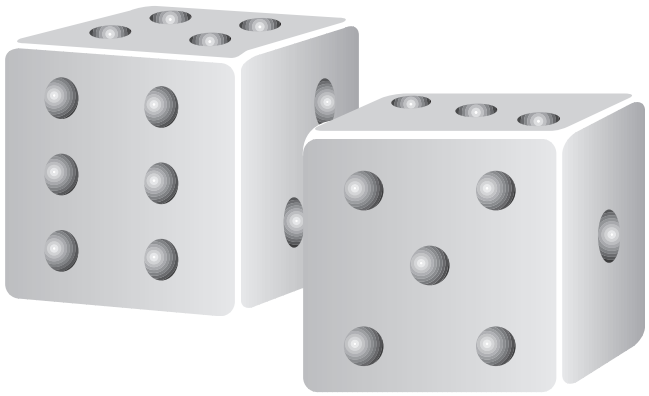
CHAPTER 2

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.

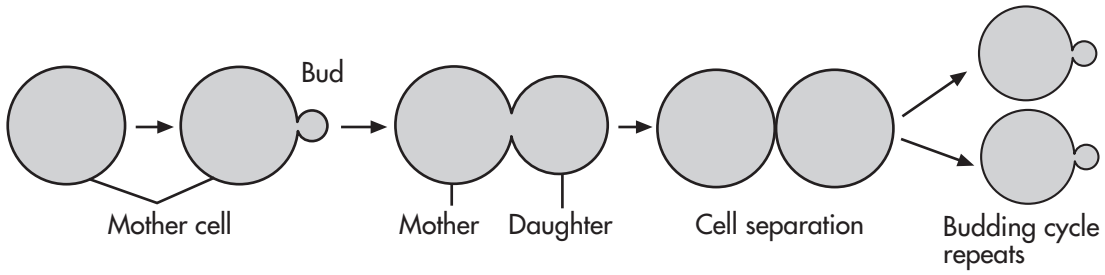
BACKGROUND

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 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)
- marking pen
- a packet of sterile toothpicks
- culture of Red, Mating type **A** yeast
- culture of Red, Mating type α yeast
- culture of White, Mating type **A** yeast
- culture of White, Mating type α 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 x 7) + (1 x 3 x 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 μl 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 μl 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

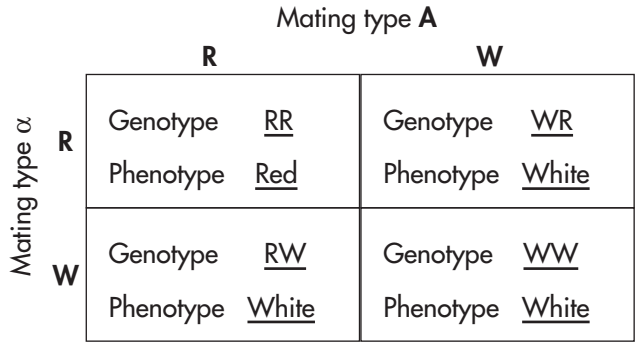
1. The YED plates get contaminated easily, so always make a few extra and emphasize the importance of working with them carefully to avoid contamination. Experience indicates that they will have much lower contamination levels if students keep the plates upside down while working with them. As mentioned 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:



2. What ratio of phenotypes did you observe as a result of the four crosses you performed?
   1 red to 3 white

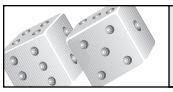
3. What does this indicate about which allele is dominant and which is recessive?
   White is dominant, and red is recessive.

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.



D.2

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 choi, 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 1aw 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	*ANL*	*anl*	(purple stems, etc.) Anl+	(anthocyaninless) Anl
Yellow-green versus dark green	*YGR*	*ygr*	(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 anthocyanin-less 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 F_1 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 F_2 seeds are mature, and the plants are dried out.
5. Week 6: The F_2 seeds are dry and the students plant the seeds.
6. Week 7: The F_2 plants germinate, and the students quickly see that (in distinction to the F_1 plants) the F_2 plants are not all alike. While many will appear wild-type (as their F_1 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 F_2 Generation

1. Students begin with Step 5 of the above outline, when they plant F_2 seeds that you have purchased and provided to them. As controls, they will also plant seeds from the parental and F_1 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 F_1 plants through an entire life cycle, pollinate the F_1 flowers, watch them produce seeds, and then harvest and plant those seeds to determine what kinds of plants will be produced in the F_2 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 F_2 seeds from the F_1 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+)	*anl/anl, YGR/YGR*	P_A
BA-15-8818	50	yellow-green (Anl+, Ygr)	*ANL/ANL, ygr/ygr*	P_B
Two packs of BA-15-8891	2 x 200	wild type (Anl+, Ygr+)	*anl/ANL, YGR*/ygr	F_1
BA-15-8888	250	segregating all four phenotypes	all of the above and below	F_2 (optional backup)
BA-15-8842	50	anthocyaninless, yellow-green (Anl, Ygr)	*anl/anl, ygr/ygr*	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 F_1 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 P_A seeds
1 envelope with 6 P_B seeds
1 envelope with 6 double-mutant seeds
1 envelope with the students own F_2 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+)	*anl/anl, YGR/YGR*	P_A
BA-15-8818	50	yellow-green (Anl+, Ygr)	*ANL/ANL, ygr/ygr*	P_B
BA-15-8890	50	wild type (Anl+, Ygr+)	*anl/ANL, YGR/ygr*	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)	*anl/anl, ygr/ygr*	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 F_1 seeds
1 envelope with 6 double-mutant seeds
1 envelope with 18 F_2 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

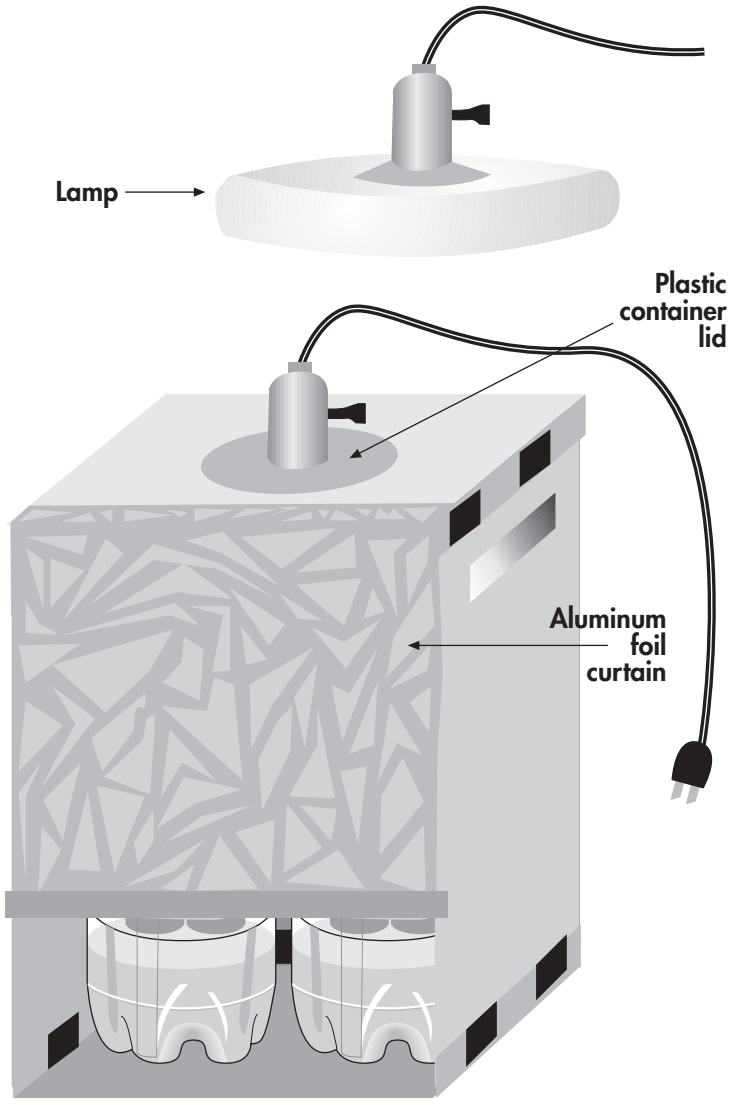


Figure 1

**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.

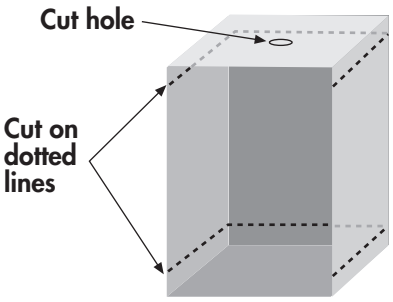


Figure 2

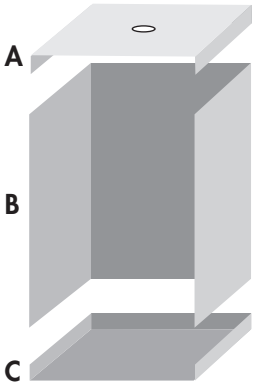


Figure 3

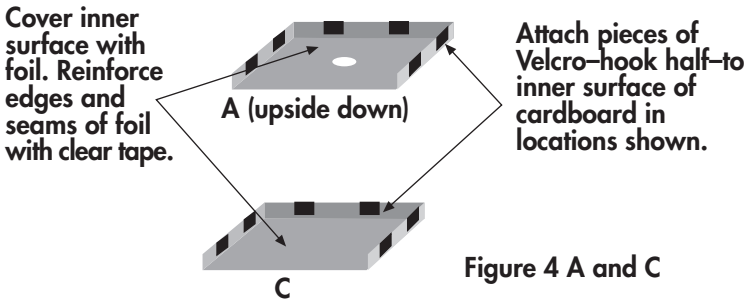


Figure 4 A and C

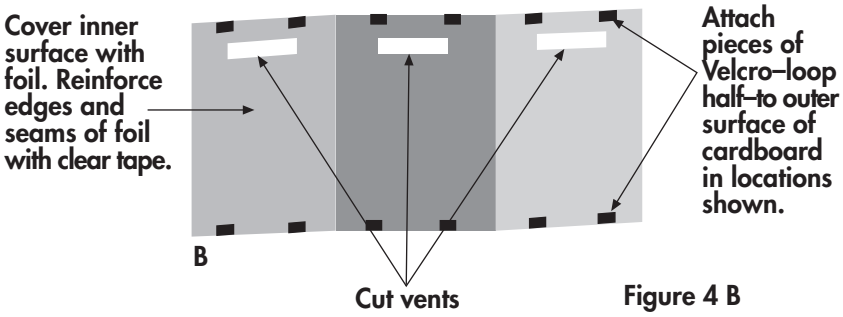


Figure 4 B

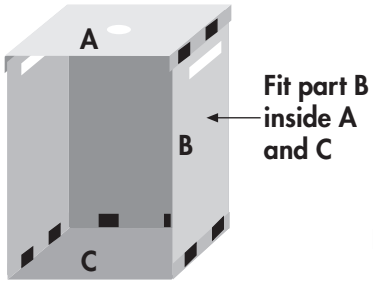


Figure 5

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

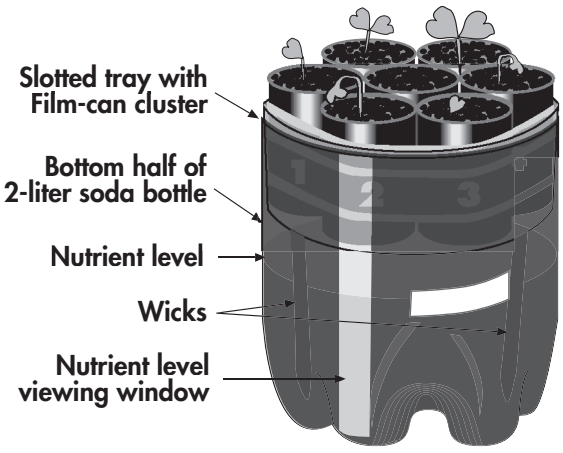
For each four student groups:

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

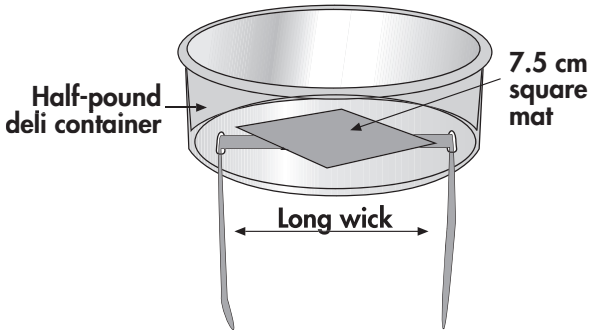
Assembly Procedure

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

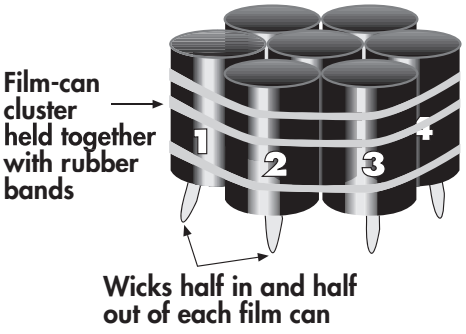


Figure 10

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 F_1 and F_2 generations), or about two liters of potting mix per four student groups. Option 2, which involves only one planting (the F_2 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 F_1 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 F_1 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 F_2 seeds (from Carolina Biological) just in case some or all of your students did not recover enough F_2 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 F_2 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 F_2 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 F_2 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 to observe their germinating seedlings daily for the first week. By the fifth day it should be possible to distinguish the phenotypes of all F_2 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), F_1(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 P_A and P_B plants and members of the F_2 generation. However, there is another trait that distinguishes the P_A and P_B 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 P_A-P_B 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 P_A (Anl) plants are almost completely hairless, whereas the P_B (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 P_A (Anl) plants are rated Hir(1), and their P_B (Ygr) plants are rated Hir(5).

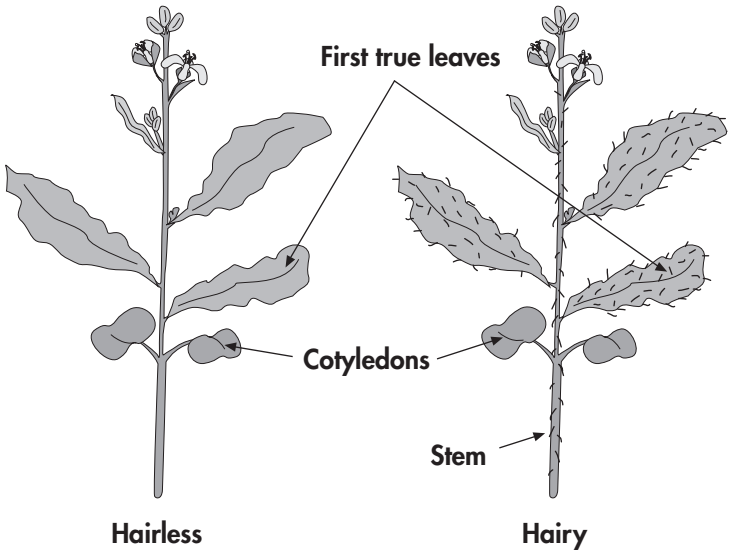

Figure 11

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 F_1 generation like the Anl and Ygr traits do?
A: No. The F_1 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 F_1 plants compare with the hairiness of the F_2 plants?
A: The F_2 plants are much more variable in their hairiness than the F_1 plants.

Q: Does hairiness cosegregate with Ygr in the F_2 generation? (That is to say, are the Ygr plants routinely hairier than the Anl plants in the F_2 generation, as they are in the parental generation?)
A: No. The hairy and yellow-green traits appear to be inherited independently.

Q: Do the F_2 plants fall into only three classes that resemble the P_A, P_B, and F_1 plants with respect to number of hairs?

A: No. The F_2 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 F_2 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 F_2 plants is caused by environmental variables that you were not aware of and could not control?
A: The P_A, P_B, and F_1 plants provide controls that appear to rule out that possibility, because they all exhibit much less variability in hairiness than the F_2 plants do. If there were uncontrolled environmental variables, there is no reason that they should affect only the F_2 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 P_B 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 F_2 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 F_3 offspring turn out to be. If the parental plants differ with respect to only one locus that influences hairiness, then both sets of F_3 plants should have the same range of hairiness as the F_2 generation did. But if there are multiple hairy genes, then the very hairy F_2 plants should produce very hairy F_3 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 P_B and F_1 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 F_1 generation?
   They are both recessive traits that are not expressed in the heterozygous F_1 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 F_2 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 F_2 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 F_2 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 F_2 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 P_A and P_B 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.fastplants.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.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of the Wisconsin Fast Plants Program in preparing this exercise. The illustrations in this exercise were adapted, with permission, from ones used by the Wisconsin Fast Plants Program in teacher workshops.