The Wonderful World of LIVING Color

Connie Scercy Wood

Introduction

The subject of biology covers a broad range of topics. Students often don't make the connections from one unit to another. What do organic compounds have to do with cells? How is active transport related to the digestive system? How does photosynthesis fit in with ecosystems? Evolution is somehow related to genetics, but how? What I've needed is a way to help students make these connections—a theme that ties them all together. I teach Biology I to ninth through twelfth grade students, some of whom are taking the class for the second time. Biology is a required course for graduation, and there is a state mandated curriculum and a state End-of-Course test at the end of the Biology I course. There are unifying concepts and strands that are part of the Standard Course of Study, such as "Form and Function", "Systems, Order and Organization", "Nature of Science", and "Science and Technology", but I must admit I have sometimes found it difficult to ensure that so many themes are incorporated into my lessons and presented in a way that leads to real understanding. In this unit I will attempt to use color as one single unifying theme to not only connects all the various topics in biology, but also the concepts and strands that are part of the curriculum.

As a high school biology teacher, I am aware of the importance and significance of color in living things and how color is sometimes used to understand the world of living organisms. The living world is full of color and depends on light from the sun, the source of color, for its existence. Some cells contain pigments, giving them color or helping them convert light energy into chemical energy. The human eye contains cone cells that help it to distinguish between different colors, although some people are born with genes that make them colorblind. Animals often display colors that are used to attract mates, or to warn off predators. Plants use color to attract pollinators. Chemical indicators can be used to detect the presence of organic compounds by changing colors. New color imaging systems reveal how the brain functions when performing certain tasks. This unit will focus on color in living things and how color is often used in their study.

Background Information

The following sections include background information necessary for a deeper understanding of how color "works" in the topics and activities to be covered in this unit.

Color and Light

Life on Earth requires energy from the sun in the form of electromagnetic radiation. The electromagnetic spectrum includes, from lowest energy to highest energy levels: radio waves, microwaves, infrared radiation, visible light, ultraviolet radiation, x-rays, gamma rays and cosmic rays. Light is a stream of particles, called photons, which travel together, and also exhibit properties of waves. The more energy the photons have, the shorter their wavelength and the more frequently they oscillate. The less energy they have, the longer the wavelength. Even though some forms of light contain more energy than others, all photons travel through a vacuum at the same speed—only slowing down as they pass through materials such as air or water.[1]

Visible light makes up only a small portion of the electromagnetic spectrum--with wavelengths from 380 nanometers to 760 nanometers—yet makes up 80% of the radiation that reaches the Earth.[2] Living things have evolved over time to make use of this resource, trapping it with chemical compounds called pigments. Plants take advantage of the energy in visible light to produce sugars, using chlorophyll and other light absorbing pigments in the process of photosynthesis. Animals use pigments in photoreceptor cells for vision.[3]

White light contains photons of all the different wavelengths of visible light, which are revealed when these different wavelengths are separated by passing white light through a prism. We perceive these different wavelengths of light as colors: red (760-647nm), orange (647 to 585nm), yellow (585 to 575nm), green (575 to 491nm), blue (491 to 424nm), and violet (424 to 380nm).[4] An object appears to have a certain color due to the selective absorbance of different wavelengths of light by the object. Light that is not absorbed is reflected into the eye of the observer.[5] If a material absorbs all wavelengths of visible light it appears black. If it reflects all wavelengths, it appears white.

The fact that all materials do not absorb all wavelengths of light is a result of the chemical composition of the material. The energy of the photons may or may not be transferred to the electron clouds surrounding the nucleus of an atom or molecule depending on the position and energy levels of the electrons and the particular energy (wavelength) of the photon. Electrons exist in discreet energy levels that can be described as clouds of various sizes and shapes. For an electron to move from one energy level to the next it must absorb or emit a precise amount of energy.[6] When the right amount of energy is absorbed, the electron will be excited to a higher energy level. If a photon of light has the right amount of energy for a particular electron to make that jump, the energy is absorbed. If there is not a good match between the amount of energy that the electrons can absorb and the energy of the photons, then those photons are rejected and depending on their wavelength, we see a particular color.[7]

Large groups of atoms which share electron clouds, such as many organic pigment molecules, need only a small amount of energy for an electron to be excited to a higher energy level, and this means they can absorb visible light. These pigments often have atoms arranged in long chains or rings of carbon, hydrogen, oxygen and nitrogen, with alternating single and double bonds. Some organic pigments have a transition metal at their center which has unfilled orbitals into which excited electrons can move up. Chlorophyll and hemoglobin are two such pigments. In addition to absorbing certain wavelengths of light (leading to their color), the resulting excitation of the electrons may be the stimulus for a chemical reaction.[8]

Understanding this interaction between the electrons of molecules and light energy will be helpful in understanding many of the ways that pigments in living things work.

Color-changing Indicators

Several color changing indicators are used to identify the presence of organic compounds. Benedict's solution is used to test for the presence of reducing sugars such as glucose and fructose. Iodine turns blue-black in the presence of starch, and biuret turns purple in the presence of amino acids from proteins.

Benedict's solution is composed of sodium carbonate, sodium citrate, and copper(II) sulfate pentahydrate. Reducing sugars—most monosaccharides-- have a free aldehyde group—a terminal double bonded oxygen. When dissolved in water, mixed with a little Benedict's solution and heated, the blue copper sulfate ($CuSO_4$) in the Benedict's solution reacts with electrons given off by the aldehyde group of the sugar to form cuprous oxide (Cu_2O), a reddish brown precipitate. Heating is required to supply the activation energy for the reaction.

$$CuSO_4 \rightarrow Cu^{2+}\text{(blue)} + SO_4^{2-}$$
$$2Cu^{2+} + \text{ Reducing Sugar (electron donor)} \rightarrow 2Cu^{+}$$
$$2Cu^{+} \rightarrow Cu_2O \text{ (cuprous oxide, a reddish brown precipitate)}$$

The color varies with the amount of reducing sugar present; from green to orange to red-brown as the amount of precipitate formed increases.[9]

Starch is a polysaccharide composed of two types of molecules. Amylose is a helical water-soluble chain of glucose molecules, and amylopectin is a branched chain of glucose molecules that are insoluble in water. Lugol's Solution, or Iodine-Potassium Iodide (I_2KI) is used to detect the presence of starch and reacts with the amylose. It is believed that the triiodide ions of the I_2KI slip inside the coiled structure of the amylase molecules. A transfer of electrons occurs that changes the light absorbing properties of the molecule, making the yellowish brown I_2KI and amylase complex appear bluishblack.[10, 11] Other sources suggest that iodine will stain other polysaccharides such as cellulose. However, when placed on onion cells, the iodine remains yellowish brown—even at the cell walls which would be made of cellulose. I have yet to find any reason for using iodine to stain onion cells except to add color and contrast.

Biuret reagent contains the same copper sulfate found in Benedict's solution, along with potassium hydroxide (to create an alkaline environment) and potassium sodium tartrate[12], so it also starts out with a blue color. Biuret is used to detect the presence of proteins. There must be at least three amino acids in the polypeptide, forming two peptide bonds.[13] A positive reaction is the result of the reduction of copper(II) ions to copper(I), which then forms a complex with the nitrogen and carbons of the peptide bonds. This new arrangement of the molecules results in a change in which wavelengths of light are absorbed and the solution changes from blue to violet. Biuret would not react with amino acids or dipeptides because there are not enough peptide bonds for the complex to form.

Bromothymol blue (BTB) is a pH indicator that changes from blue in basic solutions, to green in neutral solutions, to yellow in acidic solutions. The change in color occurs due to a change in form of the BTB from a protonated form (yellow) to a deprotonated form (blue).[14] Protonation is the addition of a hydrogen ion to a molecule; deprotonation, the removal of a hydrogen ion. So in solutions that are acidic, BTB receives hydrogen ions from the solution. In basic solutions BTB gives off hydrogen ions. The change in form results in a change in the light absorbance properties of the molecule, changing the color. BTB is often used in respiration and photosynthesis experiments. When carbon dioxide is blown into a solution of BTB through a straw, carbonic acid forms and the BTB changes from blue or green to yellow. If plants are added, which use up the carbon dioxide for photosynthesis, the BTB will slowly revert to blue as the solution becomes less acidic.

Methylene blue has many uses, as a stain, an oxidation-reduction indicator, as a treatment for malaria and psoriasis, a tissue dye, an antidote for cyanide poisoning, and as a treatment for fungal infections in fish.[15] In biology, it is used to stain cells for viewing in a microscope, and as an indicator of anaerobic respiration. Methylene blue has the chemical formula $C_{16}H_{18}N_3SCl$ in a chain of three rings. In an oxidizing environment, such as in the presence of oxygen, the compound is blue. In a reducing environment, the compound turns clear. When methylene blue is oxidized, and blue, it will be attracted to negatively charged nucleic acids, and will bind to them. So the nucleus of the cell will appear as a very dark blue color.[16]

Plant Pigments

Color in plants and algae is the result of pigment molecules which can be found in the cell walls, in solution in the cytoplasm and in the vacuole, and in plastids, such as chloroplasts and chromoplasts. The main types of pigments are the chlorophylls, carotenoids, and flavonoids. All of these molecules have structural features--central transition metal atoms, or carbon rings or chains with alternating single and double bonds—which make electrons available that can easily absorb light of various wavelengths, resulting in various colors. Some of these pigments are able to convert light energy into chemical energy—the basis for photosynthesis.

Chlorophyll is found in the chloroplasts of all plants and in algae. It has, at its core, a magnesium atom, linked to four nitrogen atoms, and surrounded by rings of carbons with alternating double and single bonds. This arrangement results in electrons being available that require very little energy to be boosted to higher energy levels.[17] Chlorophyll absorbs red and blue wavelengths of light best. Green wavelengths of light are reflected, so the molecule appears green. Two forms of chlorophyll—chlorophyll *a* and chlorophyll *b*—are the result of slight variations in the structure. Chlorophyll *a* appears yellowish-green, chlorophyll *b*, bluish-green—again, due to the slight differences in the electron configuration and what specific wavelengths of light they absorb. The color of particular types of leaves is due to the proportions of chlorophyll *a* and *b* that are present in the chloroplasts. Chlorophyll does much more than reflect green light—the light energy absorbed by these pigments is converted into chemical energy during the light reactions of photosynthesis, which is then used to produce sugars in the carbon-fixing Calvin cycle.

Carotenoids are a group of organic pigments composed of long carbon chains with alternating single and double bonds. The two main types of carotenoids are carotenes and xanthophylls, which may be found in fruits, flowers and leaves. Lycopene is a red carotenoid that gives tomatoes their color. Carotenes appear yellow or orange and are found in carrots, pumpkins, and marigolds. Xanthophyll is a yellow pigment. In leaves, both act as accessory pigments to chlorophyll. In the thylakoid membranes of chloroplasts, carotenoids, along with chlorophyll molecules are found in clusters called photosystems. When these pigments absorb a photon of light, they funnel the energy to a central chlorophyll-*a* molecule—the reaction center. Electrons in chlorophyll-*a* are boosted to higher energy levels and are donated to other molecules in the membrane which create an electron transport chain. These electrons are ultimately used in the dark reactions to reduce carbon dioxide to form sugar. The color of the carotenoids is masked by the presence of chlorophyll, until cooler temperatures and shorter days trigger a reduction in the production of chlorophyll, and its subsequent decay—revealing the carotenoids that were there all along, and which are slower to disappear.

Flavonoids are found in flowers, fruits, stems and leaves. Their role in flowers and fruits appears to be simply to provide color to attract pollinators and seed dispersing animals. The basic structure of flavonoids begins with three carbon rings called flavone, with varying side branches that affect what colors are absorbed or reflected. Color can also be affected by pH. Anthocyanins have a blue color in alkaline conditions, but appear purple or red with increasing acidity. Anthoxanthins are yellow in alkaline conditions, and turn cream as pH decreases.[18] Plants containing these pigments may produce flowers of varying colors depending on the soil pH. Hydrangea blossoms appear blue in alkaline soil, but pink in more acidic soil. The juices of red cabbage can change from red to purple to blue to green due to changes in both the anthocyanins and anthoxanthins as pH increases.[19]

Leaves that have a lot of anthocyanin appear red, masking the chlorophyll. The red pigment may provide protection from ultraviolet radiation for plants in tropical canopies and at high altitudes.[20] Leaves that are red or purple on their lower surface are often found growing in the understory where light is very dim. Most of the useful blue and red light gets absorbed by the leaves of the canopy. Anthocyanin in the lower epidermis may reflect the small amount of red light that gets through, back up through the mesophyll cells of the leaf where chlorophyll will have a second chance to absorb it.[21]

Leaves have other mechanisms for dealing with various levels of light, which can affect their color. Plants that grow in intense light will often have shiny surfaces which reflect the light and whose waxy coating reduces water loss. They may have hairs that scatter the light rays and slow water loss, or red pigments that act as sunscreens. Plants that grow in low light levels may he bumpy or have a hairy surface, so less light is reflected from their surfaces.[22]

Some plants have variegated leaves where pigments are not evenly distributed due to lack of chlorophyll in spots, air pockets under the epidermis, or concentrations of pigments such as anthocyanins in the epidermal cells. The effect may be the result of mutations, viruses, leaf damage, or certain environmental conditions. Variegation that is inherited must have been subjected to natural selection. The costs of reduced photosynthesis must have been offset by some advantage. Perhaps the leaves' mottled appearance helped them blend in to a sun-dappled forest floor, decreasing the chance that herbivores would spot them. Or maybe the variegation made the leaves appear to herbivores as they would if diseased or insect-damaged, and less appealing as a food source, and so they were avoided.[23]

Strategies

Scientific Color

Color will be highlighted throughout the year as a theme that ties all the topics covered in biology together. The first unit of the year is always on the scientific method, so to introduce the color theme, students will begin with a simple experiment to determine if there are sexual preferences for color. This activity will be based on a study of sex differences in color preference by Anya C. Hurlbert and Yazhu Ling.[24] Students will rapidly select their preferred color choice from a series of pairs of colored rectangles.

Hue preferences for males and females will be averaged and graphed. Students will then compare their choices to the data from the report by Hurlbert and Ling. This experiment will replace the basic "Do seeds need light to Germinate?" experiment I have used in the past to introduce students to the steps of the scientific method. Everyone has their favorite colors, and students are always interested in the differences between boys and girls. This experiment will grab their attention much more than the seeds and still meet the requirements for teaching the scientific method. (See Appendix, Activity 1)

Although high school students should be familiar with the metric system, I find they often need a reminder, and so I have them practice measuring using the metric system. In this acid-base activity students must accurately measure and mix solutions of various hydrochloric acid concentrations with blue food dye in order to end up with an array of test tubes in the colors of the rainbow. Too often students will just eyeball what they are measuring and fail to see the need for accuracy in their work in the lab. If they aren't careful here, the rainbow will not appear. A kit for this activity—called "Rainbow out of the Blue"--is available from Flinn Scientific.[25]

Color Chemistry

As the properties of water are discussed in the unit on biochemistry, I will include a simple activity showing how colored water moves through a flower—illustrating the capillary action of water as it is transported through xylem cells and the process of transpiration in plants. The scientific method will be reinforced with this activity by having students identify and test variables—light, temperature, wind, etc.-- that might affect the rate at which the colored water moves up the stem of the flower.

Students will also learn how color can be used to identify organic compounds using color-changing chemical indicators such as iodine, Benedict's solution, and Biuret to detect the presence of starch, sugar, and protein in a lab called McMush. This lab was published in *American Biology Teacher* in 1994 and was written by Judy Brown.[26] A typical fast food kid's meal of a hamburger, fries and cola is put in a blender and students then test a sample using the Benedict's solution, Biuret, and Lugol's solution. Positive and negative controls are used for comparison. With this activity, students will begin to see how color may be used as a tool in science. I have not had good success with the fat test, so I obtained nutrition information posters from McDonald's showing the total fat content of all their menu items. I have students predict which part of the Happy Meal will have the most fat, and then compare their prediction to the information on the nutrition poster.

Color in Cells

In the cell biology unit, students will observe cells under the microscope and look for color. They will observe cheek cells (not much color there!), red pepper, potato, flowers, the leaves of a water plant called Anacharis (*Elodea canadensis)*, and red cabbage—to try to determine where the color lies in the cells. They will observe that without staining, the cheek cells and potato cells do not have very much color. Staining with methylene blue and iodine, respectively, brings out the details in the cells when the stains selectively bind to certain parts of the cells. This makes it possible to distinguish details in cells without much color. On the other hand, some features are easily observed because they are already pigmented. The red pepper has chromoplasts containing red pigments, and the *Elodea* has relatively large, green chloroplasts. These cells' color is not throughout the cell, but only in small membrane-bound organelles.

Color and Energy

During the unit on cell energy, students will observe the pigments that plants use for photosynthesis and the pigments in the red cabbage, by using paper chromatography. Paper chromatography is a method of separating molecules by solubility, size and their attraction to the paper. Magnolia leaves work very well for this, and are available even during the winter when other leaves are gone. Fresh spinach leaves would also work well. If I get to this unit when the leaves are changing colors, students will run the chromatography on green and colored leaves and compare the pigments they find. They will see that the green leaves also contain carotenes and xanthophylls in addition to the chlorophyll. Doing this lab in conjunction with photosynthesis helps students connect the very abstract concept of photosynthesis to a living plant and the pigments involved in the process. Directions for this lab can be found in my curriculum unit, "Modeling Photosynthesis" at http://www.teachers.yale.edu/curriculum/search/viewer.php?skin=h&id=initiative_07.05.08_u .

I'm So Blue Over CO_2

Bromothymol blue (BTB) will be used in two experiments. In the first experiment, students will observe the change in color of BTB from yellow to blue as plants use carbon dioxide for photosynthesis. In the second experiment, students will observe the color change as plants in the dark carry out only respiration and the blue BTB changes back to yellow as the plants give off carbon dioxide. Students often have the mistaken idea that only animals do respiration and plants only do photosynthesis. With these two labs, they get the idea that plants actually carry out both processes. The reaction of BTB and the carbonic acid created when carbon dioxide is dissolved in the water, gives me a good chance to review pH and acidity, and use that to explain why the BTB changes color. Students can measure the pH changes with pH paper or a pH meter to confirm the results. I have included the lab handouts for these experiments in the Appendix. They are my adaptations of labs described in *Biological Inquiries.*[27] (See Appendix, Activity 3 and 4)

Methylene blue will be used in an experiment in which bacteria feeding on milk sugars in a sealed test tube that also contains methylene blue, use up the oxygen, causing the methylene blue to turn clear. Students will observe that bacteria also carry out aerobic respiration. There are several versions of this lab online. One good one is at http://www.uen.org/Lessonplan/preview.cgi?LPid=2516# . In this version, students compare the results from pasteurized and reconstituted dry milk. Ultra High temperature (UHT) milk could also be compared. The length of time it takes for the methylene blue to turn clear, is indicative of lower populations of aerobic bacteria present in the milk. My version of this activity is included in the Appendix. (See Appendix, Activity 5)

"Code o' Chrom…"

During the DNA and genetics units, students will construct color-coded paper models of DNA and analyze colored DNA chromatograms. The model pieces will use the same color code used for the base pairs in the chromatograms: red for thymine, green for adenine, black for guanine, and blue for cytosine. Students will analyze a color coded DNA chromatogram to determine the sequence of a segment of DNA. The model directions and templates are from *Recombinant DNA and Biotechnology—A Guide for Teachers*.[28] This is a wonderful resource for DNA and biotechnology activities.

Using a kit from Neo Sci—"Turning Genes On and Off"—students will investigate the effects of temperature on genes that control the color of the bacterium *Serratia marescens*.[29] This bacterium produces a red pigment, prodigiosin, at certain temperatures, but not at others. Students will culture the bacteria at different temperatures and observe the effects on the amount of pigment produced by the bacteria. It is important for students to understand the connection between genes and pigments, so that when we discuss the evolution of color in plants and animals, they will see the underlying mechanism behind evolution—the effects of natural selection on the frequency of genes.

The inheritance of eye color and colorblindness will be discussed in the unit on genetics. Students will use Punnett squares to predict the probability of inheritance of these traits. They will check their own color vision using a sample of images from the Ishihara color blindness test at http://colorvisiontesting.com/ishihara.htm .

Color Evolution

During the evolution unit, students will investigate the evolution of color in animals—the peppered moth, camouflage, mimicry, aposematic coloration—and how plants co-evolved with insects and other animals to produce brightly colored flowers and fruits. Students will play a natural selection game that shows students how camouflage is an adaptive feature. (See Appendix, Activity 2) Students will play the part of a predator searching for insects and select from pictures of insects with varying colors placed on a background of rainforest colors. They will simulate a change in the environment, by switching the background to a less colorful, drabber background, and go through the selection process again. Students will then calculate and graph the changes in the frequencies of each color. In a second variation, students will select pictures of butterflies which vary in their similarity to a more unpalatable species, representing Batesian mimicry. A great source for insect and animal images is a website called "The Coloring Spot" at http://thecoloringspot.com/. They have very nice line drawings of insects that you can copy and color in various shades to use in the acitivity. Instead of insects you could do this same activity with any of the animals they have—frogs, antelope, or fish. I recommend laminating the pieces once they are colored, so they can be used over and over. I will use either colorful wrapping paper, or fabric for the background. Either of these can be found in a wide variety of "natural" looking patterns and colors to simulate a particular type of environment.

Another good example of how color has evolved in animals is demonstrated in a PBS Nature video on the evolution of the dog—"The Rise of the Dog"—part of the Nature series, "Dogs that Changed the World". The video tells the story of how selective breeding for docile traits in foxes being raised for their fur, led to a change in the coat color after a relatively short time. The foxes became easier to handle over several generations, but their coat color changed and took on the appearance of domesticated dogs, because the genes for behavior and coat color were linked. This was not exactly what the furriers wanted, but is a great example of how selection for one trait (in this case, artificial selection) can also result in selection for other linked genes. This video is online at the Nature website: http://www.pbs.org/wnet/nature/lessons/from-wolf-to-dog/video-segments-dogs-that-changed-the-world/4800/, under the title, "From Wolf to Dog". A video guide is included in the Appendix. (See Appendix, Activity 6)

Throughout the semester, students will keep a running color story board. At the beginning of the semester, they will create an accordion foldable made from different colored paper. An example of this kind of foldable can be seen at http://www.catawba.k12.nc.us/c_i_resources/Accordion.pdf . It folds up easily for storage when not in use, but can hold lots of information. As we go through the curriculum, students will add notes, pictures, and color connections to their rainbow foldable about topics covered that deal with each color. For example, on their green page they might add things about photosynthesis, ecology, and maybe a camouflaged insect hiding on the page. On the purple page they might include a diagram of iodine forming a purplish-black color as the iodine diffuses into a dialysis tube containing starch. A drawing of a purple flower with the parts labeled, and a bee pollinating the flower, attracted to its bright color, might also appear on the purple page. In addition, as ideas from different units are added to the pages, students will be asked to link the ideas with labeled arrows—like a concept map—in order to make connections between the various concepts taught throughout the course. At the end of the semester, they should have a unique tool to use as we review for the final exam.

Notes

[1] Hazel Rosotti, *Why the World Isn't Grey* (Princeton: Princeton University Press, 1983), 20-21.

[2] Penelope A. Farrant, *Color in Nature—A Visual and Scientific Exploration (*London: Blandford, 1997), 2.

[3]Marco Ferrari, *Colors For Survival—Mimicry and Camouflage in Nature* (New York: Barnes and Noble Books, 1997), 20.

[4]Rossotti, *Why the World Isn't Grey*, 22.

[5]Farrant, *Color in Nature*, 3.

[6]Ibid., 4.

[7]Rossotti, *Color in Nature,* 39-40.

[8]Farrant, *Color in Nature*, 5.

[9]AP Biology, "Chapter5 Macromolecules Lab" http://74.125.47.132/search?q=cache:fciKsk3ajccJ:apbio.savithasastry.com/Units/Unit%25201/labs/macromol_lab08.doc+benedict%27s+test&cd=45&hl=en&ct=clnk&gl=us

[10] Wikipedia, "Starch", http://en.wikipedia.org/wiki/Starch.

[11]Elmhurst College, Virtual Chembook, "Starch-Iodine" http://www.elmhurst.edu/~chm/vchembook/548starchiodine.html

[12]Wikipedia, "Biuret Reagent", http://en.wikipedia.org/wiki/Biuret_reagent

[13] R. Van Lanen, "Simple Tests for Amino Acids and Proteins", http://chemistry.olivet.edu/classes/chem100/pdf/Labs/Simple%20Color%20Tests%20for%20Amino%20Acids%20and%20Proteins%20Lab.pdf

[14]Absolute Astronomy.com, "Bromothymol Blue", http://www.absoluteastronomy.com/topics/Bromothymol_blue#encyclopedia

[15]Wikipedia, "Methylene Blue", http://en.wikipedia.org/wiki/Methylene_blue.

[16] American Chemistry, "Methylene Blue, Part 1: The Biologist's Dye"
http://www.americanchemistry.com/s_chlorine/science_sec.asp?CID=1252&DID=4730&CTYPEID=113

[17] Farrant, *Color in Nature*, 73.

[18]Rossotti, *Why the World Isn't Grey*, 88.

[19]Ibid., 88-89.

[20]Farrant, *Color in Nature,* 77.

[21]Ibid., 82.

[22]Ibid., 55.

[23]Ibid., 76.

[24]Anya C. Hurlbert and Yazhu Ling, "Biological components of sex differences in color preference," Current Biology 17, no.16 (2007): R623-R625.

[25] Flinn Scientific, "Rainbow out of the blue",
http://www.flinnsci.com/store/Scripts/prodView.asp?idproduct=15609&noList=1

[26] Judy Brown , "McMush," *American Biology Teacher* 56, no.8 p492-95 (Nov-Dec 1994).

[27] Martin Shields, *Biological Inquiries:Standards-Based Labs, Assessments and Discussion lessons* (New York: John Wiley and Sons, Inc, 2006).

[28]Helen Kreuzer and Adrienne Massey, *Recombinant DNA and Biotechnology—A Guide for Teachers, 2nd ed.,* (Washington, D.C.: ASM Press, 2001).

[29]Neo Sci, "Turning Genes On and Off Lab Investigation",
http://www.neosci.com/catalog.asp?sid=411050405&showID=131&content=cn_showitem

Bibliography

Absolute Astronomy.com. “Bromothymol Blue.” http://www.absoluteastronomy.com/topics/Bromothymol_blue#encyclopedia (accessed September 5, 2009).

American Chemistry. “Methylene Blue, Part 1: The Biologist's Dye.“ http://www.americanchemistry.com/s_chlorine/science_sec.asp?CID=1252&DID=4730&CTYPEID=113 (accessed September 5, 2009).

AP Biology. “Chapter5 Macromolecules Lab.” http://74.125.47.132/search?q=cache:fciKsk3ajccJ:apbio.savithasastry.com/Units/Unit%25201/labs/macromol_lab08.doc+benedict%27s+test&cd=45&hl=en&ct=clnk&gl=us (accessed September 5, 2009).

Brown, Judy. “McMush.” *American Biology Teacher* 56, no.8 p492-95 (Nov-Dec 1994). Identification of organic compounds in a Happy Meal.

Caro, Tim. *Antipredator Defenses in Birds and Mammals.* Chicago: University of Chicago Press, 2005. Great book on predator-prey relationships and the adaptations prey species have evolved to survive predation.

Elmhurst College, Virtual Chembook. “Starch-Iodine” http://www.elmhurst.edu/~chm/vchembook/548starchiodine.html (accessed September 6, 2009).

Farrant, Penelope A. *Color in Nature—A Visual and Scientific Exploration.* London: Blandford, 1997. Fantastic book that contains amazing pictures and really good scientific explanations.

Ferrari, Marco. *Colors For Survival—Mimicry and Camouflage in Nature.* New York: Barnes and Noble Books, 1997. A scientific picture book–the best of both worlds. I’ve never such good examples of camouflage and mimicry.

Flinn Scientific. “Rainbow out of the blue.” http://www.flinnsci.com/store/Scripts/prodView.asp?idproduct=15609&noList=1 (accessed November 15, 2009). They used to give out the directions for this lab free as Flinn Fax. Now they sell it as a kit.

Hurlbert, Anya C., and Yazhu Ling. “Biological components of sex differences in color preference.” Current Biology 17, no.16 (2007): R623-R625.

Kreuzer, Helen, and Adrienne Massey. *Recombinant DNA and Biotechnology—A Guide for Teachers, 2nd ed.* Washington, D.C.: ASM Press, 2001. A great resource to have for teaching DNA and biotechnology. Filled with teacher friendly information and activities.

Lanen, R. Van. “Simple Tests for Amino Acids and Proteins.” http://chemistry.olivet.edu/classes/chem100/pdf/Labs/Simple%20Color%20Tests%20for%20Amino%20Acids%20and%20Proteins%20Lab.pdf (accessed September 5, 2009).

Neo Sci. “Turning Genes On and Off Lab Investigation.” http://www.neosci.com/catalog.asp?sid=411050405&showID=131&content=cn_showitem (accessed November 15, 2009). Source for the lab kit described in my lesson plans.

Rosotti, Hazel. *Why the World Isn’t Grey*. Princeton: Princeton University Press, 1983. Really good explanations for all things that deal with color.

Shields, Martin. *Biological Inquiries:Standards-Based Labs, Assessments and Discussionlessons.* New York: John Wiley and Sons, Inc, 2006. An excellent resource for biology teachers.

Wikipedia. “Biuret Reagent.” http://en.wikipedia.org/wiki/Biuret_reagent (accessed September 5, 2009).

Wikipedia. “Methylene Blue.” http://en.wikipedia.org/wiki/Methylene_blue (accessed September 6, 2009).

Wikipedia. “Starch.” http://en.wikipedia.org/wiki/Starch.(accessed September 5, 2009).

Websites for Teachers and Students

http://thecoloringspot.com –insect and other animal coloring pages

http://colorvisiontesting.com/ishihara.htm --online Ishihara color-blind test sampler.

http://www.athro.com/evo/gen/genefr2.html --Inheriting Eye Color: an interactive site for eye color inheritance involving 2 genes and 3 alleles—brown, green , blue.

http://www.uen.org/Lessonplan/preview.cgi?LPid=2516# --site for the milk and methylene blue lab

http://www.pbs.org/wnet/nature/lessons/from-wolf-to-dog/video-segments-dogs-that-changed-the-world/4800/. --*Nature* website with video of “The Rise of the Dog”.

http://www.catawba.k12.nc.us/c_i_resources/Accordion.pdf --show examples of accordion style foldables.

Appendixes

Activity 1—Sex differences in color preferences

Why do little girls seem to like the color pink, while little boys seem to hate it? Do boys hate it simply because it is often associated with girls, or are there basic differences in what colors are preferred by males and females? Color vision is perceived by three kinds of cone cells in the retina of our eyes. Each type is sensitive to different wavelengths of visible light—blue, red or green. The colors we see are the result of stimulation of different combinations of these three types of cone cells. But it is the brain which interprets the signals sent from the cone cells. It is the brain where associations between the color of an object, our past experiences and perhaps some innate responses to that color are made. Do the brains of male and females work differently? Do they respond differently to certain colors?

Problem: Do males and females have different preferences for certain colors?

Hypothesis: If males and females are shown different colors to compare and asked to select which color they prefer, then____
____

Independent variable:____
Dependent variable:____
Constants:____
____
____

Materials: Set of 7 color cards in 7 different hues
Procedure: Each person will be presented with a yellow card and an orange card and must choose which one they prefer. The yellow is then presented with each of the other colors and the preference is recorded on the data sheet. Orange is then presented for comparison to the other color cards in the same manner, and the preferences recorded.

Color preference data for __________________________ Gender: male/female

	Yellow	Orange	Red	Purple	Blue	Turquoise	Green
Yellow	----------						
Orange	----------	----------					
Red	----------	----------	----------				
Purple	----------	----------	----------	----------			
Blue	----------	----------	----------	----------	----------		
Turquoise	----------	----------	----------	----------	----------	-----------	
Green	----------	----------	----------	----------	----------	----------	----------

Tally the number of times each color won:

Yellow:____ Red:____ Blue:____ Green:_____

Orange:____ Purple:____ Turquoise:____

Repeat this for each individual to be tested.

Now rank the colors by preference. (If any colors tied, rank the one higher that was preferred when the two were compared together.):

Female Color Rankings: (1=highest; 7=lowest preference)

Name:	1	2	3	4	5	6	7
"Maria"	T	G	Y	O	B	P	R

Male Color Rankings:

Name:	1	2	3	4	5	6	7

To find the average rankings for each gender, add up the place rankings for all females and then for all males: (Example: If yellow was ranked 4 by Maria, 2 by Flo, and 7 by Keyana, then Y=13/3=4.3)

	Females	Average Ranking:		Males	Average Ranking:
Yellow:	_______	_______	Yellow:	_______	_______
Orange:	_______	_______	Orange:	_______	_______
Red:	_______	_______	Red:	_______	_______
Purple:	_______	_______	Purple:	_______	_______
Blue:	_______	_______	Blue:	_______	_______
Turquoise:	_______	_______	Turquoise:	_______	_______

Green: ______ ______ Green: ______ ______

Graph the average rankings for males and females for the hue values of the colors.

Conclusion:

1. What do the results tell us about color preferences for males and females? Are there any gender differences in those preferences?

2. What might be a reason for the preferences if there are any, or if there are no significant preferences for all members of a group, what might account for that?

3. What might be some possible sources of error or bias with this test? How might the procedure be changed to reduce these sources of error?

4. In an experiment done by Anya Hurlbert and Yazhu Ling, British females, aged 20-26 preferred colors in the reddish-purple region, and showed a sharp decline in preference for colors in the greenish-yellow region. Males in the study preferred blue-green colors, but without any large declines in preference for other colors. How do our class results compare to this study?

Activity 2--Colorful Adaptations

Some animals blend in so well with their environment that if you were looking right at them, you might not be able to see them. Their color and form are perfectly adapted for the environment they live in. This camouflage helps them hide from predators, or, from their prey!
List some animals that use camouflage as a defense: ______________________________
____

But, how did they get that way? How does evolution work to change the color of a population? Remember Darwin's four main points:

1. There is variation in a population.
2. More offspring are born than will survive.
3. There is competition for limited resources.
4. Those with favorable variations will get the resources, survive, reproduce, and pass on those favorable variations to their offspring.

Materials: 20 Paper insects of various colors plus extras for offspring
Rainforest-colored background

Procedure: Spread out your population of insects across the background. Record the number of each color in your data table. You and your partners will take turns being a predator that eats these insects. To do this, you will turn away from the background, then spin around and pick up one of the insects very quickly. The next person does the same, and you continue to take turns selecting insects until you have “eaten” ten. For each insect that survived your attack, add one insect of the same color to simulate reproduction.

Record the colors of all the surviving insects and their offspring.

Repeat the procedure two more times.

Data: Surviving Insects Over Time

Color	Generation 1	Generation 2	Generation 3	Generation 4	% Change

Calculate the % change in the number of each color variation:

$$\% \text{ change} = [(\text{Final} - \text{Initial}) \div \text{Initial}] \times 100$$

Repeat this experiment with a different background:

Data: Surviving Insects Over Time

Color	Generation 1	Generation 2	Generation 3	Generation 4	% Change

Calculate the % change in the number of each color variation:

$$\% \text{ change} = [(\text{Final} - \text{Initial}) \div \text{Initial}] \times 100$$

Use the data to visually represent the changes from the first generation to the last generation in both environments, with a graph.

Conclusion: Write a paragraph summarizing what the data from this simulation tells us about how camouflage may have evolved. Consider the following questions in your response, along with any other observations you made.
Did this population of insects change over time? What caused the change? What colors were favorable in each environment? Why would the outcomes be different if the population you began with was the same each time? In which environment was the change greater?

Evaluation: Discuss how this simulation was and was not effective at demonstrating natural selection. What are some of its limitations?

Activity 3

Do Plants Use Carbon Dioxide? (adapted from an activity in Biology Inquiries by Martin Shields)

WHAT DO **YOU** THINK?
What do plants need to live? ____
What do they need to do photosynthesis? ________________________________
Do plants consume CO_2 or release it when they are in the light, photosynthesizing?

BACKGROUND INFORMATION—Just so you'll know:
Bromothymol blue (BTB) is an indicator that turns from blue to yellow in the presence of CO_2. It will turn back to blue if the CO2 is taken away.

What do you think would happen to the BTB if you blew into it? ____________________
Why?____

1. Put on your goggles! Set up 2 flasks. Add 100 ml of water to two 150ml flasks. Add 21 drops of BTB to both flasks. Using a straw, blow gently into both flasks until they turn yellow. **Be careful—don't blow too hard!**

What did you add to the flasks by blowing into them? ______________________

2. The teacher will set up 2 flasks with BTB, blow into them until they turn yellow, and leave one in the light and cover one with foil. (No plant will be added.) Do you think the BTB in either flask will change back to blue? GIVE A REASON!

____

3. Put a piece of Elodea into both of your flasks. Leave one flask in the light. Cover the other flask completely with foil so that no light can get into it. Leave for at least 24 hours.

What will it mean if the BTB in the flasks turns back to blue? _________________

What will it mean if the BTB in the flasks stays yellow? _____________________

4. Predict what you think will happen to the BTB in the flask with the Elodea in the light and EXPLAIN WHY: ____

5. Predict what you think will happen to the BTB in the flask with the Elodea in the dark and EXPLAIN WHY: ____

RESULTS!

Flask	Initial Color	Final Color
A. BTB + Light		
B. BTB + Dark		
C. BTB + Light + Plant		
D. BTB + Dark + Plant		

Draw in the plants and use colored pencils to show the before and after results:

BTB + Light + Plant (Before) BTB + Light + Plant (After) BTB + Dark+ Plant (Before) BTB + Dark + Plant (After)

CONCLUSIONS…Hmmm…

6. Did being in the light or the dark change the BTB without a plant back to blue? ______

7. So is the CO_2 you blew into the flask still there? ______________________________

8. Do plants consume CO2 when they are in the light photosynthesizing? How do you know?____

9. Do plants consume CO2 when they are in the dark? How do you know?

Activity 4

Do Plants Do Respiration? (adapted from an activity in Biology Inquiries by Martin Shields)

WHAT DO **YOU** THINK?

What do plants do with the food they make? ______________________________________

When do plants do photosynthesis? _________________When can they not do photosynthesis? ___________

When would they be able to do respiration? ______________________________________

When would they be doing only respiration? _____________________________________

BACKGROUND INFORMATION—Just so you'll know:

Bromothymol blue (BTB) is an indicator that turns from blue to yellow in the presence of CO_2. It will turn back to blue if the CO_2 is taken away.

PROCEDURE:

1. The teacher will set up 2 flasks with blue BTB, and leave one in the light and cover one with foil. (No plant will be added.) Do you think the BTB in either flask will change to yellow? GIVE A REASON!

____

2. Put on your goggles! Set up 2 flasks. Add 100 ml of water to two 150ml flasks. Add 21 drops of BTB to both flasks. (No blowing this time!)

3. Put a piece of Elodea into both of your flasks. Leave one flask in the light. Cover the other flask completely with foil so that no light can get into it. Leave for at least 24 hours.

What will it mean if the BTB in the flasks turns yellow? ____________________

What will it mean if the BTB in the flasks stays blue? _______________________

4. Predict what you think will happen to the color of the BTB in the flask with the Elodea in the light and EXPLAIN WHY: ____

5. Predict what you think will happen to the color of the BTB in the flask with the Elodea in the dark and EXPLAIN WHY: ____

RESULTS!

Flask	Initial Color	Final Color
A. BTB + Light		
B. BTB + Dark		
C. BTB + Light + Plant		
D. BTB + Dark + Plant		

Draw in the plants and use colored pencils to show the before and after results:

BTB + Light + Plant	BTB + Light + Plant	BTB + Dark+ Plant	BTB + Dark + Plant
(Before)	(After)	(Before)	(After)

CONCLUSIONS…Hmmm…

6. Did being in the light or the dark change the BTB without a plant to yellow? ________ So was any CO_2 added to that flask while it sat in the light or in the dark? ____________

7. From your results, do plants release CO_2 when they are in the dark and respiring? ________How do you know? ____

9. If plants consume CO_2 during photosynthesis and release it during respiration, then explain the results for the flask in the light. are the plants doing more photosynthesis or more respiration? How do you know? ____

Activity 5

Bacteria…Got Milk?! (adapted form an activity presented at Biology Alliance)

Our milk today is pasteurized, thanks to a process developed for other foods by Louis Pasteur. Pasteurization kills most bacteria that cause disease and cause milk to spoil when the milk is heated up to 161degrees Fahrenheit for 15 seconds. Although this process kills most of the bacteria, some microbes may survive, or some may be reintroduced during handling and processing of the milk.

Bacteria feed on the milk sugar, lactose--breaking it down using oxygen in aerobic respiration:

SUGAR + OXYGEN → CARBON DIOXIDE +WATER +ENERGY

Methylene blue (MB) changes from blue to clear when no oxygen is present—weird, huh?

PROCEDURE:

1. To prove that there are bacteria in fresh milk, your teacher has applied a small sample of pasteurized milk to an agar plate and incubated it over night to allow any bacteria present to grow. Were there bacteria in the milk? ______________________

2. Now fill a test tube with milk. Add one drop of Methylene blue to the test tube and close the test tube with a stopper to prevent any additional oxygen from getting in.

What is in the test tube at the start of this experiment?

____
____
____
____

3. What do you predict will happen to the color if the bacteria use up all the oxygen?

____

4. The control group will be a stoppered test tube with water (no bacteria) and 1 drop of Methylene blue. What do you predict will happen to the Methylene blue in this test tube?

____

Why? ____

The test tubes will be left out at room temperature and the color observed each day.

RESULTS!

Test Tube	Initial Color	Final Color
A. MB + WATER		
B. MB + MILK+ BACTERIA		

CONCLUSIONS…Hmmm…

5. Did the MB in the test tube with just water change colors? _______ Why or why not?

____

6. From your results, were there bacteria using oxygen in the milk? __________________

How do you know? ____

7. How many days did it take for the milk bacteria to use up all the oxygen? ___________

8. How do you think lowering the temperature would affect the amount of time it takes for the bacteria to use up all the oxygen doing respiration? ________________________
____

Activity 6

Dogs That Changed the World—the evolution of the dog.

1. Dogs evolved from wolves that were looking for food in ________________________

2. Wolves who had the (shortest / longest) flight distance when humans came around were most likely to get more food.

3. The first proto-dogs were wolves whose brain, teeth, and body were all increasingly smaller because they were getting food from ______________ and ________________.

4. In Siberia, foxes being raised for their fur were aggressive and hard to handle. When scientists selected foxes to breed who were more tolerant of humans, not only did their behavior change, their _________________ changed.

5. Charles Darwin believed that evolutionary changes happened gradually over long periods of time. The fox breeding experiment showed that dogs could have evolved ______________________________

6. Dogs quickly became important to people. In Mexico, hairless dogs were considered sacred. This mutation also caused them to be missing _________________.

7. The hairless dogs are thought to have healing power. How does the woman in the video use the hairless puppies to help her pain?
____

8. If a hairless mother has puppies, are they all hairless? __________________

9. Do wolves bark like dogs? ____

10. Why would people have selected for barking in dogs?_________________________

11. What behavior in wolves is like the guarding behavior in the Dobermans?
____

12. Dogs have at least 2 senses that are much better than in people. They are _______________ and _____________________.

13. When early people spread over the earth, they ________________________________.

14. One scientist collected mitochondrial DNA from dogs all over the world and using that information found that all dogs started from ancestors that came from ______________________________________

15. Three thousand years ago, the Inuit people migrated to the ___________________ with their dogs, who had been used to a much warmer environment.

16. The Inuit use their dogs to ____
____
____

17. Dogs are the best animals at reading human __________________________________

18. Herding dogs can take directions from their owners __________mile away.

19. They use their ________________ instincts to herd the sheep. The sheep recognize the predator-like behavior of the dogs and ________________ together.

20. What are some advantages to the sheep farmer to use the dogs to herd his sheep?
____
____