Science News

IN HIGH SCHOOLS | EDUCATOR GUIDE

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The Artistry of Animal Coloration

SOCIETY FOR SCIENCE & THE PUBLIC

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EDUCATOR GUIDE

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About this Issue

The articles highlighted in this educator guide summarize new findings in animal coloration. "Color me dino" explores inferences scientists may be able to make about dinosaur biology, lifestyle and habitat based on what are thought to be pigment pouches found in fossilized remains. But scientists must first agree whether these are actually pigment pouches or just the remains of bacteria involved in the animals' decay. The article examines the experiments scientists are conducting and the technology used to analyze samples. Students can focus on a particular phenomenon of interest mentioned in the article or examine the cross-curricular intersections that bring biology, chemistry and technology together. "Protein paints chipmunks' stripes" also explores pigmentation, but focuses on one way fur coloration patterns may have evolved in mammals. Along with a main focus on the biology of pigments, this guide invites students to think about how molecules interact with light to produce colors and includes an activity focused on extracting pigments from plants using a low-tech technique.

Connections to Curricula

- Cell structure and function
- Color and pigmentation
- Camouflage
- Light reflection and absorption
- Scientific discourse
- Modeling based on data
- Testing equipment
- Experimental design
- Chromatography

What's in this Guide?

- Article-Based Observation: These questions focus on reading and content comprehension by drawing on information found in the article "Color me dino." Questions focus on scientists' disagreement about whether they've found fossilized pigment pouches and how scientists may further test their ideas.
- Quest Through the Archives: With Internet access and your school's digital access to *Science News*, your students can use this short section to explore fossils, camouflage and coloration as reported by *Science News* since 1922.
- Cross-Curricular Discussion: The questions and extension prompts connect to the articles "Color me dino" and "Protein paints chipmunks' stripes" and encourage students to think about animal coloration from a biological, chemical and physical perspective. The section culminates in engineering and experimental design concepts. The section is divided roughly by subdiscipline for educators who would like to focus on one particular topic area. The extension prompts are either more topic specific or more conceptually advanced.
- Activity: The activity Analyzing Plant Pigments Using Paper Chromatography teaches students a common protocol for extracting and identifying pigments in plants. In this guided experimental design experience, students first learn the process and then create their own experiment using what they've learned. The same process can be used to extract pigments from dyes, markers and other substances.

Standards Alignment

Next Generation Science	Common Core
Biological Evolution: Unity and Diversity: HS-LS4-1, HS-LS4-2, HS-LS4-4, HS-LS4-5	ELA Standards: Reading Informational Text (RI): 1, 2, 4, 7
From Molecules to Organisms: Structures and Processes: HS-LS1-1, HS-LS1-2	ELA Standards: Writing (W): 2, 7
Energy: HS-PS-3-1, HS-PS-3-2	ELA Standards: Speaking and Listening (SL): 1, 4
Waves and their Applications in Technologies for Information Transfer: HS-PS4-4	ELA Standards: Reading for Literacy in Science and Technical Subjects (RST): 1, 2, 3, 4, 5, 7, 8, 9
Engineering Design: HS-ETS1-2	ELA Standards: Writing Literacy in History/Social Studies and Science and Technical Subjects (WHST): 2, 7, 8, 9

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Article-Based Questions

Directions: After reading the article “Color me dino,” answer these questions:

1. Using the article, define the term *paleo color*. What do you think paleontology means based on your answer?

2. The article mentions that microscopic structures have been found inside fossilized skin and feathers. What did scientists first think about the identity of these structures?

3. How did Jakob Vinther and his colleagues explain the microscopic structures found in a 125-million-year-old fossil feather? Define the term for these structures and state what type of information they provide scientists.

4. According to the article, what is the significance of determining the true identity of the microscopic structures found in these fossils?

5. Scientists are still debating the identity of the microscopic structures. State an additional test that was suggested to provide new data about the debated topic. Name a challenge of using that test.

6. Explain the techniques Jakob Vinther’s team used to make suggestions about possible *Psittacosaurus* habitats.

7. Come up with a #hashtag that summarizes this article and send it to @ScienceNews. Include the second hashtag #SNHSdinos so your class can track each other’s ideas and vote on the best one.

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Responses to Article-Based Observation

1. **Using the article, define the term *paleo color*. What do you think paleontology means based on your answer?** Possible student response: *Paleo color is the study of pigmentation from fossilized remains and what that pigmentation can reveal about how ancient animals lived, behaved and interacted with their surroundings. Paleontology is the study of fossilized remains to determine characteristics of past organisms and environments.*
2. **The article mentions that microscopic structures have been found inside fossilized skin and feathers. What did scientists first think about the identity of these structures?** Possible student response: *These structures were thought to be the remains of bacteria and maybe bacteria that decomposed the deceased animal.*
3. **How did Jakob Vinther and his colleagues explain the microscopic structures found in a 125-million-year-old fossil feather? Define the term for these structures and state what type of information they provide scientists.** Possible student response: *Jakob Vinther and his colleagues proposed that the microscopic structures were melanosomes. Melanosomes are structures that produce and store melanin pigments. The shape of the melanosome is linked to the color in birds and mammals.*
4. **According to the article, what is the significance of determining the true identity of the microscopic structures found in these fossils?** Possible student response: *If these structures are melanosomes, they will be interesting for the field of paleo color. If dinosaur pigmentation and patterning can be determined, then scientists could infer quite a bit about the life of dinosaurs, including clues about behavior, habitat and evolution.*
5. **Scientists are still debating the identity of the microscopic structures. State an additional test that was suggested to provide new data about the debated topic. Name a challenge of using that test.** Possible student response: *One additional test mentioned in the article was to look for signs of bacteria using pyrolysis gas chromatography-mass spectrometry. But the technique destroys the sample.*
6. **Explain the techniques Jakob Vinther's team used to make suggestions about possible *Psittacosaurus* habitats.** Possible student response: *The researchers made a model of Psittacosaurus with the coloration patterns they expect from their examination of melanosomes in the fossil. They also made a gray model of Psittacosaurus and photographed it under various lighting conditions — inverting the coloration to create the optimal camouflage pattern for those conditions. Comparing the fossil coloration to the optimal camouflage patterns created with the gray models allowed the researchers to infer a possible habitat for Psittacosaurus.*
7. **Come up with a #hashtag that summarizes this article and send it to @ScienceNews. Include the second hashtag #SNHSdinos so your class can track each other's ideas and vote on the best one.** Possible student response: *#pigmentsmakepaleocolorpossible??*

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Quest Through the Archives

Directions: After reading the articles "Color me dino" and "Protein paints chipmunks' stripes," use the archives at www.sciencenews.org to answer these questions:

1. Find another article about fossilized dinosaur remains. Was this fossil older or younger than the fossilized feathers discussed at the end of "Color me dino?"

2. Both articles include references to the form of camouflage called countershading (on *Psittacosaurus* and rodent belly fur), when an animal's pigmentation is lighter on areas typically in shadow and darker on areas that are typically exposed to light. What is the earliest article you can find that gives an example of a different type of camouflage? State the type of camouflage.

3. Have there been other *Science News* articles referencing the possible identification of pigments in fossilized remains? Explain by citing a specific article.

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Responses to Quest Through the Archives

1. **Find another article about fossilized dinosaur remains. Was this fossil older or younger than the fossilized feathers discussed at the end of "Color me dino?"** Possible student response (answers will vary): www.sciencenews.org/article/parasites-wormed-way-dino%E2%80%99s-gut. *"Parasites wormed way into dino's gut" references a 77-million-year-old hadrosaur fossil that may have been infected with parasitic worms. This fossil is younger than the 120-million-year-old feather fossil described in "Color me dino."*

2. **Both articles include references to the form of camouflage called countershading (on Psittacosaurus and rodent belly fur), when an animal's pigmentation is lighter on areas typically in shadow and darker on areas that are typically exposed to light. What is the earliest article you can find that gives an example of a different type of camouflage? State the type of camouflage.** Possible student response: www.sciencenews.org/archive/nature-ramblings-praying-mantis. *The 1929 article "Nature Ramblings: Praying Mantis" refers to a praying mantis as having "leaf-and-stick camouflage." This type of camouflage can be categorized as a protective coloration.*

3. **Have there been other *Science News* articles referencing the possible identification of pigments in fossilized remains? Explain by citing a specific article.** Possible student response: www.sciencenews.org/blog/science-ticker/green-was-ancient-snakes-signature-color. An article from earlier this year describes scientists who identified pigment structures linked to bright green coloration in well-preserved 11.2-million- to 8.7-million-year-old snakeskin.

Cross-Curricular Discussion

After students have had a chance to review the articles "Color me dino" and "Protein paints chipmunks' stripes," lead a classroom discussion based on the questions that follow. You can copy and paste only the questions that apply to your classroom into a different document for your students. Blackline Master 3 is a printable document that contains a chart related to the Physical Sciences Extension Prompt.

BIOLOGICAL SCIENCES

Discussion Questions:

1a. Ask students to think about the purpose of camouflage. What is it and how does it help organisms survive? [*Students might think about camouflage for defense; it can help animals hide in plain sight. Camouflage might be based on color alone or patterns of coloration, such as countershading*.] Ask students to explore different types of camouflage found in nature (they can find images online or take their own) and why the camouflage is useful in each case. This could lead to a discussion about natural selection and how an offspring with beneficial coloration might survive better than others. If environmental conditions change, offspring with different colorations might be selected for, as in the case of the peppered moth. Have your students read the related *Science News* article titled "Jumping gene turned peppered moths the color of soot," or, depending on their Lexile level, the version at *Science News for Students*, "How a moth went to the dark side."

What is the purpose of camouflage? How does it help organisms survive? What are some different types of camouflage found in nature? Can you find examples? Explain how each is useful. How might natural selection favor camouflage?

1b. Ask students what they know about human skin and hair pigmentation. Have students watch the Howard Hughes Medical Institute's video titled "How we get our skin color." The video outlines the physiology of skin pigmentation and defines the purpose and function of melanosomes. Ask your students if their skin or hair varies in color in different situations [*like after being out in the sun*]. After watching the Howard Hughes Medical Institute's video, ask students to explain the role of melanocytes based on information provided in the film. [*Within melanocytes, membrane-bound organelles called melanosomes produce and store melanin pigments.*] Ask students what factors determine overall skin color. [*Some factors that affect overall skin color are: the number of melanosomes, amount of melanin, and type of melanin present.*] How does melanin potentially protect DNA inside skin cells? [*Melanin absorbs specific wavelengths of radiation from sunlight which could decrease the UV radiation hitting DNA inside a nucleus. This radiation can cause mutations in the DNA.*]

What gives human skin or hair a particular color? Does your skin or hair vary in color under certain conditions? How does melanin interact with UV radiation? How can this interaction potentially offer a protective benefit to cells?

Extension Prompt:
1c. Explain that melanocytes come from a special group of cells, known as neural crest cells. Encourage students to research the types of cells that form from neural crest cells [*cartilage and bone of the cranium and face, smooth muscle, and some cells of the nervous system*] or the impact that these cells have on development of an organism. You might want to have students read another *Science News* article, "Domesticated animals' juvenile appearance tied to embryonic cells," to explore how possible selection for neural crest cells with defects may have led to common physical traits in domesticated animals [*selection for cells with defects may affect coat color, jaw size, facial characteristics and so on*].

Research the group of cells known as neural crest cells. How are they related to melanocytes? What types of cells form from neural crest cells during development? What animals do and don't have neural crest cells and how can the cells help us understand evolution?

PHYSICAL SCIENCES
Discussion Questions:
2a. In "Protein paints chipmunks' stripes," scientists describe how an increased concentration of a protein appears to interfere with pigment production. Review with students that the molecular mechanisms that drive these changes are unknown to the researchers. Still, this can be a launching point for a discussion about how differing the concentrations of reactants can affect the rate of a chemical reaction. [*Typically, as the concentration of reactants increases, the rate of a reaction increases due to the increased number of reactant collisions per unit time.*] Are there any other substances that generally affect the rate of a chemical reaction? [*Catalysts increase the rate of a chemical reaction by lowering the activation energy. Inhibitors prevent catalysts from interacting properly with reactants and therefore, slow the rate of a reaction.*] Have students draw an energy coordinate diagram for a general exothermic reaction and explain how the shape of the graph changes in the presence of a catalyst. [*The University of Texas outlines an example in the first graph here.*]

How does varying the concentration of reactants in a chemical reaction generally affect the reaction rate? When present, what other substances can affect the rate of a chemical reaction? Explain. Draw an energy coordinate diagram for a general exothermic reaction. Explain how your graph would change in the presence of a catalyst.

2b. Discuss the nature of pigments as substances that absorb light. Ask your students how the absorption of some wavelengths of light relates to the colors that we see. [*The colors that we see are the wavelengths of light that are reflected. Based on a pigment's chemical structure, the molecule absorbs and reflects specific wavelengths of light.*] You might want to review the electromagnetic spectrum. Explain that pigments have unique absorption patterns or spectra. Have students research a pigment. They should determine the molecular structure of one pigment molecule and explain how it allows us to see the color we see. [*Examples of pigments can be found on The WebExhibit by Idea titled "Pigments through the Ages" (have students find a pigment for which a chemical formula is available). An example of an absorption spectrum of several plant pigments can be found halfway down the page on Estrella Mountain Community College's page on photosynthesis*]. Can students find examples of animals that have an outward color appearance that is not directly related to pigments? [*Some animals have structures that scatter light to make them appear a particular color. Peacock feathers, for example, aren't blue because of pigment, but instead their feather structures scatter light to create the blue appearance.*]

How would you define a pigment? How do pigments interact with visible light and how does that relate to the colors we see? Research a particular pigment. Draw its molecular structure and determine how it interacts with light. In what plants or animals does this pigment exist? Find an example of an animal that has an outward color that is not directly related to pigment. Explain how the animal's physical structure interacts with light to produce that particular appearance.

Extension Prompt:
2c. Scientists use a variety of techniques to analyze animal pigmentation. Divide students into groups to research the equipment and techniques referenced in the articles (gas chromatography-mass spectrometry, Raman spectroscopy, scanning electron microscope and transmission electron microscope). Ask students to share the information they found about the equipment and the technique. What is each technique most commonly used for? Provide students with a few examples and ask which technique they would use in the given situation. Use the chart on Blackline Master 3 to allow students to organize their responses. [*For example, they have a tissue sample from mussels found at the mouth of the harbor. They want to find out if metals coming off of brake pads from vehicles are getting into the water. Which technique would they use to test the mussel tissue samples to look for potential metals? GC-mass spectrometer.*]

ENGINEERING AND EXPERIMENTAL DESIGN
Discussion Question:
3a. Have your students ever tried looking at cells under a microscope? If they haven't, they can prepare their own slides using this simple procedure for onion and cheek cells. Can students see and identify any organelles? Ask students why scientists develop more and more powerful tools for looking at specimens. Have students examine the degraded snakeskin images (on Page 25 of "Color me dino"). According to "Color me dino," how does the transmission electron microscope (TEM) help distinguish between bacteria and melanosomes? [*Observing tissue using a TEM produces images in which melanosomes appear dark and bacteria look translucent.*]

Prepare your own slides and look at cells under a microscope. What do you see? Can you draw the features of the cells? Which organelles are easy to identify? Why do you think scientists develop more powerful tools for looking at specimens? Examine the degraded snakeskin images on Page 25 of "Color me dino." What parts of the cells can you see? Compare the images. How does the transmission electron microscope help to distinguish between bacteria and melanosomes?

Extension Prompts:

3b. Discuss the idea of making inferences based on deductive and inductive reasoning. [*An inference is a conclusion reached by reasoning through evidence.*] Explain that this exercise will require students to make inferences in order to create and analyze fictional melanosome data. Have students draw or describe a fictional animal focusing on the animal's coloration (encourage them to stick to reddish brown, black or iridescent hues). Along with the animal's coloration, have students hypothesize a likely habitat for the animal. [*For example, a black and gray striped snake that is typically found in forests and marshes.*] Next, give students a small piece of paper (about 3 by 5 inches) to design their animal's "transmission electron microscope (TEM) skin picture" (similar to the picture given in the article). Students should first determine what section of skin is highlighted in their skin picture. Next, they should determine the total number and type of microstructures, as well as the general concentration pattern of microstructures within their skin picture. [*For example, 20 eumelanin melanosomes and 15 bacteria. Eumelanin melanosomes are clustered in the bottom third of the picture and bacteria are scattered throughout.*] Once designed, ask students to draw out their skin picture. Have students switch skin pictures with another student, count melanosomes and bacteria, infer a possible coloration pattern for their classmate's animal, and predict the animal's potential habitat. Students can compare their inferences to their classmates' original ideas.

What is your fictional animal's coloration pattern (stick to reddish brown, black or iridescent hues)? What is a typical habitat for your animal? Using the melanosome shape, type and color chart on Page 26 of "Color me dino," you will design your own "transmission electron microscope (TEM) skin picture" for your fictional animal. First, answer the following questions: What section of skin is pictured? What type of melanosomes does the skin contain? What are the numbers of melanosomes and bacteria? What is the general distribution of melanosomes and bacteria?

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Techniques for Analyzing Pigmentation

Directions: Do research to fill in the portion of the table that relates to your group's assignment.

Equipment	Description	Examples of when to use it
GC-Mass Spectrometer		
Raman Spectrometer or Raman Microscope		
Scanning Electron Microscope		
Transmission Electron Microscope		

Activity: Analyzing Plant Pigments Using Paper Chromatography

Class time: 1 to 4 class periods, depending on parts of the experiment performed and depth of analysis
Day 1: Paper chromatography experiment to learn the general procedure
Day 2: Students design their own experiment
Days 3 and 4: Students perform their experiments and analyze data

Purpose: To extract pigments from one or more plants, beginning with spinach, and use this technique in the study of plant pigments. Also, to develop, test and analyze related hypotheses.

Notes to the teacher: Students can explore the basics of this technique and then develop their own experimental design, based on their background knowledge, to test a different hypothesis. Creating the spinach solution before class begins will decrease the amount of in-class time needed to perform the initial chromatography experiment.

General background on technique: Chromatography (Greek for "to write in color") is the physical process of separating compounds in a mixture based on each compound's ability to move within a mobile phase in a definite direction through a stationary phase. In 1903, Russian botanist Michael Tsvet used a column of calcium carbonate to extract plant pigments from a petroleum ether and chlorophyll solution, creating the chromatography process. The same method can be applied to extract plant pigments using paper chromatography. Plants have many pigments, each of which is a molecule that absorbs and reflects light. When a slurry of pigments is prepared and placed on the paper (the stationary phase) and the paper is placed in an acetone-vinegar solution (the mobile phase), the pigment molecules are carried up the paper and distributed. Separation is based on their attraction to the stationary phase (paper) and the mobile phase (solvent), as well as on their molecular weight. Generally, the least massive molecules will travel the fastest and those with the greatest mass will travel at a relatively slower rate. By measuring the distance a pigment travels under controlled conditions (the R_f-value), that pigment may be identified.

Color-producing pigments like eumelanin and pheomelanin can be found in animals, as described in "Color me dino." Plants also have color-producing pigments, such as chlorophyll a (blue-green) and xanthophyll (pale yellow-green), that are crucial for the light reactions of photosynthesis. Plants have various types of pigment groups and these produce the varied colors we see in fruits, vegetables and greenery. Other plant pigments such as flavonoids often play a role in the chemical defense of plants and are toxic to many herbivores and pathogens. Some block ultraviolet (UV) radiation that can damage cell proteins and DNA. Carotenoids are a category of pigments known for their bright red, yellow and orange colors and have antioxidant properties.

Materials for each pair of students:

- Blackline Master 4
- Chromatography or filter paper
- Scissors
- Ruler
- 600 mL cup or beaker
- Pencil
- About 5 fresh spinach leaves, finely chopped
- Cheesecloth or strainer
- Mortar and pestle
- 3 mL disposable pipette or dropper
- Acetone (5 mL)
- Small beaker or small vial with lid
- Solvent of 92% vinegar 8% acetone
- Gloves and safety goggles
- Calculator (optional)

Directions:

1. Introduce students to the categories of pigments. If time allows, students might research the colors that various pigments create and where they are found:

   Some familiar plant pigments of interest in nutritional and pharmaceutical research:

Pigment	Color	Found in
Anthocyanins	blue/purple/red	berries, grapes, red peppers, beets, eggplant, plums
Beta-carotene	orange/yellow	carrots, pumpkin, sweet potatoes, citrus, papaya, melon, squash
Curcumin	yellow	turmeric
Lutein	yellow/orange	kale, broccoli, spinach
Lycopene	red	tomatoes, watermelon, red grapefruits
Zeaxanthin	yellow	corn

2. Cut chromatography paper: Show students how to prepare their chromatography or filter paper. If your paper doesn't come in strips, then show students how to cut the paper into strips that are long enough to reach from the top of their beaker to just above the bottom, wrapping around the pencil to hang freely. Be sure edges are straight and try not to touch the paper with bare hands, as that will transfer body oils.
3. Pause for a safety moment: Check student allergies and moderate materials accordingly. Remind students not to taste any substances and to use materials only as directed. Students should wear safety goggles and gloves. After handling the materials, students should wash their hands thoroughly. Be sure there is proper room ventilation because acetone is a volatile substance.
4. Prepare the spinach: Place the finely chopped spinach leaves into a mortar and add about 5 mL of acetone. Thoroughly grind with the pestle. Add more acetone as needed, but your goal is a dark concentrated solution. Pour the solution through a funnel lined with cheesecloth and drain into a small vial or beaker (squeeze the cloth to get the highly concentrated portion of the solution).
5. Pipette transfer: Using a pencil, draw a reference line about 1 to 2 cm from the bottom of the chromatography paper. Create a dot of spinach solution on the middle of the reference line (you should have an intense green color) by adding one drop at a time, allowing it to dry, then adding another until the dot is highly concentrated.
6. Remind students to read the remaining directions on Blackline Master 4 carefully. If you want to show them the next steps (this takes the moment of discovery away, but provides clear direction), add some solvent solution (92% vinegar 8% acetone) to the bottom of the beaker so the tip of the chromatography

paper is submerged but your dot is not (Step 5 on Blackline Master 4). Leave the beaker untouched for about 10 to 20 minutes. Point out that students should stop their experiment before the solvent reaches the very top of their filter paper. Give them time to practice their technique.

7. Demonstrate how to calculate the R_f-value (measure the distance from the reference line to the middle of the new location of the pigment and divide by the distance from the reference line to the solvent near the top of the paper). Since everyone did the same basic process, you can have students post their results and compare. Ask why some of their results might be different [*experimental error, different amount of time for solution to separate out, etc.*]. Discuss which pigment traveled farthest and why students think so. Which pigments could they identify based on their R_f-values? For those pigments not listed on the reference chart provided on Blackline Master 4, how could students identify them? How did the pigments look after they were separated versus when they started? Why use a solvent instead of water to separate the pigments? Why stop the chromatogram before it reaches the top of the paper? What could make it difficult to identify each pigment [*pigments overlap on the paper, for example*]? What could you do to fix such a problem [*change the ratio of vinegar to acetone in the solvent, for example*]?

8. Now students can make the experiment their own: Based on the background and initial test, students may have questions that they'd like to answer. Ask students to list variables they might like to test in a different chromatography experiment [*type of plant, part of plant, temperature of sample and whether it is cooked or fresh, solvent type, solvent strength, type of paper, distance traveled over time*]. Discuss the importance of isolating variables and have students work in teams to identify how they will test a new variable. Depending on the time you'd like to invest, a matrix of variables can be set up and teams can be assigned to tests so that each variable is tested at least three times.

Example matrix with possible variables to test:

Variable to test:	**Team 1**	**Team 2**	**Team 3**	**Team 4**	**Team 5**
Fresh vs. cooked spinach	x	x			x
Spinach leaf vs. oak tree leaf		x	x	x	
Spinach leaf vs. spinach stem	x	x	x		
Solvent (vinegar and acetone): 92% vinegar vs. 50% vinegar			x	x	x
Chromatography paper vs. coffee filter	x			x	x

9. Students should formulate a hypothesis based on the variable they are testing.

10. Students need to consider how many times they need to repeat their experiment in order to be confident of their results. They can use any of a variety of power analysis calculators to help them or you can print out a table for students to use manually. Match the table with the statistical analysis you'd like your students to use, such as the t-test.

11. Give students time to design their experiment using Part 2 of Blackline Master 4 and identify what materials they'll need. You might want to check their written work before they proceed or have groups orally share their experimental designs before giving them the go-ahead.

12. Give students time to perform their own experiments, collect data and analyze it. Groups can then share their data orally, as group posters or lab reports.

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Analyzing Plant Pigments Using Paper Chromatography

PART 1: INTRODUCTION TO SPINACH CHROMATOGRAPHY

Directions: Practice the paper chromatography process

1. Prepare a data table similar to the one shown in Step 8 below.
2. Put on your gloves so you don't transfer the oils from your skin to the chromatography paper. (This is known as contaminating the sample and will affect your results.) Cut your chromatography paper so it is the right length for the beaker or cup you will be using.
3. Place about 5 leaves of chopped, fresh spinach in a mortar and add 5 mL acetone. Use the pestle to release the pigment from the leaf. You want a very intense color solution.
4. Using a pencil, draw a reference line about 1 to 2 cm from the bottom of the chromatography paper.
5. Use a pipette or dropper to transfer a drop of solution to your chromatography paper so that the middle of the dot is on the reference line. Let the drop dry, then add another to the same spot. Repeat until the spot is very dark (about 20 drops). Only add the next drop when the last is dry (you can blow on it gently).
6. Prepare your chromatography paper so it hangs from a pencil (or dowel) just above the bottom of your beaker (as shown in Figure 1).
7. When you're ready to go, add solvent solution (92% vinegar 8% acetone) to the bottom of the beaker so the tip of the chromatography paper is submerged, but your dot is not. Leave the beaker untouched for about 10 to 20 minutes. Watch as the solvent (mobile phase) moves up the paper (stationary phase) and observe what happens to the pigment dot over time. Remove the chromatography paper from the solution just before the solution reaches the top of the paper (where it touches the pencil). Using a pencil, mark the final solvent line and the location of each pigment as seen in Figure 2 (these get harder to read later when the paper dries).
8. Use your ruler to measure the distance each pigment traveled compared to the reference line and record your data on a table like this one:

FIGURE 1: BEAKER AND PAPER SETUP

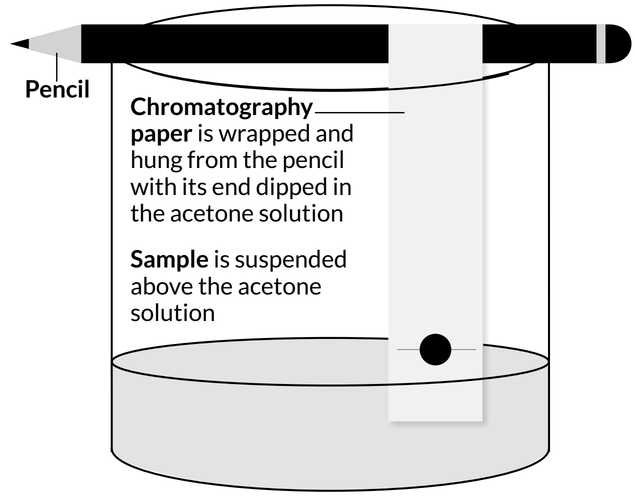
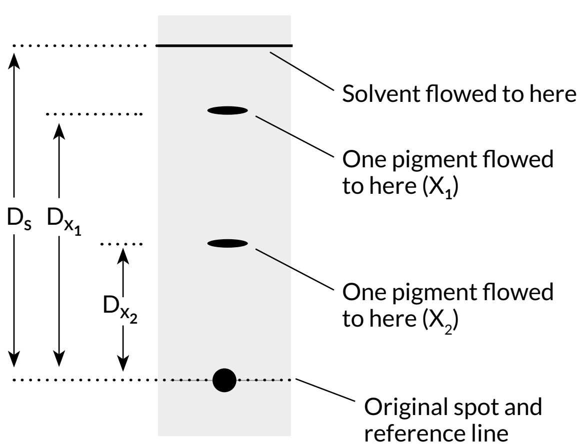


FIGURE 2: LABEL YOUR PAPER



Pigment band	Band color	Distance solvent traveled (mm)*	Distance pigment traveled (mm)	Calculate R_f-value	Possible identity of pigment
1					
2					
3					
Continue as needed					

* this value will be the same for all entries

9. Calculate your R_f-value:
   R_f = distance pigment traveled (mm) / distance solvent traveled (mm)

10. Use the table below to help you identify the pigments in your sample:

R_f value	Visible color	Pigment type
.98	Yellow	Carotene
.86	Yellow	Xanthophyll
.80	Red	Xanthophyll
.67	Dark gray	Phaeophytin a
.60	Light gray	Phaeophytin b
.50	Yellow	Xanthophyll
.48	Light blue-green	Chlorophyll a1
.46	Dark blue-green	Chlorophyll a
.30	Light yellow-green	Chlorophyll b1
.25	Dark yellow-green	Chlorophyll b
.15	Yellow	Xanthophyll

Source: http://www.dpcdsb.org/NR/rdonlyres/7799127C-23A2-4E9B-9E0E-7E8A9095428D/105157/pigmentslab.pdf

PART 2: PRE-LAB: DESIGN YOUR OWN EXPERIMENT

Directions: Use the prompts below to help you plan your experiment. Once you've planned the experiment, have your design checked as directed by your teacher and then get started.

1. What do you want to focus on? Do you want to compare the results of different plants, the same plant under different conditions or a different composition of acetone and vinegar solvent?

2. What is the question you want to explore?

3. Identify the variables you want to test in order to answer your question:

4. Write a hypothesis based on your variables and question.

5. What materials will you need to perform your test?

6. Data collection: What data will you collect? How will you collect it? How many times will you need to repeat your test? How will you organize your data table? Make your data table:

7. What statistical analysis will you use on your data?

8. What procedure will you use? Be as specific as possible.

PART 3: POST-LAB: ANALYZE YOUR DATA

Directions: Use the prompts below to analyze your data.

1. Based on your data and the statistical test you selected, what story does your data tell about your plant's pigments? Explain.

2. Did you prove or disprove your hypothesis?

3. How will you visually display your data and results? Be sure to label any visuals and provide a key for necessary information.

4. What errors do you believe existed in your experiment? How could you minimize or remove the potential error in future experiments?

5. As you examine your data, what new questions arise? How could you test for those?