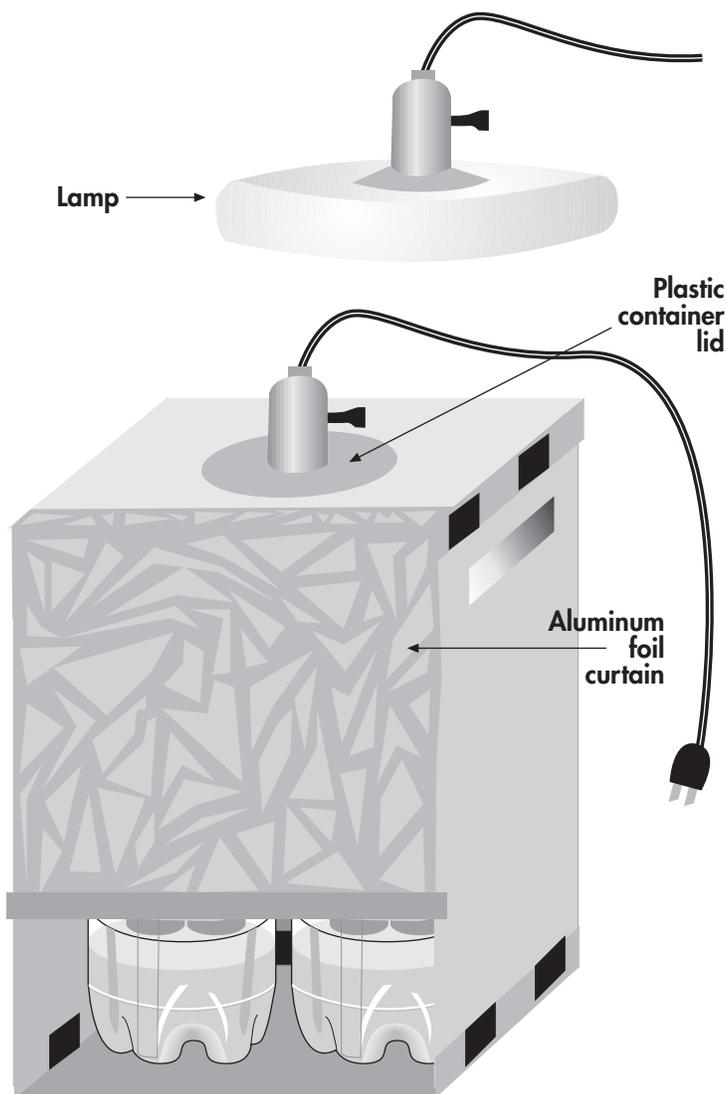


Figure 1

**A Lights of America 30 watt

Circlight (model # 2630) or 32 watt light (model # 2730) is good; a GE 39 watt Energy Saver (Product Code 18739) is better. Such lights are sold at many discount and hardware stores. If you cannot find such a light locally, the 30 watt model is available (at considerably higher price) from Carolina Biological (Cat. # BA-15-8999 or BA-15-8997).

Construction Procedure

1. Discard the lid of the copy-paper box. Check the rest of the box carefully; if any flaps are loose, reglue them and allow the glue to dry.
2. Stand the box on end. Using the marking pen and the quarter, draw a circle in the middle of what is now the top of the box (Fig. 2) and on the plastic lid. Use the utility knife to cut out both circles carefully.
3. Make cuts 2.5 cm from each end, all around the box (Fig. 2), to separate both ends (A and C) from the rest of the box (B) (Fig. 3).
4. Lay piece B flat. Cut a 3 x 14 cm vent slot on each section of piece B 3 cm from the top edge (Fig. 4 B).
5. Attach 2.5 cm pieces of Velcro tape — loop half — to the outer surface of piece B in the locations shown (Fig. 4B).
6. Attach 2.5 cm pieces of Velcro tape — hook half — to the inner surfaces of pieces A and C (Fig. 4 A and C) in positions such that the hook pieces will make contact with the loop pieces on piece B when the box is reassembled (Fig. 5).
7. Apply glue stick to the inner surfaces of all three cardboard pieces, and cover all these surfaces with aluminum foil, shiny side up.

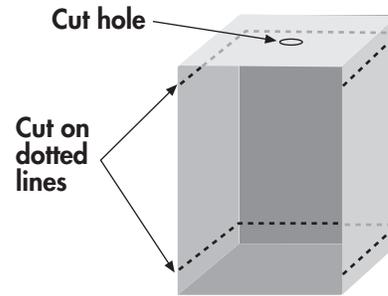


Figure 2

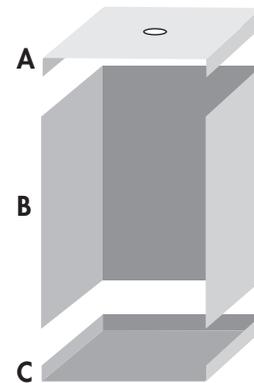


Figure 3

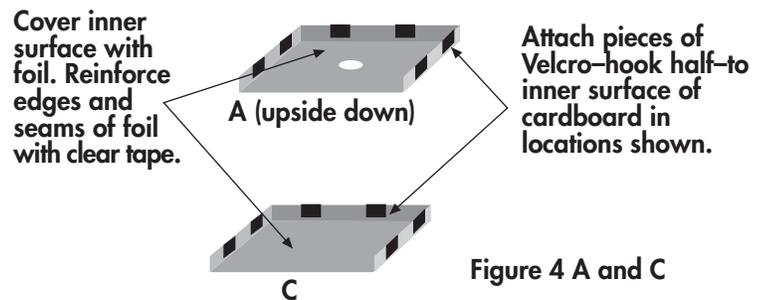


Figure 4 A and C

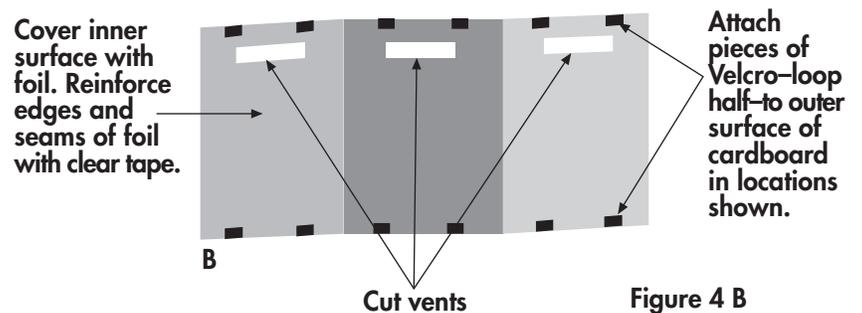


Figure 4 B

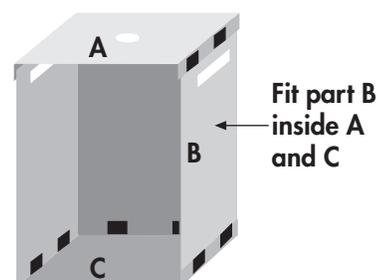


Figure 5

8. Cut away the foil that covers the circular hole in piece A and the vent holes in piece B.
9. Use glossy clear tape to reinforce all the seams and edges of the foil, plus the bend regions of piece B.
10. Fold piece B and insert it into the end pieces, A and C, to reassemble the box (Fig. 5). Press Velcro tapes together to stabilize box.
11. Insert the lamp base through the hole in piece A from the inside. Slip the plastic lid with a hole in it over the lamp base. Screw the lamp into the socket (Fig. 1).
12. Cut a piece of aluminum foil 40 cm long. Reinforce the sides and middle of this foil with clear tape. Apply glue stick to both side of a paint stirrer, attach the stirrer to one end of the foil, then roll it over so that it is covered with foil on both sides.
13. Tape the other end of the foil (shiny side in) to the top of the box so that it forms a curtain over the top portion of the opening (Fig. 1). (It should not extend all the way to the bottom of the box.)
14. Cover the 20 x 26 cm piece of cardboard with foil, shiny side out. Reinforce the edges and seams of the foil with clear tape. (This piece of foil-covered cardboard will be placed between the plants and the foam blocks that prop up the plants.)

B. ASSEMBLING A FILM-CAN GROWTH SYSTEM (FIG. 6)

Whether you are implementing Option 1 or Option 2, you will need one film-can growth system for each group of four students. Four such growth systems are easily accommodated in one plant lighthouse.

Materials

For the entire project:

- 1 roll of narrow masking tape
- 1 can of quick-drying flat-black spray paint
- 1 4d finishing nail
- 1 pair of wire cutters
- 1 piece of wooden dowel ~10 cm long
- 1 drill with 5/64 and 3/16 drill bits
- 1 bunsen burner, alcohol burner, or other flame
- 1 pair of forceps
- 1 white correction pen or bottle of correction fluid

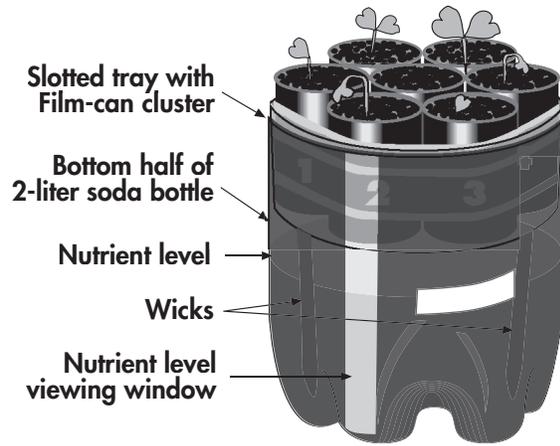
For each four student groups:

- 4 2-liter soda bottles
- 4 half-pound deli containers (Such containers are available at no cost to educators at many supermarket deli counters.)
- 4 1 x 36 cm strips of capillary mat (Capillary matting is available from horticultural supply companies, but often only in very large quantities. Pellon™ from a fabric store will serve the same purpose; the heaviest available grade of Pellon is recommended.)
- 4 7.5 cm squares of capillary mat
- 4 1 x 8 cm strips of capillary mat

- 28 black 35 mm film cans (35 mm film cans are available in large quantities at no cost at most film-processing counters or kiosks.)
- 12 #64 rubber bands (#64 rubber bands are often used by mail carriers to bundle mail, and thus are available at no cost in many offices.)
- 20 cm of 9 or 10 mm O.D. Tygon or clear aquarium tubing
- 1 single-edge razor blade

Assembly Procedure

1. Nutrient reservoir. Peel the label off a 2-liter soda bottle after heating it with hot tap water or air from a hair dryer. Cut the bottle in two about 13 cm from the bottom. Discard the top piece and treat the bottom piece (which will become the nutrient reservoir) as follows:
Attach a vertical strip of tape to one side. Spray the outside with several thin layers of flat black paint until it is nearly opaque. When the paint is dry, remove the tape to create an observation window (Fig. 6.). (The black paint is to suppress algae growth in the reservoir.)
2. A nail poke. Remove the head of the finishing nail with wire cutters. Drill a 5/64 inch hole in one end of the dowel. Force the blunt end of the nail into the hole (Fig. 7).
3. Slotted tray. Heat the nail poke in a flame and use it to melt two slots (2 x 12 mm) on opposite sides of the bottom of a half-pound deli container.



Completed growth system
Figure 6

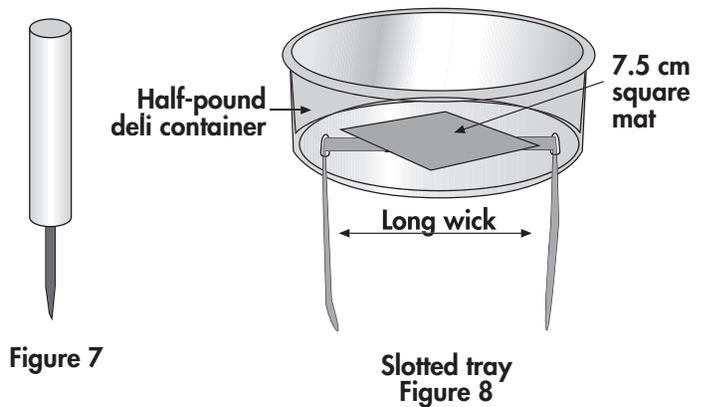


Figure 7

Slotted tray
Figure 8



Figure 9

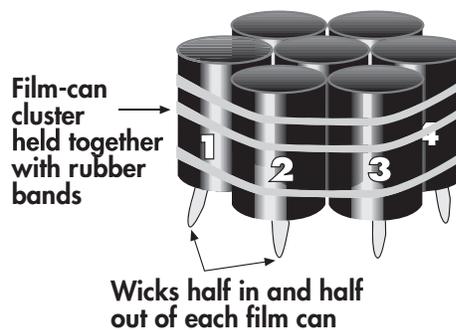


Figure 10

Thread a 1 x 36 cm piece of capillary mat through the two slots in the deli container so that it hangs down about the same distance on each side (Fig. 8). Place a 7.5 cm square of capillary mat in the bottom of the tray.

Place the slotted tray in the nutrient reservoir.

4. Film can cluster. Cut a 1 x 8 cm strip of capillary mat on a 45° angle to produce seven diamond-shaped wicks about 1 x 2 cm in size (Fig. 9).

Drill a 3/16 inch hole in the bottom of each film can. Using forceps, insert one wick in the bottom of each film can, leaving about half of each wick protruding.

Cluster seven film cans and secure them with two or three #64 rubber bands. With the correction pen or brush, number the six outer cans with numerals 1 through 6 (Fig. 10). The central can will be #7 but need not be marked.

5. Completed growing system. Place the film-can assembly into the slotted tray and place the slotted tray in the nutrient reservoir.

C. PREPARING THE POTTING MIX

Each film-can growth system requires about 250 ml of potting mix. Thus, for each planting you need about a liter of mix for every four student groups. Option 1 of the exercise requires two plantings (for the F₁ and F₂ generations), or about two liters of potting mix per four student groups. Option 2, which involves only one planting (the F₂ generation), requires only about one liter of potting mix per four groups.

Measure into a large pail or bucket equal volumes of a peat-based soilless planting formula (such as Peatlite, RediEarth, or JiffyMix) and medium vermiculite. Mix thoroughly by hand. Add water to moisten the mixture somewhat but not enough so that it clumps.

D. PREPARING NUTRIENT SOLUTION

You will need about 400 ml of 1/8 X Peter's Professional Fertilizer per film-can growth system.

Label a 1-liter soda bottle *1 X Peter's Stock Solution*. Add one soda-bottle capful of Peter's fertilizer (right out of the bag) to that bottle, add a liter of tap water and mix. Next, label a 2-liter soda bottle *1/8 X Peter's Nutrient*. Add 250 ml of water to the bottle, draw a line at the meniscus with a marking pen, and label the line *250 ml*. Pour out the water and pour in 1 X Peter's Stock Solution up to the 250 ml mark. Add 1750 ml of water. Two liters of 1/8 X Peter's Nutrient is adequate for four student groups, and more can quickly be made from the 1 X stock, as needed.

E. PREPARING WATER BOTTLES

Water bottles (one per student group) can be made from plastic soda bottles (16, 20, or 24 oz.) that have plastic caps. Heat the nail poke in a flame and melt a small hole in the cap. Fill the bottle with water, cap it, turn it upside down, and squeeze it to obtain a stream of water that will not disturb seeds or seedlings.

F. PREPARING SPLIT-RING TIES (NEEDED FOR OPTION 1 ONLY)

Use the single-edge razor blade to cut off 3 mm rings from the piece of Tygon tubing (also called *clear aquarium tubing*). Then use a pair of scissors to split each ring open, dropping finished rings into a container as you go.

OPTION 1 PROCEDURE

Detailed procedures are given on student pages S121-133.

Remember to (a) give the students only the Phase 1 instructions at the beginning, (b) have them plant only the F₁ seeds, and (c) refer to these seeds simply as Wisconsin Fast Plant seeds, because it would be premature to discuss any of the genetic aspects of the experiment at this time.

Seed-planting day for Phase 1 should be on either a Monday or Tuesday, so that the seedlings can be watered from above on three successive school days. Planting should take the students about 30 minutes.

Phase 1 Schedule

Well in advance: Construct plant lighthouses and film-can growth systems. If possible, test the growing system in advance for at least a week by following the student instructions.

Just in advance: Assemble all growing materials. In order to minimize the amount of clean-up, keep the potting mix in a single large container in a central location and have the students fill and level their film cans there. Provide a large spoon, a small spoon, and a ruler (or other straight edge) next to the potting mix.

Day 1 (on a Monday or Tuesday): Hand out the Phase 1 instructions, the film-can growing systems, and the F₁ seeds. Oversee the planting of the seeds.

Days 2-4: Remind students to water their film cans with their water bottles.

Every day: Have students check the nutrient levels in their reservoirs and add 1/8 X Peter's as necessary to keep the level up near (but not above) the bottom of the slotted tray. It is a good idea to add the 1/8 X Peter's to the film can tray and allow it to run through the slots to the reservoir; this will assure that the capillary matting remains saturated. Make sure that all reservoirs are full at the end of the day before weekends and holidays.

Days 4-7: Have students transplant some seedlings if necessary and/or remove extra seedlings, so as to end up with two healthy plants per film can.

Day 11 or 12: Have students assemble bee sticks.

Days 13-17: Have students cross-pollinate plants with open flowers on at least three successive days.

Days 17-35: Remind students to observe seed pod development at intervals. If time permits, you may want to dissect a few seeds at intervals, so students can view the embryos developing inside the seeds with a dissection microscope.

Day 35+ (approximately 20 days after last pollination): Have students remove their plants from the water so that the seed pods can dry out.

Day 38-40+: After the seed pods are thoroughly dry, have students harvest the seeds according to instructions on the student pages. Supply small envelopes for storing the seeds.

Phase 2 Schedule

Fast Plant seeds differ from most other seeds by not having any required dormant period before they will germinate. So after the seeds have been dried, you may begin the second phase of this experiment any time you wish. It is a good idea to purchase a supply of F₂ seeds (from Carolina Biological) just in case some or all of your students did not recover enough F₂ seeds to execute Phase 2 as outlined.

Remember that students did not get Phase 2 instructions initially, so give them to the students now.

It is important to begin Phase 2 on a Monday so that students will be able to observe their germinating seedlings daily for the first week. By the fifth day it should be possible to distinguish the phenotypes of all F₂ plants and record the results. Nevertheless, have students refill their water reservoirs before leaving for the weekend, so that they can recheck their results early in the following week.

You need to provide a mechanism for students to give a standardized name to the mutant phenotypes they will observe. There are at least three ways to do this:

¥ **Good:** You tell the class that the name for the mutant phenotype seen in the P_A seedlings is *anthocyaninless*, and that the name for the phenotype seen in the P_B seedlings is *yellow-green*.

¥ **Better:** You make color copies of the pictures of Fast Plant mutants in the Carolina Biological catalog and let the students use these to decide what phenotypes their plants exhibit. Alternatively, you can make your own Fast Plant photos as follows: When seedlings are 4 to 5 days old, snip off one seedling of each genotype at ground level. Use glue stick to attach the seedlings to a sheet of paper. Label each genotype. Quickly (before the plants wilt) copy the page on a color copier. This works surprisingly well.

‡ **Best:** If you did not grow a complete set of Fast Plant mutants at the beginning of the genetics section, (as suggested under *Hints and Troubleshooting*) do so now. Plant all of your mutant seeds (one type per film can) when the students are planting their F₂ seeds and label each type of mutant.

It is a good idea to check that each group can distinguish the anthocyaninless yellow-green (Anl/Ygr) double mutants from the two kinds of single mutants. This distinction is important in determining whether their F₂ plants behaved according to the expectations of Mendelian genetics.

You also need to provide a mechanism for combining and tabulating the class data. A good way of doing this is to use a strip of butcher paper to prepare enlarged versions of Tables 1.B and 2.B that have columns in which all groups can enter their data, plus a column for class totals.

OPTION 2 PROCEDURE

Detailed procedures are given on student pages S141-145. Note that the work sheets for Option 1 Phase 2 are also to be used for Option 2. However, they have not been duplicated at the end of the Option 2 student pages.

It is important to begin this experiment on a Monday, so that students can observe their germinating seedlings daily for the first week. By the fifth day it should be possible to distinguish the phenotypes of all F₂ plants and record the results. Nevertheless, have students refill their water reservoirs before leaving for the weekend, so that they can recheck their results early in the following week.

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HINTS AND TROUBLESHOOTING (BOTH OPTIONS)

1. The Wisconsin Fast Plants can help you dramatize the concept of heritable variation in visible traits at the very beginning of the genetics unit.

If you have space available in a plant lighthouse two weeks before you begin the genetics unit, consider setting up a growth system with a different variant in each film can. If you are doing Option 1, your students' Fast Plants should be nearly mature (and wild-type in phenotype) by that time. Thus, they should be able to recognize the various phenotypic differences quickly and easily. This would be a good time to let them in on the big secret: namely, that even though all of the plants they have been growing appear to be normal (wild-type), they are actually carrying invisible versions of two of the mutant traits visible in your seedlings. (But even Option 2 students should be able to see the visible differences among these plants and become intrigued by their heritable basis.)

In short, you can use the Fast Plants at the beginning of the genetics section to stimulate student interest in understanding how visible traits are controlled, hidden from view in one generation, and then caused to reappear in another generation.

If you decide to go this route, you should already have seeds of four distinctive genotypes to plant in four of the film cans: namely, the P_A (Anl), P_B (Ygr), F₁(wild-type) and the double mutant seeds (Anl/Ygr). Additional mutant seeds you should consider purchasing for use in the other three film cans include rosette (Cat. # BA-15-8815), elongated internode (Cat. # BA-15-8824) and AstroPlants (Cat. # BA-15-8835).

If you cannot grow a complete set of demonstration mutants before the beginning of the genetics section, consider doing so when the students are starting Phase 2 (or Option 2) of the exercise.

2. On the page where the construction of a plant lighthouse was introduced, it was noted that if you already have a six-tube fluorescent light bank (such as Carolina Biological Cat. # BA-15-8998) you may grow the Fast Plants under it, even though its light output is on the marginal side. (A four-tube bank of this sort does not provide adequate light, however, and should NOT be used.) Growth under a six-tube bank is improved if (a) the fluorescent tubes are replaced every two years, (b) aluminum foil curtains

(weighted at the bottom) are attached to all four sides of the light bank, and (c) adjustable supports are placed under the plants to keep their growing tips no more than 10 cm from the lights.

It was also noted that if you already have a collection of the styrofoam quads and other growth system components that Carolina Biological sells for growing Fast Plants, you may use them instead of the film-can growth system described in this unit. However, you should consider setting up at least one film-can growth system for comparison purposes. You might even want to ask one student group to set up both kinds of systems and to make careful comparisons of plant performance in the two systems.

3. A follow-up exercise — or a couple of potential science fair projects. The focus of the Fast Plant experiments so far has been on the two recessive single-gene traits that distinguish the PA and PB plants and members of the F₂ generation. However, there is another trait that distinguishes the PA and PB plants. It appears before the seedlings are a week old and becomes very clear by the time the plants are eight to ten days old. Challenge your students to figure out what it is. They will need to use good illumination and a good hand lens to see this PA-PB difference — and even then they may have great trouble seeing it. But once they figure out what the trait is, it will jump out at them! By the way, an eyepiece from a microscope, used backwards, makes an excellent hand lens for use in such studies.

The answer (which students should be allowed to discover for themselves) is that the PA (Anl) plants are almost completely hairless, whereas the PB (Ygr) plants are hairy; that is (like most plants), they have many hairlike outgrowths on their leaves and stems (Fig. 11).

The function of such plant hairs is not yet fully understood, but it is thought that they serve such purposes as deterring insect predators, interfering with wind flow (thereby decreasing the rate of water loss from leaves), and increasing the efficiency of light absorption.

Hairiness is not an all-or-none trait. It is a **quantitative variable**. That is, plants differ widely in the degree to which they express the trait. The standard designator for the hairy phenotype is Hir, (from *hirsute*, the Latin word for *hairy*), and Fast Plants range from Hir(0) (completely hairless) to Hir(9) (extremely hairy). Your students PA (Anl) plants are rated Hir(1), and their PB (Ygr) plants are rated Hir(5).

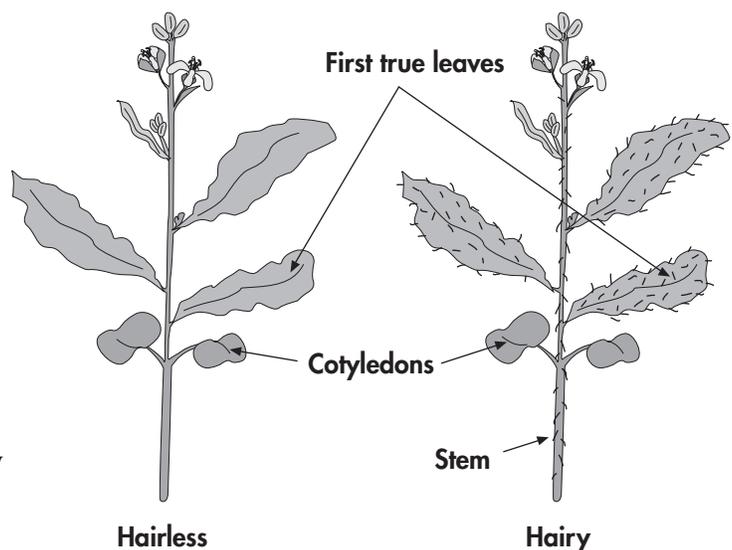


Figure 11

Below are a few of the simpler questions that you might pose to your students, and some sample answers that they should come up with if they merely observe their plants carefully.

Q: Does the hairy trait disappear in the F₁ generation like the Anl and Ygr traits do?

A: No. The F₁ plants are somewhere in between the two parental types in hairiness.

Q: What does this say about the heritable basis of the hairy trait?

A: It appears to be a heritable trait that exhibits co-dominance, or incomplete dominance.

Q: How does the hairiness of the F₁ plants compare with the hairiness of the F₂ plants?

A: The F₂ plants are much more variable in their hairiness than the F₁ plants.

Q: Does hairiness cosegregate with Ygr in the F₂ generation? (That is to say, are the Ygr plants routinely hairier than the Anl plants in the F₂ generation, as they are in the parental generation?)

A: No. The hairy and yellow-green traits appear to be inherited independently.

Q: Do the F₂ plants fall into only three classes that resemble the P_A, P_B, and F₁ plants with respect to number of hairs?

A: No. The F₂ plants are much more variable in hairiness than that.

Q: What does this imply about the heritable basis of the hairy trait?

A: Hairiness probably is controlled by genes at more than one *hairy* locus. (If the P_A and P_B plants differed by only one allele at one locus, then the F₂ plants should fall into three distinct classes representing the heterozygote and the two homozygotes; but careful inspection will reveal that they do not.)

Q: How can you rule out the possibility that the variation you see in the F₂ plants is caused by environmental variables that you were not aware of and could not control?

A: The P_A, P_B, and F₁ plants provide controls that appear to rule out that possibility, because they all exhibit much less variability in hairiness than the F₂ plants do. If there were uncontrolled environmental variables, there is no reason that they should affect only the F₂ plants.

Below are some questions of a quantitative nature that you might pose to your students. To answer these questions, students would have to count hairs and perform various statistical tests.

Q: Are the number of hairs on one part of a hairy plant (such as the edge of the first true leaf) correlated with the number of hairs on another part (such as the surface of that leaf or the edge of the second leaf)?

A: Yes. But obtaining this answer probably would be a highly instructive statistical-analysis exercise, something with which the math teacher might help.

Q: How much variation is there in the number of hairs on equivalent structures (such as the first true leaves) in a group of plants that are presumably similar genetically (such as the PB plants)?

A: There is no single or simple answer to this question, but it could lead a motivated student into an extended study of natural variability and the way that such variability is evaluated statistically.

There are other interesting questions that you might pose to your students. These questions could be answered only by performing additional crosses between their various plants and/or growing additional plants of known genotype under different conditions. Some of these questions could be used for great science fair projects. Just a couple of examples:

Q: How would you perform a scientific test of your hypothesis (mentioned above) that the hairy trait is controlled by genes at more than one locus?

A: The best way to test this hypothesis would be to separate out the F₂ plants with the least hairs and the most hairs, cross-pollinate each kind with another plant of the same type, and see how hairy their F₃ offspring turn out to be. If the parental plants differ with respect to only one locus that influences hairiness, then both sets of F₃ plants should have the same range of hairiness as the F₂ generation did. But if there are multiple hairy genes, then the very hairy F₂ plants should produce very hairy F₃ offspring, and vice versa. Repeating this kind of selective breeding for another generation or two could provide a great study of the effect of selection on plant evolution.

Q: How could you test the hypothesis that the number of hairs produced by a plant is controlled by both its genotype and the environment to which it is exposed during development?

A: The best way to test this hypothesis would be to grow plants of two different but fairly uniform genotypes (such as the PB and F₁ plants) under a range of environmental conditions and see how these conditions affect the number of hairs produced. Environmental factors to be tested might include such things as light intensity, or light color, and the concentration of fertilizer and/or salt in the nutrient reservoir.

ANSWERS TO THE QUESTIONS ON THE WISCONSIN FAST PLANTS WORK SHEET STUDENT PAGES 137-140

1. What are the two mutant traits that distinguished your P_A and P_B plants from one another and from wild-type Wisconsin Fast Plants?
*The P_A plants are anthocyaninless mutants.
The P_B plants are yellow-green mutants.*
2. If the mutant traits exhibited by the P_A and P_B generation are heritable, why didn't those two traits appear in their progeny in the F₁ generation?
They are both recessive traits that are not expressed in the heterozygous F₁ plants.
3. Based on your explanation above, what would you predict that the ratio of wild-type to mutant individuals for each of these two traits in the F₂ generation? Explain.
3:1 The probability that an individual will receive a recessive allele from each of its heterozygous parents is one in four. Thus, 1/4 of the offspring should have the recessive phenotype.
4. Above each of the tables below, record how many F₂ plants germinated and grew large enough that their phenotypes could be determined with confidence. Then in the right hand column of each table record how many of these F₂ plants had each of the indicated phenotypes.
All groups should have different data in Table 1.A, but the same data in Table 1.B.
- 5-7. With respect to the P_A trait, how does the ratio of wild-type to mutant individuals that you predicted in Question 3 compare to the ratios of wild-type to mutant individuals that you reported in tables 1 A and B?
The predicted wild-type-to-mutant ratio was (in each case) 3:1

All groups should have different ratios for their own plants, but the same ratio for the class plants.

It should be very interesting to see how your students decide what constitutes a significant difference between predicted and observed ratios, and how much confidence they have in their ability to make such judgments.

This should provide you with an excellent opportunity to discuss the role of statistical analysis (as opposed to gut feelings) in making such decisions with respect to scientific observations.
8. Record the observed phenotypes of the F₂ plants with respect to combinations of P_A and P_B traits.
Again, all groups should have different data for their own plants, but identical data for the class.

9. In the table below, compare the ratios of the four possible combinations of PA and PB traits that you and your class observed with the ratios that are predicted for this kind of dihybrid cross. In each case, set the number of double-mutant plants to one.

The predicted ratio is 9:3:3:1, but all groups should have different ratios for their own data and identical ratios for the whole class.

- 10-11. Are the differences between the predicted and the observed ratios in the above table significant?

Again, it should be very interesting to see how your students decide what constitutes a significant difference between predicted and observed ratios, and how much confidence they have in their ability to make such judgments.

If, for some reason, you have decided not to make unit 2.E a class assignment, encourage your students to study it voluntarily, to find out about one widely used method of deciding whether differences between predicted and observed experimental results are significant.

REFERENCES

1. *Catch the Spiral: Cycle Through Life With Fast Plants* (2000). (ISBN 0-7872-6910-7) Kendall/Hunt Publishing Company, Dubuque, Iowa. Tel. 800-228-0810; Fax 800-772-9165; www.kendallhunt.com 25 explorations in plant biology. Developed by Dr. Paul H. Williams and other members of the Wisconsin Fast Plants Program, College of Agriculture and Life Sciences, University of Wisconsin-Madison.
2. *CrGC Information Catalogue*(1997). Crucifer Genetics Cooperative, University of Wisconsin-Madison, Madison Wis. Tel. 608-263-2634; Fax 608-263-2626; www.fast-plants.cals.wisc.edu/crgc/crgc.html This catalog and information brochure from the Crucifer Genetics Cooperative (which is closely associated with the Wisconsin Fast Plants Program) lists seeds for a much wider variety of *B. rapa* mutants, as well as several other species of crucifers.
3. *Wisconsin Fast Plants Poster*. Carolina Biological. Tel. 800-227-1150; www.carolina.com This colorful 24 x 36 poster illustrates the rapid development and complete life cycle of the Fast Plants.

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CHAPTER 2

**Passing Traits from
One Generation
to the Next**

SECTION E

**How Are
Genetic
Results
Evaluated
Statistically?**

Chapter 2: Section E Background

MANY BIOLOGY STUDENTS (and not a few biology teachers!) suffer “math insecurity.” Many of them probably would just as soon go through life not thinking about any mathematical issues more complicated than counting their change at the pizza parlor. However, being able to make quantitative judgments, in addition to qualitative ones, is an important part of being a scientifically literate, responsible citizen. Obviously, you cannot be expected to completely counteract the math insecurities of all of your students and turn them all into enthusiastic statistical whiz kids while teaching them genetics. But you can conceivably help lower the barriers to thinking quantitatively outside of the math classroom, if you approach this exercise as a fun and easy way – rather than a painful but necessary way – to determine whether all the time and effort that they put into the Fast Plants experiment yielded results consistent with Mendel’s “laws” of heredity!



Introduction to Using Statistics to Evaluate Genetic Explanations

STUDENT PAGES 148-149

LESSON OVERVIEW

This lesson is a reading assignment to prepare your students to work with the concepts behind a Chi square analysis. It introduces them to the problem of evaluating genetic results statistically with a “real life” situation that they should have no problem identifying with.



Too Many White Kittens? Using Chi Square (χ^2) to Find Out

STUDENT PAGES 150-151

LESSON OVERVIEW

This is a guided practice activity on working with the Chi square analysis formula. The students will learn the concepts behind each step of the Chi square process in a non-threatening exercise.

TIMELINE

It will take an average student about 15 minutes to read sections E.1 and E.2 carefully enough to understand the concepts involved.

How to Perform a Chi-Square Test on Any Data Set

STUDENT PAGES 152-155

LESSON OVERVIEW

This is a guided practice activity on working with the Chi square analysis formula. The students will learn the concepts behind each step of the Chi square process.

TIMELINE

Assuming that the Fast Plant data for the class have already been tabulated and made available, calculation of the χ^2 and p values should not require more than 15-20 minutes.

ANSWERS TO THE QUESTIONS ON THE WISCONSIN FAST PLANTS™ CHI SQUARE WORKSHEET

There will be two sets of correct answers for the first Chi square analysis, but all students should get the same result for the second Chi square analysis. If you are going to have the students perform the analyses in class, it would be a good idea to work out the correct answers for yourself in advance, once you have the class data. Blanks are left below for this purpose.

Part One, A Simple Trial Run

The mutant trait I am analyzing by Chi square is *anthocyaninless*

Total number of F₂ plants obtained by the class: _____.

Multiply the total number of F₂ plants by the expected frequencies (3/4 and 1/4) to get the expected numbers of plants in each category. You probably will get non-integral numbers.

Phenotype	Expected #	Observed #	Difference	Difference ²
Wild-type				
Mutant				

$$\chi^2 = \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}} \text{ summed for all classes}$$

(Remember: “classes” here refers to classes of plant phenotypes, not classes of students!)

χ^2 : _____ p: _____ Use the table on page S143 to determine the value of p.

The mutant trait I am analyzing by Chi square is *yellow-green*

Total number of F₂ plants obtained by the class: _____.

Phenotype	Expected #	Observed #	Difference	Difference ²
Wild-type				
Mutant				

Again multiple the total number of F₂ plants by the expected frequencies to get the expected numbers of plants in each category. You may get non-integral numbers.

$$\chi^2 = \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}} \text{ summed for all classes}$$

χ^2 : _____ p _____

Conclusion: *Presumably both Chi square analyses in part one will indicate that the data are consistent with the hypothesis that the mutant trait in question is due to a recessive allele at a single locus (which we know on the basis of prior studies is the case for both traits). If your advance analysis indicates that one or both of the Chi square analyses do not lead to this conclusion, you will need to be prepared to lead a discussion of alternative hypotheses. (One of which might be that certain groups failed to classify certain of their plants correctly!)*

Part Two, Chi Square Analysis of the Two Phenotypes at Once

COMBINED DATA FOR THE ENTIRE CLASS

TOTAL NUMBER OF F₂ PLANTS: _____

'a' phenotype	'b' phenotype	Frequency expected*	Number expected	Number observed	Difference	Difference ²
Wild-type	Wild-type					
Mutant (aa)	Wild-type					
Wild-type	Mutant (bb)					
Mutant (aa)	Mutant (bb)					

*You can obtain these frequencies by either (a) using the product-of-probabilities method, (b) using a Punnett Square, or (c) reviewing similar calculations you made for previous exercises.

χ^2 : _____ p _____

Conclusion: *Presumably the Chi square analysis here will indicate that the data are consistent with the hypothesis that the 'a' and 'b' loci assort independently. Once again, if your advance Chi square analysis does not lead to this conclusion, you will need to be prepared to lead a discussion of alternative hypotheses. (One of which might be, once again that certain groups failed to classify certain of their plants correctly!)*