What Caused the Ghostly Leaves? Inquiry-Based Investigation of the Genetics and Molecular Biology of Corn Albinism

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Introduction

The trait of "albinism" (chlorophyll-deficiency) in maize results in shockingly white seedlings, and can be produced by a variety of mutations (Harpster, 1984). Chlorophyll deficiency can result from mutation of any of the fifteen enzymes in the chlorophyll biosynthesis pathway. Because chlorophyll appears to regulate thylakoid development, other proteins such as the light-harvesting complex or various CO_2-fixing proteins can also be affected. (Harpster, 1984). Each of these mutations has a specific effect on the proteinaceous content of the cytoplasm, as observed by gel electrophoresis. We have used maize seeds from a monohybrid cross for one of these mutations to develop an inquiry-based laboratory exercise that explores basic genetics and gene regulation, while exposing students to electrophoresis—a basic technique of biotechnology.

This exercise spans two weeks in lab, and was developed for non-majors with little or no biology background. Before beginning the lab, students should be familiar with the Central Dogma of biology (one gene generally leads to the production of one protein), the basic concepts of Mendelian genetics (monohybrid crosses, phenotypic ratios, and basic mechanisms of inheritance), and possibly the general idea of gene regulation (not all genes are used in all cells, and some genes control whether others are used).

In this exercise, students examine the genetics and inheritance of the trait of albinism in corn. Students examine two-week-old corn plants grown from a monohybrid cross, in which the recessive gene is for chlorophyll deficiency. The nature of the original cross is not revealed to the students. Students form hypotheses about the cause of the albino trait, including the number of genes involved, how they are inherited, what the gene (and its resulting protein product) does, and what the effect on the protein composition will be. Typically students need some help with this step; instructors may find it helpful to model several hypotheses (such as the examples given in our exercise below), so that students feel more comfortable constructing a simple one of their own.

To test their hypotheses about the green vs. white trait, students extract full cellular protein from green and white corn leaves (Niedzlek-Feaver, 1995), and separate those extracts through gel electrophoresis. Students compare the protein banding patterns for white versus green corn to determine how many proteins are seem to be different. This suggests how many genes may be altered to create the trait.

To further test and revise their hypotheses, students complete simple Mendelian genetic analysis. Students count the number of plants displaying each trait and calculate phenotypic ratios for white versus green leaves. They use these data and Punnett squares to examine inheritance. This allows further testing of their hypotheses: were one, two, or more genes involved? As a side benefit, students may also examine the effects of sample size by comparing ratios calculated from different numbers of plants (plants from a single lab group versus a full class versus all lab classes combined).

From this lab, students learn the basics of genetics, gel electrophoresis, gene regulation, and sample size in an inquiry-based format. This lab is intended for non-science majors, and has been streamlined to occur over two, two-hour lab periods in consecutive weeks—but can easily be adapted for more advanced classes. Such adaptations could include (1) more extensive involvement in reagent and sample preparation, (2) more involvement in preparing, running, and loading the gel, and (3) examination of the original literature on chlorophyll deficiency, to determine which specific gene is involved.

Generally, we have students count the plants and extract protein during the first week's laboratory session, culminating with the loading of samples and running of the gel by a teaching

assistant. Students' cellular extracts are saved until we confirm that the gel ran properly. During the second week's lab class, students analyze their data: they view protein gels to see how many bands differ between white and green corn. They then test their hypotheses further by calculating phenotypic ratios and comparing them to the results of a few simple Punnett squares.

The albino maize seedlings used in this exercise are homozygous recessive for the "lemon-white" mutation (lw). They are the offspring of a monohybrid cross, and thus show the green and white traits in a 3:1 ratio. You may notice that white seedlings die rapidly, and that overall protein content appears to be lower in the white leaves (see Instructor's Notes, Figure 1). This is because these white seedlings hydrolyze some protein for biosynthesis as their carbohydrate supply from endosperm is depleted (Seltmann, 1955). As a result, white seedlings begin to die within 12-15 days, and must be harvested promptly.

Student Outline

What Caused the Ghostly Leaves?
Using a Molecular Strainer to Answer Genetics Questions

A few weeks ago you planted some corn seeds, and have probably noticed them sprouting at the back of our lab room. You have probably noticed these seedlings have an unusual trait: some of the plants have shockingly white leaves! Now it's time for you to do the work of a scientist: What do you think causes the leaves to be so white? You may already have a guess. What we do know is that the white leaves don't contain chlorophyll—the bright green molecule used in photosynthesis. How could a gene cause this?

Remember, each gene usually results in one protein. Some proteins are enzymes and make all the substances found in a cell. Each enzyme performs only one specific task, such as building chlorophyll from simpler substances. There are at least fifteen different enzymes that help to build chlorophyll. Not all proteins are enzymes, though. Other proteins may do work for the cell, such as photosynthesis. For example, a group of proteins called the "photosynthetic reaction center" (PRC) each bind hundreds of chlorophylls and collect solar energy for photosynthesis. Still other "regulatory" proteins coordinate the tasks of the cell by turning genes on and off as needed—for example there may be proteins that regulate the genes that produce each chlorophyll-building enzyme or PRC protein. There are also enzymes that help the cell dispose of worn out molecules, and there may be some that degrade chlorophyll. Every one of these proteins could affect the production and/or presence of chlorophyll in the cell, and each one of them results from a gene.

Think about what gene (or genes) might be different in the white and green plants, and what proteins are different between those plants. What do you think causes the difference between the white and green plants? Take a moment now to write a simple hypothesis that could explain the absence of chlorophyll:

HYPOTHESIS: *Albino corn has white leaves because* _____

_____

_____

HINT: *You know that genes create proteins, and that proteins can result in traits. Traits can be shaped by a single gene, or by multiple genes. Try to weave these ideas into your hypothesis. Remember, any hypothesis that can be tested is a "good" one.*

How could knowing more about the specific proteins in the corn leaves help you test your hypothesis? Come prepared to lab with some ideas to discuss with your classmates and instructor.

With your classmates, you will come up with some hypotheses to test. For each one, you will predict how the mixture of proteins would differ in green or white leaves. By the end of the lab, you'll be able to test some of those predictions! We'll test those predictions even further during next week's lab, on Mendelian genetics.

It will help a lot if you think about how a change to one gene will affect the proteins found in a cell. One way to think about it is that genes are a lot like recipes: each gene provides instructions for building a particular "dish" (or protein)—whether it be a dessert, a main-course, or even a homebrewed remedy for the common cold. The DNA found in the cell is like a popular cookbook: it contains many different recipes (or genes). The same cookbook (or set of genes) is found in every cell in the plant. Not all recipes are used by every cell, however. Each cell—like a household—uses only the recipes it needs to function. Mutations are like changes to a recipe that make it unreadable (no protein produced) or make the results inedible (a protein is produced, but doesn't work). A regulatory gene would be like a recipe that is required by other recipes—for example a recipe for bread. Without bread, we could not make stuffing, sandwiches, garlic toast, or other dishes—likewise, when a regulatory gene malfunctions, a larger set of genes may be unused or overused.

To help you get started, a simple hypothesis (not necessarily a correct one) is listed below:

Hypothesis	**How would protein mixture differ in white vs. green leaves?**
White and green leaves are genetically the same; color is due to environment.	*Both will contain the exact same mixture of proteins.*
White leaves contain a new gene that makes a chlorophyll-destroying enzyme.	*Both leaves will have the same mix of proteins, plus one more in white leaves.*

Cells Contain Many Molecules—Including Protein

Cells contain a rich, dense mixture of materials, including proteins, DNA, membranes, nutrients, and waste products. The precise mixture varies, depending on the genetic makeup of the cell as well as its current environment. One of the challenges of modern biology is to sort out those mixtures in order to study a single gene or single protein. Even if we can isolate just a single type of molecule—say, all the proteins in the cell—there are still thousands of different variations present. A single cell contains many different proteins: several types of enzymes, structural materials like the cytoskeleton and spindle fibers, proteins that regulate the timing of processes by turning genes and proteins on or off (think of these as molecular traffic cops), and proteins that form pores in

membranes around the cell to allow transport. Locating just one type of molecule can be like finding a needle in a haystack.

Gel Electrophoresis is a Molecular Strainer

Fortunately, there is one technique used in biotechnology and molecular biology labs that helps us sort out the unwanted "hay" from the precious needles that interest us. "Gel electrophoresis" is a method for sorting out molecules, based on various properties like size, shape, and positive or negative charge. It works a bit like a kitchen strainer that you might use for cleaning foods in the sink.

Think about how a strainer works: you dump in a bunch of radishes, and pour water over them. The water runs over the food and carries away small particles like dirt and sand. The water and small particles leave through the holes in the strainer. The radishes, however, stay put because they are too large for the holes. The size of the objects and the size of the holes are important: the same strainer we use for radishes might not work well at all for washing rice because the holes are too large. Now, imagine filling one strainer with rice, and one with water. Both rice and water are able to exit the holes, but water runs out much faster. That is because individual water molecules are much smaller than the strainer holes; rice grains are a lot larger than water molecules, and can only fit through the holes one way. Smaller objects run through a strainer faster than larger ones.

A similar set of principles operates in gel electrophoresis. Mixtures of molecules (e.g. proteins) are placed at one end of a slab of gel, and filtered through it. Smaller molecules move through it faster than larger ones. Although we cannot see them, the gel slab is full of tiny holes and channels. As the molecules filter through the gel, tiny molecules move faster than large ones. Unlike a strainer, a gel isn't just a single layer of holes—it is a slab of tiny meshwork… a little like a set of strainers stacked one on top of the other. We also don't wait for molecules to exit from the gel—we often stop them in midstream to see which substances have migrated faster than others.

Mixtures of molecules are loaded at the top of the gel, and later—after they travel through the gel—can be viewed as "bands" or stripes in the gel. Each band contains proteins that have the same size and shape. Often a single band contains only one type of protein. If we loaded a mixture of all the proteins in a cell, we would see many, many bands on the gel—corresponding to the many proteins used by the cell. This is what we'll be viewing in the upcoming lab.

You'll notice that the gel apparatus is plugged into a power supply. This is another important difference between gel electrophoresis and a simple strainer. It would take much too long for molecules to move through the gel with only the force of gravity to pull them along. Instead, an electric voltage is applied across the gel—with a positive charge at one end, and a negative charge at the other. The mixture of molecules is often either charged naturally, or treated so that it becomes charged. Our protein samples, for example, are mixed with detergent (in this case sodium dodecyl sulfate, or SDS) which coats each molecule and gives it a negative charge. We set up the gel with a positive electrode at the far end, so that the negatively charged proteins will be pulled along through the gel faster. Tiny proteins will travel farther and faster than large ones. A similar method can be used to separate pieces of DNA (e.g. individual genes), and is used to view specific fragments during DNA fingerprinting.

PART 1: PLANTING CORN SEEDS

Materials

6 Corn Seedlings
Prepared Seed Starter Tray
Toothpicks
Labeling Tape

1. You will be given a seed starter tray with six separate sections. You will also be given six corn seeds. Plant each of the seeds in each section of the tray and cover with soil, but no deeper than 1 cm.
2. Write your group number and lab section number on a piece of labeling tape and wrap it around the toothpick so that the writing is clearly visible. Make it look like a flag.
3. Put a toothpick in one of the six sections of your planting tray, so you will know which tray is yours.
4. Your instructor will show you how to put your starter tray into a larger tray with the other groups from your lab section.
5. Once all groups have placed their starter trays into the large tray, your instructor will water them, and place the tray onto the plant cart, where they will be left to grow for two weeks.

COLLECTING DATA FOR MENDELIAN ANALYSIS

In preparation for Mendelian analysis, you will need to record some information about the corn plants you grew. We're doing this now, before we grind the leaves up and destroy the plants!

Materials

Your group's Plant Starter Tray from two weeks ago

1. Look at the six plants you planted during the previous class. You should see both green and albino (white) plants. (If some seeds didn't germinate, you may have less than six plants. That's okay.)
2. Count the number of green plants and albino plants and record the number of each in Table 1.
3. You will need this data later.

Table 1. Your Group's Data (1pt)

	GREEN (NORMAL CHLOROPHYLL)	**ALBINO (NO CHLOROPHYLL)**
Total Number of Plants		
Numbers For Ratio		

PART 3: EXTRACTING PROTEIN FROM CORN LEAVES

Materials

Corn Plants
Mortar and Pestle
50 ml Beaker
1 Tube with 1.5 ml Grinding Buffer (Tube "G" available from your instructor)
Disposable Plastic Graduated Pipets
Microcentrifuge Tube Rack
1 Pipet Pump
1 Tube of 0.1 ml Laemmli Sample Buffer (Tube "L" available from your instructor)
Microcentrifuge
Digital Balance
Ice Pack (soft)
1- 6x6 piece of Miracloth
1 Tube with 0.5 ml Sodium Bisulfite (Tube "S" available from your instructor)
Marking Pen
1 Clean Microcentrifuge Tube
1 – 1 ml Pipet (with 0.1 ml graduations)
Boiling Water Bath
Ice Bucket

INSTRUCTIONS: *It is VERY IMPORTANT that you **keep the sample cold** throughout the entire process! To prevent enzymes on your skin from degrading the samples, **wear gloves throughout the procedure**. Be careful of measurements – it is important that your measurements be as EXACT as possible.*

1. *Place your mortar and pestle into the ice bucket in the lab until it is very well chilled (at least 5 minutes).*
2. Your instructor will assign you either the normal green corn plants or the white albino corn plants to use.
3. Cut the leaves from the plant you were assigned. (*Only cut healthy looking leaves, and do not include the stem.)*
4. Weigh out between 0.5-1 grams of leaves. Do not include stems or roots.
5. Remove the mortar and pestle from the ice bucket. (To avoid contamination and degradation of your sample, do not handle it too much and try to keep it cold!)
6. Wipe off any moisture on the mortar and pestle very thoroughly.
7. Place the mortar on the ice pack. Smash it down to keep good contact between the two.
8. Put the leaves into the mortar.
9. Use a fresh, clean plastic TRANSFER PIPET to add the grinding buffer from Tube "G" AND the sodium bisulfite from Tube "S" to the mortar. (If your instructor prefers, you may tip the liquid into the mortar.)
10. Grind the leaves in the grinding buffer and sodium bisulfite until you have produced a *very smooth* paste. *(Do not rush!)*
11. Remove the mortar from the ice pack and place the 50 ml beaker on the ice pack.
12. Place the 6"x6" square of Miracloth over the beaker, and filter the slurry from the mortar through the Miracloth into the beaker. *(squeeze the cloth gently to get all the liquid, but not leaf debris, into the beaker.)*
13. Throw the used Miracloth away in the regular trashcan.
14. Use a fresh, clean PLASTIC TRANSFER pipet to transfer EXACTLY 1.00 ml of the filtered liquid into a clean microcentrifuge tube **(*WARNING –it is important that all groups do this in order for the centrifuge to balance!)*** If your instructor prefers, you may re-use tube G.

15. Label the microcentrifuge tube well, with your group and section number, and whether you used green or white leaves. *(Label your tube on the lid only!)*
16. **Keep your tube on ice**, until your instructor is ready to load the centrifuge. This allows time for slow groups in your class to catch up.
17. Your instructor will help each group load the centrifuge with the tubes. They must be balanced equally.
18. The tubes must spin at 14,000rpm for 10 minutes.
19. After the tubes are finished spinning, be extremely careful *not to mix the contents up again – try not to shake or disturb the contents of the tube in any way.*
20. You will notice a pellet of solid material at the bottom of the tube. This is the material that you do not want. Therefore, you must remove some of the liquid from above the pellet. This is called the supernatant.
21. Use your 1ml pipet and pipet aid to remove **EXACTLY 0.1 ml (100 µl)** of the supernatant from the tube and place it into the tube of Laemmli Sample Buffer (Tube "L") that your instructor had given you. Then, pipet up and down to mix the two thoroughly. **(CAUTION: Laemmli Sample Buffer has had 2-mercaptoethanol added to it, which is very toxic and has a strong sulfur smell like rotten eggs. Make sure you are wearing gloves and goggles when dealing with this buffer.)**
22. Label tube "L", which now contains your sample, <u>*on the lid of the tube only*</u>. (Write "G" if you used green leaves, and "W" if you used white leaves – and also write your group number)
23. Very carefully place the tube into the floating foam rack in the boiling water bath, so that only the bottom of the tube touches the water. *(Do not completely submerge the tube, this will cause the lid to pop off and water to dilute your sample, causing poor results)*
24. Allow it to float in the boiling water for 5 minutes.
25. After 5 minutes, transfer your tube directly to ice. Use forceps!
26. Your instructor will later set up the Protein Gel apparatus, first by loading a 15% polyacrylamide gel into the chamber, and then filling the chamber with Running Buffer.
27. Your instructor will then load your sample into one of the wells at the top of the gel.
28. There are 15 wells in the gel, the first one is left blank, so that you know which way is right side up on the gel when attempting to read it later. *(Your instructor will load the gel with samples from green and white leaves and will record where each sample is loaded.)*
29. The gel will the run at 200 V for 35 minutes.
30. Your instructor will then place your gel into Coomassie Blue Stain overnight, and then place it in distilled water to remove any excess stain until next week's class.

PART 4: READING YOUR PROTEIN GEL

1. Today you will observe the results of your Protein Gel.
2. Do your best to analyze your class' gel, and answer the following questions based on your results.

Questions

1. Give an approximate estimate of how many bands you saw, and explain how you got that estimate. What feature of the cell does this illustrate?

2. Results and Analysis:
   a. Describe the pattern of bands you saw in the gel, as well as any differences between white and green corn leaves. A simple sketch and some words would be useful.

   b. What was your hypothesis? Is it supported or contradicted by your results? Explain.

3. What further information would help you determine the cause of white leaves? Explain.

Mendelian Genetic Analysis of Albino Corn

You have some hypotheses about the trait of white leaves in corn, and from your gel you have seen how proteins are affected by this trait. How many genes do you think are involved? This is a hypothesis that can be checked by analyzing how the genes are inherited, a technique known as Mendelian genetic analysis.

This week we will use the data you recorded about numbers of plants, in order to determine how many genes are involved in producing white leaves.

Materials

Your group's data from Table 1.

1. Find the numbers of green and albino plants you recorded in Table 1.
2. Your instructor will combine data from the whole class. Record the class data in Table 2.
3. Determine the ratios for both group and class data to the smallest whole numbers. Record them in the last column of each table. (To calculate a ratio, choose the phenotype with the smallest number and divide all the others by that number. Ask you instructor if you need help.)
4. Before you move on to answer the questions, consider your hypotheses from the gel electrophoresis lab. Do you think that albino corn results from one gene, two genes, or several?

   If necessary, revise your hypothesis:

Table 2. Your Class's Data (1pt)

	GREEN (NORMAL CHLOROPHYLL)	ALBINO (NO CHLOROPHYLL)
Total Number of Plants		
Numbers For Ratio		

Table 3. Data From All Lab Classes
(Your instructor will provide this data, you will need it to answer the questions)

	GREEN (NORMAL CHLOROPHYLL)	ALBINO (NO CHLOROPHYLL)
Total Number of Plants		
Numbers For Ratio		

Questions

1. From the gel electrophoresis lab, how many genes do you think are involved in causing albino leaves? Explain, using data from the gel.

2. Based on the numbers of plants, which gene(s) do you think might be dominant—the genes for green leaf color, or for white leaf color? Explain your hypothesis, using the available data. (Hint: the parents of these plants were all green.)

3. Let's test a hypothesis: what if the gene for the formation of chlorophyll in corn plants has two alleles: E and e? One produces green plants with normal chlorophyll, and the other creates albino (white) plants unable to produce normal chlorophyll. You will determine which is dominant.
   a. Write out the Punnett square for the mating Ee x Ee:

   b. From your Punnett square, what is the ratio of phenotypes for an Ee x Ee mating? dominant:recessive= (EE and Ee):(ee)=_____:_____

4. Analyze the class data in Table 2.
   a. Compare your ratios from Table 2 to the ratios for the Ee x Ee cross (above). Is your data consistent with leaf color resulting from a single gene? Is green (normal chlorophyll production), or white (no chlorophyll) dominant? Explain by citing your *data.*

   b. Using your conclusions (above) write out the phenotypes for the following gene combinations:

GENOTYPE	PHENOTYPE
EE	
Ee	
ee	

5. When you do genetic studies, sample size is important. If you are looking at a large enough "sample" of offspring, your data will match the Punnett squares almost perfectly. Compare your group's data to: (1) the data from the whole class and (2) the data from all classes. Comment on the effects of sample size.

6. *Though we did not observe this in class today,* corn plants also have a gene for height. The allele "T" is dominant and gives tall plants. The "t" allele gives short (dwarf) plants.
   a. What *geneotype* will give short plants?

   b. List *all* the genotypes that would give short *green* plants:

7. Consider the mating EeTt x eett.
   a. Write out a Punnett square for this mating.. Ask your instructor to check the way you've set up your square before you fill it out:

b. Write out the genotypes and their phenotype ratios from your Punnett square (above) in the following table:

	PHENOTYPES			
	GREEN TALL	**GREEN SHORT**	**ALBINO TALL**	**ALBINO SHORT**
GENOTYPES (From Punnett square above)				
RATIOS (From Punnett square above)				

8. Write out a Punnett square for the mating EeTt x EeTt. Ask your instructor to check the way you've set up your square before you fill it out:

9. Analyze your square to fill in the table below.

	PHENOTYPES			
	GREEN TALL	**GREEN SHORT**	**ALBINO TALL**	**ALBINO SHORT**
GENOTYPES				
RATIOS				

10. Given your two Punnett Squares above, what mating could have created the following data? Give the ratios, and explain the reason for your choice.

1021 green tall, 1000 green short, 999 white tall, 1011 white short

11. The albino trait in corn is lethal. These plants cannot photosynthesize, so they die once they use up the food reserves stored in the seed. Because of this, the white plants do not reproduce. Which genotype (s) will be lethal?_____ Can the *allele* for albinism be inherited by a healthy adult? How?

Notes for the Instructor

Growth of Corn Seedlings

Corn seedlings must be grown approximately 2 weeks before the lab exercise begins. Younger seedlings have insufficient leaf material for protein extraction; older white seedlings die

from nutrient deficiency. Watch for necrosis (browning) at the tips of white leaves as a warning sign that plants must be used promptly.

We grow seedlings using small, disposable pots filled with ordinary potting soil. We use seed-starter trays from Ward's, cut into sections containing 6 pots—one per table of students. Seeds are planted by students to a depth of 1 cm. These are maintained under grow lamps and kept continuously moist.

Protein Gels

We generally destain gels in plain water, and store them in small disposable plastic containers. These easily keep for a week until the next lab class meeting.

There are two main differences in the protein banding pattern for white versus green corn (Figure 1). First, bands are less intense in white corn, due to reduced protein levels. This results because these seedlings digest their own proteins for a nutrient source in the absence of photosynthesis. (The resulting buildup of nitrogen compounds accounts for the necrosis of leaf tips in white corn.) Secondly, one or two large molecular weight bands near the top of the gel are missing in white corn. These may correspond to ribulose bisphosphate carboxylase and the light harvesting complex (Harpster, 1984).

Discussing Results with Students

It helps to have students predict the appearance of gels for a few simple scenarios before beginning. We draw a gel with many bands on the chalkboard, and ask students to describe how it would change if one gene were inactive (erase one band), if a new gene were present (add one band), if all the same proteins were produced but one was inactive (no changes), or if a regulatory gene activating several other genes were damaged (many bands disappear). It may also help to remind students that not all genes are used in every cell all the time. As a teaching exercise, one might draw a hypothetical gel for proteins in "leaf cells" and ask them how results would differ for cells taken from roots, stems, etc.

Nonscientists often find all this talk of proteins to be rather abstract, thus it can be useful to make the discussion as concrete as possible. It can be useful to choose one band and give it a name, e.g. the photosynthetic reaction center or a "cellulose-building enzyme," and tell students what that protein does in the plant. Remind them that without that protein, the function would not occur.

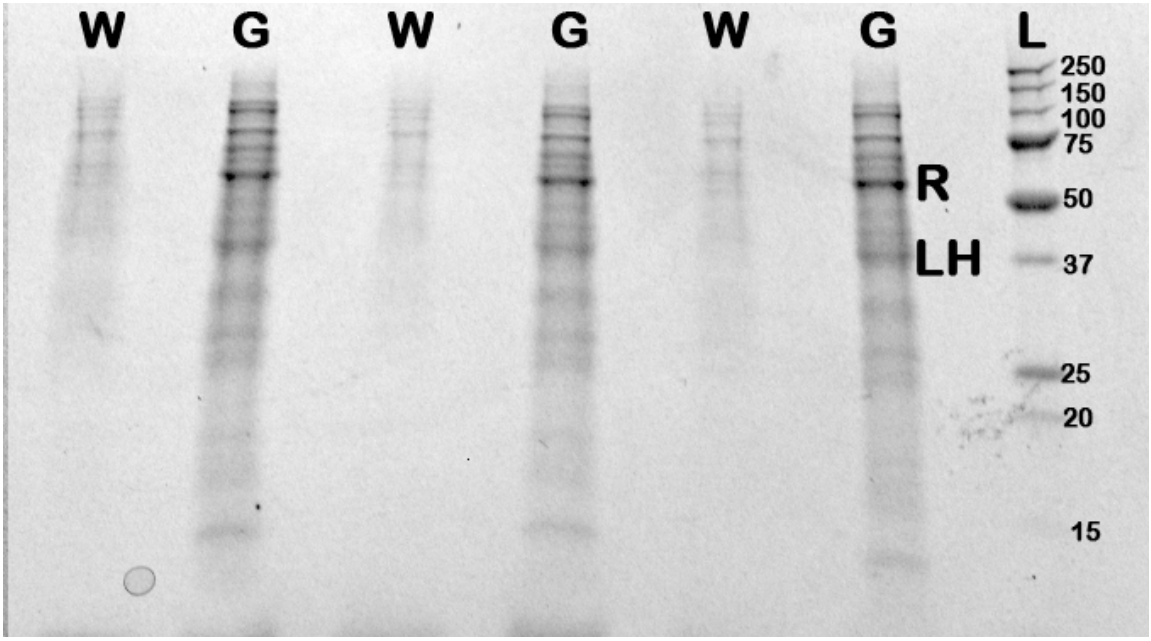

Figure 1. Typical results of protein gel electrophoresis of green and white seedlings. Protein content of green (G) and white (W) leaves harvested from 12 day old corn seedlings and separated by 15% SDS polyacrylamide gel electrophoresis. A molecular weight ladder (L) is shown for reference. Tentative identifications of ribulose bisphosphate carboxylase (R) and light harvesting complex (LH) are based on Harpster (1984).

Although the commercially available albino corn seeds we use contain a mutation that affects chlorophyll synthesis, the individual enzyme is not visible on our protein gels. A likely explanation is that this enzyme occurs at relatively low abundance in the cell. Instead, we see downstream effects on either the synthesis or stability of the photosynthetic proteins. This is a fine opportunity to discuss gene regulation and protein stability with students: a change to a single gene can have far-ranging effects. For teaching purposes, however, any differences students notice in the gels can be used to test hypotheses and promote discussion. Sometimes students see intensity differences in other protein bands—these may be artefacts or genuine differences in expression.

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About the Authors

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Appendix: Materials, Reagents, and Suppliers

Materials for Protein Extraction and Gel Electrophoresis

Item	# Needed per Group	Comments & Info	Source, Catalog #
Mini-PROTEAN 3 protein gel electro-phoresis chamber and basic power supply	1 per class (6 groups)	Bio-Rad offers discounts on equipment used for educational purposes.	Bio-Rad
Tris-HCl Ready Gel precast gels, 15% acrylamide	1	12% gels will also work	BioRad, 161-1121
protein molecular size ladder		optional; can run against a single protein sample (e.g. Emporase) for crude comparison	BioRad, 161-0363
digital balance	1		
small mortar and pestle	1	pre-chill in ice bucket before class begins	
gel ice packs, pre-chilled	1	packs must be soft to surround mortar during use	available at Wal-Mart or similar store
50 mL beaker	1		
MiraCloth	1	cut in 6"x6" squares	VWR, 80058-394
disposable plastic graduated transfer pipets	1	1 mL or larger	
marking pens	1	must be able to label microcentrifuge tubes	
microcentrifuge tube racks	1		

Item	# Needed per Group	Comments & Info	Source, Catalog #
1 mL pipets, graduated by 0.1mL	1	plastic or glass are OK; may substitute 100 µl micropipette and tips	
pipette pump	1	must fit 1 mL pipette	
boiling water bath	1	beaker with boiling chips or glass beads on hot plate	
microcentrifuge	1	must run at 14,000 rpm for 10 mins	
ice buckets	1	may be small; styrofoam shipping boxes work fine	
micropipetter	1	20 µL size	
pipette tips	1	≤20 µL, gel-loading tip	
Ziplock® plastic containers with lid, sandwich size	1	used to stain/destain gels	

Living Materials

Item	# Needed per Group	Comments & Info	Source, Catalog #
green and albino corn seeds (3:1 ratio, monohybrid cross)	six seeds	Grow 12-14 days only; white seedlings die of nutrient deficiency after approximately 2 weeks.	Wards, 86W8085 or Carolina, 177130
albino corn seeds	as needed	Can be used for extra tissue as needed; store in separate location so as not to confuse students.	Carolina, 177100

Reagents

Item	Comments/Instructions	Source, Catalog #
Laemmli sample buffer	Add 50 µL beta-mercaptoethanol per 950 µL buffer before using. Mix and store in freezer as 100 µL aliquots.	BioRad, 161-0737
10X Tris-Glycine-SDS buffer	Dilute 10X for electrophoresis running buffer.	BioRad, 161-0732
sodium bisulfate	10% solution (w/v); store in refrigerator as 0.5 mL aliquots.	
Bio-Safe Coomassie Blue stain		BioRad 161-0786

Grinding Buffer Recipe

KCl	1.11 g
10% SDS soln.	15 mL
sucrose	225 g
Tris-Base	9.1 g

Add all ingredients to 1000 mL; add water to bring up to 1500 mL. Transfer 1.5 mL aliquots to microcentrifuge tubes and store in refrigerator until immediately before use. Recipe provides sufficient buffer for approximately 1500 students.