

Chapter 13

Phenotypic Variation in Plants

Lawrence Blumer

Department of Biology
Morehouse College
830 Westview Dr. SW
Atlanta, Georgia 30314
(404) 658-1142, FAX (404) 522-9564
LBlumer@Morehouse.edu

Larry is Associate Professor of Biology at Morehouse College, where he joined the faculty in 1990. He teaches courses in ecology, environmental biology and introductory biology for non-majors. He received his BGS (1974), MS in Zoology (1978) and Ph.D. in Biological Sciences (1982) from the University of Michigan. His research interests are on behavioral and evolutionary ecology of animals, particularly the reproductive social behavior of fishes. This laboratory study was developed with the support of NSF grant DUE-9250962 in the Instrumentation and Laboratory Improvement Program.

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Introduction

The laboratory study presented here was developed for an upper-level undergraduate ecology laboratory course, and has been used in that course, each semester, for the past three academic years. It has been my experience, that students have difficulty with the concept that the variation we observe in nature can have two separate causes. Understanding that both genetic and environmental variation can cause differences between organisms is essential for understanding the relationship between ecology and evolutionary processes. The goal of this laboratory study is to permit students to identify the causes for variation in a plant species, and to see for themselves that both environmental differences and genotypic differences can be the source for phenotypic differences.

This study focuses on a mustard plant, *Brassica rapa*, in which distinct, genetically different varieties are available, but it could be adapted for experiments with animals (insects) or bacteria that can easily be manipulated in a laboratory. Students perform an experiment to determine the causes for phenotypic differences between varieties of *Brassica rapa* that would be observed in these plants in nature. Students raise three different varieties at two temperatures, and under two light intensities under controlled environmental conditions. Height, weight (and other physical measures), and concentrations of chlorophylls and anthocyanins are measured on each plant variety from each treatment after as little as two weeks growth. We find that the characteristics of a given variety of plant change with light intensity and temperature which indicates that environmental differences can cause phenotypic variation. However, significant differences between plant varieties are observed under each environmental condition suggesting genetic differences are responsible for much of the observed phenotypic variation. This hypothesis can be tested indirectly by performing electrophoresis on protein extracts from each plant variety grown under the same conditions. Students observe that both environmental and genetic differences can cause variation in plant characteristics, and these causes for variation can be separated from each other experimentally.

The Student Outline is written with the assumption that the laboratory period is three hours. Data collection can occur during a three-hour period, but the data analysis and the optional protein electrophoresis require an additional laboratory period. I have students start the seeds for this study two weeks prior to the data collection. Preparing the materials and sowing the seeds takes one hour. The data analyses I suggest students perform are most easily conducted with a computer statistical analysis program such as StatView (see Appendix B). This program is available in both Macintosh and Windows (DOS) formats, and is very easy to use. The chi-square tests (for categorical variables) and analysis of variance tests (for continuous variables) are not described here. An excellent description of these tests can be found in Brown and Downhower (1988). The Brown and Downhower (1988) text

is out-of-print, but their Appendix on Analysis of Data could be reprinted with permission of the publisher (Sinauer Associates) for a nominal fee.

This study could be made shorter (and less demanding) by eliminating the sections on chlorophyll concentration and protein electrophoresis, and conducting only the anthocyanin analysis and collecting morphological-structural data. Collection of just the morphological-structural data would make this study appropriate for lower-level undergraduates in an introductory course or in a non-majors course.

Materials

Seeds of three varieties of *Brassica rapa* (40 seeds of each variety/student group)
 Quads (polystyrene cubes with four cells) (15/student group)
 Quad wicks (60/student group), soil (seed starter type) to fill quads
 Plant growth chambers, environmental incubators, or light banks on tables
 (four conditions needed): 15° C (cold room) and 25° C with full light (~150 $\mu\text{E}/\text{m}^2/\text{sec}$),
 and full-light and half-light (~75 $\mu\text{E}/\text{m}^2/\text{sec}$) at 25° C
 Thermometers (4), quantum radiometer (optional)
 1% HCl in methanol (100 ml/student group), or acetone (100 ml/student group), glass waste
 bottles for solvents
 Mortar and pestle (glass, 4 oz) (one/student group), clean sand
 Quartz and polycarbonate cuvettes, spectrophotometers (2 or more for a class of 16)
 Forceps, small sharp scissors, and mm rulers (2 of each/student group)
 Glass test tubes (10 ml), green rubber stoppers (#000) (12/student group) and test tube rack
 Syringes (3 cc) (non-sterile luer lok) and syringe filter disks (25 mm 0.2 μm non-sterile
 nylon, Nalgene #196-2020) (12/student group)
 Gloves and safety glasses, tape and marking pens, aluminum foil
 Pasteur pipettes (glass) (40/student group) and rubber bulbs
 Graduated pipettes (5 ml glass) (2/student group) and pipette helper
 Microfuge tubes (1.5 ml) (24/student group) and microfuge tube rack
 Microcentrifuge
 Top loading electronic balance (0.01 g accuracy)
 Plastic weighing boats (3" square) (12/student group)

Materials for Protein Electrophoresis

2 \times protein treatment buffer (2 ml/student group)
 (0.125M Tris-Cl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol)
 Vertical electrophoresis chamber and power supply
 Precast polyacrylamide SDS gels
 Dry bath for 1.5 ml microfuge tubes set at 100° C, timer

Notes for the Instructor

There is some risk of this experiment being either too theoretical or appearing to be a cookbook straw man if students are not familiar with the importance of phenotypic variation and heritability in the evolutionary process. Students should not be told about the commercial source of the *Brassica* seeds nor that the varieties are true breeding, since the purpose of this study is to evaluate the nature of phenotypic variation. This is why I use acronyms (TA, TC, SA) for each variety and I provide seeds to students in clear film cans labeled only with the variety acronyms.

Plant growth need not be done in expensive growth chambers, although precise control of environmental conditions is an advantage if "room temperature" in your laboratory fluctuates widely. A

cold room at 15° C and a room temperature area with the same levels of fluorescent lighting (banks of six tubes set 15 cm above the quads), and banks of six fluorescent tubes and three fluorescent tubes both at room temperature should work fine. However, adequate light is essential for the full expression of anthocyanin pigments.

Research grade spectrophotometers (such as the Beckman DU-600 which I use in this study) are not required to perform the pigment concentration analysis, this part of the study could be done with Spectronic 20 units. The pigment extraction and spectrophotometry are not essential for this study and could be eliminated if time is short or spectrophotometers are unavailable.

It is essential that the plants grow at least two weeks and ideally three weeks before the collection of data. The soil must either be regularly watered or a continuous wick system used to keep the soil wet (see Appendix B: Sources of Materials). Should some quad cells fail to yield seedlings one week after sowing, resow seeds in the empty cells. Yes, this will increase the variability among plants within a treatment, but it is important that the students have some materials with which to make measurements.

Details on the preparation of materials and sources are given in Appendix B.

Student Outline

Introduction

Understanding the sources of phenotypic variation in organisms is central to the understanding of natural variation and the responses of organisms to their environment. Variation within a species of plant or animal is very common over a geographic range. Variation in size, shape, coloration, behavior and physiology may be a product of current environmental differences between sites (phenotypic plasticity), a product of heritable differences (genotype differences = ecotypes) between the subpopulations at different sites, or a combination of both. The classical methodology for determining the causes of variation is reciprocal transplants or transplants to a common environment. Transplanting individuals possessing different traits to a constant environment or performing cross transplants between natural sites is a means of evaluating the relative importance of environmental and genetic variation in producing the observed phenotypic variation. The finding of persistent differences between subpopulations independent of environmental conditions suggests that genetic variation underlies observed phenotypic variation. For example, a species of yarrow, *Achillea millefolium*, grows in a wide variety of habitats in California ranging from sea level to more than 3000m elevation. Plants at a given altitude have different height and biomass compared to plants from other altitudes even when seeds of plants from different sites are grown under the same conditions at sea level (Clausen, Keck, and Hiesey, 1948). This result indicates that the observed phenotypic variation among the California *Achillea* is ecotypic, caused by genotypic differences between populations.

In this experiment, phenotypic variation in a vascular plant species will be evaluated for both morphological-structural traits (for example, total biomass, internodal length, and total plant height), and biochemical traits (for example, total chlorophyll and anthocyanin concentrations). We will work with varieties of rapidly growing mustard plants, *Brassica rapa*, (Williams, 1989).

Chlorophylls, of course, are the principal photoreceptor pigments in plants, located in the chloroplasts. We can quantify the concentration of chlorophylls from different samples by evaluating the absorption of light at the specific wave lengths at which peak absorption occurs. The absorption of light increases with pigment sample concentration. Anthocyanins are a class of flavonoids, three ring secondary plant compounds, that produce orange to blue colors in leaves, stems, roots, flower petals, and fruits of many plants (Harborne, 1988). There are more than 260 different anthocyanin compounds and these pigments may serve a wide range of functions such as protecting plant cells from

ultraviolet light, attracting insect pollinators, and acting as anti-herbivore chemical defenses (Harborne, 1988). Anthocyanin concentrations can be quantified in the same manner as the chlorophylls using a spectrophotometer to evaluate light absorption (Harborne, 1973).

Hypotheses and Predictions

H₀: Variation between the plant varieties results from genetic differences between them.

Prediction: A given variety will have a constant phenotype between environments and will remain distinct from other plant varieties.

H₁: Variation between the plant varieties results from environmental differences between the sites where these varieties were growing in nature.

Prediction: Phenotypes will change with environmental conditions.

H₂: Variation between the plant varieties results from both genetic differences and environmental variation.

Prediction: Intermediate results of the two predictions above.

Methods

Growing Plants from Seed

Seeds from three varieties of the mustard plant, *Brassica rapa*, will be used in this experiment. Information about the source habitat for each variety is not available, but the phenotypes of the source plants are known. Variety TA is from plants that are known to grow tall and contain anthocyanin, variety TC is from plants that grow tall and do not contain anthocyanin, and variety SA is from plants that grow short and have anthocyanin pigments. You will sow seeds two or three weeks prior to the collection of data on the plant phenotypes. Seeds will be planted in plastic quads containing four cells each. You and your partner will prepare a total of 15 quads. Place a paper wick in each quad cell (4/quad) and gently pull each wick to ensure that the wick remains sticking out the bottom of the quad when the quad is placed on a flat surface. Fill each quad with prepared soil mixture and wet the soil with water (using spray bottle) until the soil has absorbed water and water drips from the bottom of the quad. Sow two seeds (of a given variety) in each quad cell. You and your partner, will sow seeds in 15 quads, five quads of each of the three varieties of *B. rapa* (Table 1). Place two seeds on the soil surface in a quad cell, press into the soil with the tip of a pencil to just below the soil surface and cover lightly with soil. One quad sown with seed of each variety will be placed in each of five experimental treatments (below) and allowed to germinate and grow.

Temperature Effects: TA, TC, and SA seeds will be grown at 15° C and 25° C with approximately the same light intensities in each of two environmental incubators. The soil in every quad will be kept continually moist by using a self watering wick system. No fertilizer will be used.

Light Intensity Effects: TA, TC, and SA seeds will be grown at full-light and at half-light intensities with the same temperatures, 25° C in each environmental incubator. The soil in every quad will be kept continually moist by using a self watering wick system. No fertilizer will be used.

Protein Polymorphism: TA, TC, and SA seeds will be grown at full-light and 25° C in an environmental incubator for the purpose of preparing gross protein extracts for electrophoretic analysis. The soil in every quad will be kept continually moist by using a self watering wick system. No fertilizer will be used.

Table 13.1. Quad assignments to treatments and varieties.

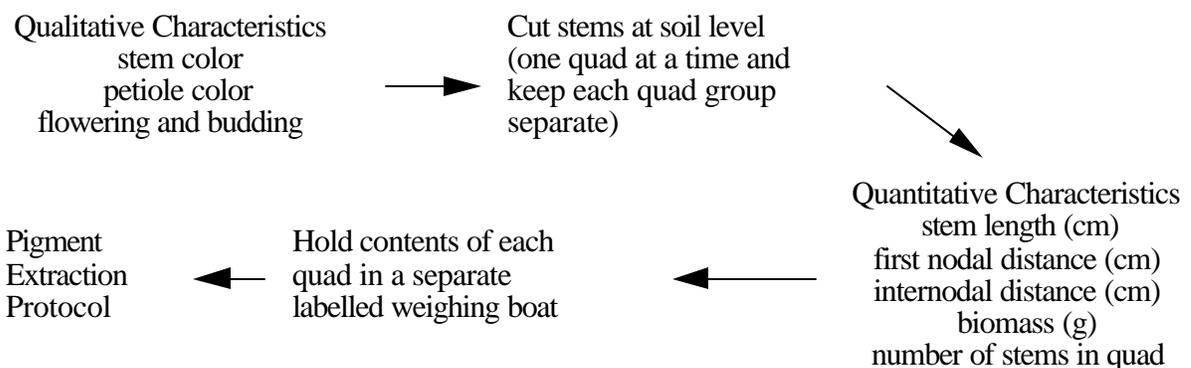
Treatment						
Variety	15 C	25 C	Full-Light	Half-Light	Protein Prep	Totals
TA	1 Quad	1	1	1	1	5
TC	1	1	1	1	1	5
SA	1	1	1	1	1	5
Totals	3	3	3	3	3	15 Quads

One week after sowing the seeds, thin seedlings (cut stem, remove and dispose extra seedlings) leaving one seedling per cell. We will use a quantum radiometer to check the intensity of photosynthetically active radiation (PAR) in each environmental incubator. Record the observed PAR for each treatment ($\mu\text{E}/\text{m}^2/\text{sec}$) and measure and record the temperature in each incubator.

Morphological-Structural Data

The plants grown in the two light treatments and the two temperature treatments will be evaluated for a variety of morphological characteristics. Two or three weeks after sowing the seeds, describe the plants from each variety and treatment qualitatively: stem color (green, purple, white), petiole color (green, purple, white), and flowering and budding (yes or no) (see Figure 1 for protocol flow chart).

Figure 13.1. Protocol flow chart for collection of morphological-structural data. Evaluate the plants from each quad separately.



Working with one quad at a time, cut each plant from one quad at the soil level, then measure stem length (mm) above soil level, distance from soil to first node (mm), and internodal distance (mm between the two nodes closest to the soil) (Figure 2).

Calculate the mean value for each of the measurements for the plants from each quad. Weigh the plants from one quad together. Record the total biomass and the number of plants (normally four) from each quad, so the mean biomass per plant stem can be calculated. A suggested data record format is given below (Table 2a and b). Record data in your laboratory notebook using this format, and later transfer the data to the class computer files (see section on Statistical Data Analysis). Keep the plants

from each quad separate from the plants from other quads. Use labeled plastic weighing boats for the plants from each quad. You and your partner will have one quad of each variety per treatment (four quads of each variety total). You will be informed in class which pigment extraction to perform, half of the class will perform the chlorophyll analysis and the other half will perform the anthocyanin analysis. All four plants from one quad will be used to prepare a single pigment extraction. You and your partner will perform pigment extractions and quantify the pigment concentration from plants of each of the three varieties grown under each of the four environmental conditions (twelve combinations).

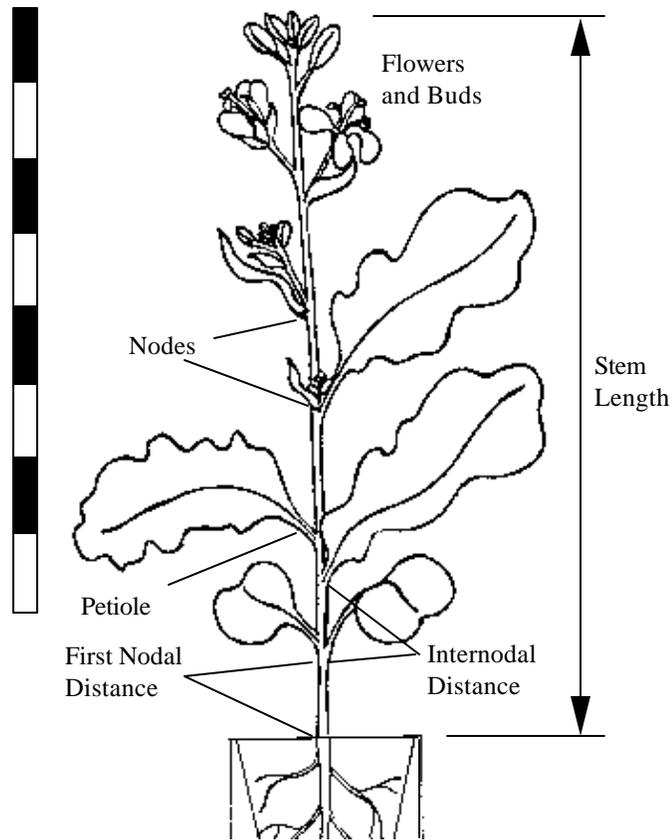


Figure 13.2. Morphology of 13-day old *Brassica rapa* (after Williams, 1989). Note that individual plants may vary from this example. Scale units are cm.

Table 13.2a. Laboratory notebook data recording format for morphological data. The Student Group # will be the same for your entire data set, but that information will be required for the class data file. Note that this is identical to the format that will be used for the class data file (see section on Statistical Data Analysis). An example entry is shown in italics.

Student Group #	Treatment	Variety	Stem Length	First Node	Internodal	Flowering	Stem Color	Petiole Color
<i>1</i>	<i>twenty-five</i>	<i>TA</i>	<i>73</i>	<i>12</i>	<i>17</i>	<i>no</i>	<i>purple</i>	<i>purple</i>

Table 13.2b. Laboratory notebook data recording format for biomass and pigment extraction absorbance data. You and your partner will perform a pigment extraction and collect absorbance data on either anthocyanin (abs. antho. 530) or on chlorophyll (abs. chloro. 415 and abs. chloro. 662). Note that this is identical to the format that will be used for the class data file (see section on Statistical Data Analysis). An example entry is shown in italics.

Student Group #	Treatment	Variety	Total Mass (g)	# of Plants	Abs. Antho. 530 nm	Abs. Chloro. 415 nm	Abs. Chloro. 662 nm
<i>1</i>	<i>twenty-five</i>	<i>TA</i>	<i>0.6</i>	<i>4</i>	<i>0.756</i>		

Pigment Extraction And Analysis

Wear safety glasses and gloves. Perform all acetone extractions in the hood. You will need to know the total biomass of the plants that you use to make each extraction. The following protocol flow chart (Figure 3) summarizes the extraction procedures for both anthocyanins and chlorophylls detailed below.

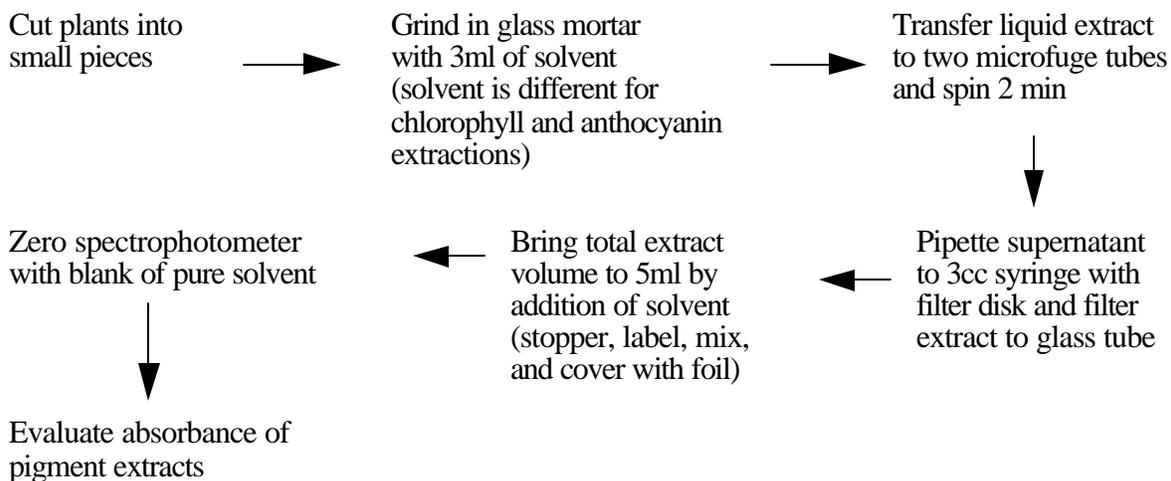


Figure 13.3. Protocol flow chart for pigment extraction and analysis. Prepare a separate pigment extract for plants from each quad.

Anthocyanins: Cut four plants from one quad into small pieces, then grind plant material with a glass mortar and pestle containing a pinch of sand and 3 ml of 1% HCl methanol. Pipette or pour into two microfuge tubes (make sure the tubes are balanced, contain the same volumes, and place them in opposite positions in the centrifuge rotor), and spin 2 minutes. Fit a 3 cc syringe with a filter disk (remove the cap, if present, from the luer-lok end of syringe barrel and twist on the filter disk. Pull the syringe plunger from the barrel and position the end of the filter disk over a small glass test tube. Carefully transfer supernatant (using a clean glass Pasteur pipette) to the 3 cc syringe fitted with the filter disk and force supernatant through filter into the glass test tube. Adjust the total extract volume in the test tube to 5 ml. Stopper the tube, label with tape, mix by shaking, and cover with foil to minimize exposure to light. Rinse the mortar and pestle with water and dry before preparing the next sample of plants. Disposable polycarbonate cuvettes are to be used to evaluate the anthocyanin extractions and methanol blank. Use 1% HCl methanol as the blank in the spectrophotometer. The spectrophotometer program ANTHO will measure absorbance in the visible range at 530nm. Be sure to turn-on the VIS

lamp before attempting to read the blank. After you have obtained the absorbance values for your samples, turn lamp off. Rinse cuvettes with methanol and pour all methanol waste and methanol extractions in the Methanol Waste Bottle. You will be able to calculate the absorbance values per mg of fresh biomass used in each extraction after you enter your data in the class data file (see section on Statistical Data Analysis).

Chlorophylls: Grind four plants with a glass mortar and pestle containing a pinch of sand and 3 ml of acetone. Pipette or pour into two microfuge tubes (make sure the tubes are balanced, contain the same volumes, and place them in opposite positions in the centrifuge rotor), and spin 2 minutes. Fit a 3 cc syringe with a filter disk (remove the cap, if present, from the luer-lok end of syringe barrel and twist on the filter disk. Pull the syringe plunger from the barrel and position the end of the filter disk over a small glass test tube. Carefully transfer supernatant (using a clean glass Pasteur pipette) to the 3 cc syringe fitted with the filter disk and force supernatant through filter into the glass test tube. Adjust the total extract volume in the test tube to 5 ml. Stopper the tube, label with tape, mix by shaking, and cover with foil to minimize exposure to light. Rinse the mortar and pestle with water and dry before preparing the next sample of plants. Use the quartz cuvettes with your instructor supervising (handle the quartz cuvettes with care, they are very expensive). Acetone will melt disposable polycarbonate plastic cuvettes. Use acetone as the blank in the spectrophotometer. The spectrophotometer program CHLORO will measure absorbance in the visible range at 415nm and 662nm. Be sure to turn-on the VIS lamp before attempting to read the blank. After you have obtained the absorbance values for your samples, turn-off the VIS lamp. Rinse cuvettes with acetone and pour all acetone waste and acetone extractions in the Acetone Waste Bottle. You will be able to calculate the absorbance values per mg of fresh biomass used in each extraction after you enter your data in the class data file (see section on Statistical Data Analysis).

Protein Electrophoresis (optional)

The following protocol flow chart (Figure 4) summarizes the procedures for protein electrophoresis sample preparation.

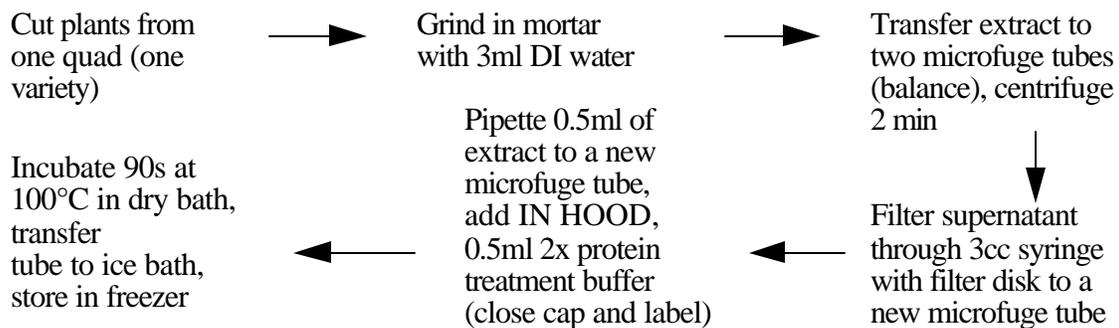


Figure 13.4. Protocol flow chart for protein electrophoresis preparation. Prepare a separate protein preparation for plants from each variety.

Grind four plants from one quad with a glass mortar and pestle containing a pinch of sand and 3 ml of deionized water. Pipette or pour into two microfuge tubes and spin 2 minutes. Carefully remove supernatant to a 3 cc syringe fitted with a filter disk and force supernatant through filter into a new microfuge tube. Wear gloves and eye protection for this next section. Pipette 0.5 ml of supernatant to a clean 1.5 ml microfuge tube and add an equal volume of 2x protein treatment buffer (0.125M Tris-Cl,

4% SDS, 20% glycerol, 10% 2-mercaptoethanol) in the hood. Close the microfuge cap, label the cap with the plant variety and your group number. Incubate tube in a dry bath for 90 seconds at 100° C, then cool in ice bath. Thoroughly wash the mortar and pestle after each extract is made so cross contamination of samples is minimized. Place the microfuge tube samples in the freezer to hold for electrophoresis.

Your instructor will help you load a gel with your samples and start the electrophoresis of samples from each plant variety. A polyacrylamide gel is used for this vertical electrophoresis. The proteins will separate by molecular weight with the low molecular weight molecules moving down the gel farthest. Samples containing known molecular weight components will be used in some gel lanes so the molecular weights of your plant extract proteins can be evaluated. Your instructor will assist you in staining the gel so separated proteins will be visible. Look for differences between the plant varieties in the position of protein bands which would indicate differences in protein molecular weight.

Do the three varieties exhibit differences in protein electrophoresis? If protein differences are observed, does this indicate genotypic differences between the varieties?

Statistical Data Analysis

Enter your data in the StatView class data files that your instructor has created on the computers in the laboratory (Table 3a and b). Compare traits, physical and biochemical, between treatments within each variety, and between varieties within each treatment. Perform chi-square tests on the categorical variables (leaf color, stem color, and flowers), and an analysis of variance (ANOVA) with Scheffe' comparisons on the continuous variables. Your Instructor will explain how these statistical tests are performed with the StatView program. Do all the traits respond to environmental variation in the same way? What is the cause for the differences between the three varieties of plants, genotypic variation, environmental variation or both?

Table 13.3a. StatView Data File "Variation-Morpho". A data spread-sheet form similar to that shown below is already present on the laboratory computers. "Group" is a categorical variable containing the name assigned to identify you and your partner in the data file (type the number "1" for "Group One", "2" for "Group Two"). "Treatment", "Variety", "Flowering", "Stem Color", and "Petiole Color" are all coded categorical variables. Stem "Length", distance to "First Node", and "Internodal" distance are all continuous variables measured in millimeters.

Group	Treatment	Variety	Length	First Node	Internodal	Flowering	Stem Color	Petiole Color
Group One	Twenty five	TA	73	12	17	no	Purple	Purple
Group One	Fifteen	TA	15	5	0	no	Purple	Purple
Group One	Full light	TA	140	6	10	yes	Purple	Purple
Group One	Half light	TA	58	15	19	no	Green	Green

Table 13.3b. StatView Data File “Variation-Pigments”. A data spread-sheet form similar to that shown below is already present on the laboratory computers. “Group” is a categorical variable containing the name assigned to identify you and your partner in the data file (type the number “1” for “Group One”, “2” for “Group Two”). “Treatment”, and “Variety” are coded categorical variables. “total mass”, “# of plants” per quad, “abs. antho. 530” (extract absorbance at 530nm), “abs. chloro. 415”, and “abs. chloro. 662” are all entered as continuous variables.

Group	treatment	variety	total mass (g)	# of plants	abs antho 530	abs chloro 415	abs chloro 662
Group One	Fifteen	TA	.21	3	.754	.	.
Group One	Twenty five	TA	.60	4	.378	.	.
Group One	Full light	TA	.94	3	.756	.	.
Group Two	Fifteen	TA	.16	3	.	2.309	1.643
Group Two	Twenty five	TA	.76	4	.	3.696	3.091
Group Two	Full light	TA	.43	4	.	3.550	2.582

Acknowledgments

Figure 2 is reprinted with the permission of Carolina Biological Supply Company, Burlington, North Carolina 27215, and the Wisconsin Alumni Research Foundation, Madison, Wisconsin 53707.

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APPENDIX A

Expected Results and Pigment Spectrophotometry Scans

In general, the TA plants will grow tall and exhibit anthocyanin in stems and leaves, TC plants will grow tall and show no evidence of anthocyanin, and SA plants will grow very short and exhibit anthocyanin. These general characteristics are seen consistently under all environmental conditions suggesting that the differences between varieties have a genotypic basis. However, environmental conditions do modify the phenotypes exhibited within a given variety. Very low light conditions can almost completely inhibit the expression of anthocyanin. Chlorophyll concentrations are also influenced by light conditions, but differences between varieties under a given environmental treatment are typically small.

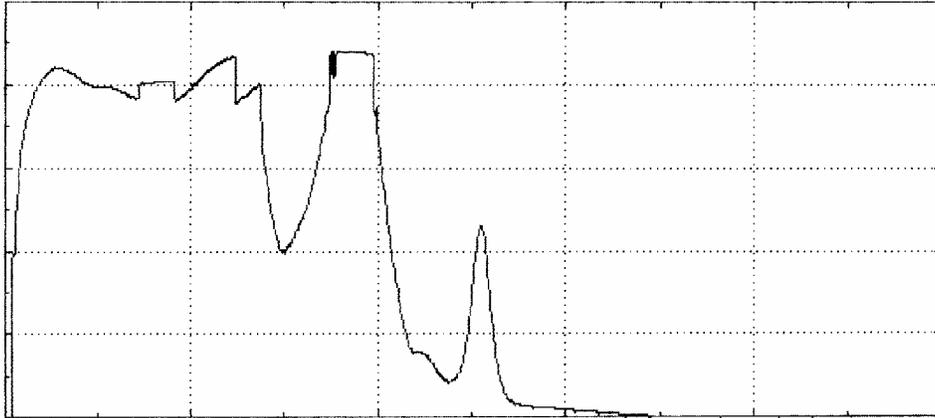
Low temperature typically retards growth and low light typically causes stem elongation but the specific effects of a given condition will not be the same in each variety nor will the effects be the same for each trait measured.

Spectrophotometry over a range of wavelengths (190nm - 1100nm), on methanol and acetone extracts from plants grown under high light conditions indicate the best wavelengths of detecting differences in anthocyanin and chlorophyll concentrations respectively (Figures 5 and 6). These absorbance versus wavelength scans verify that 530nm is an appropriate wavelength for detecting anthocyanin, and 415nm and 662nm are appropriate wavelengths for detecting chlorophylls.

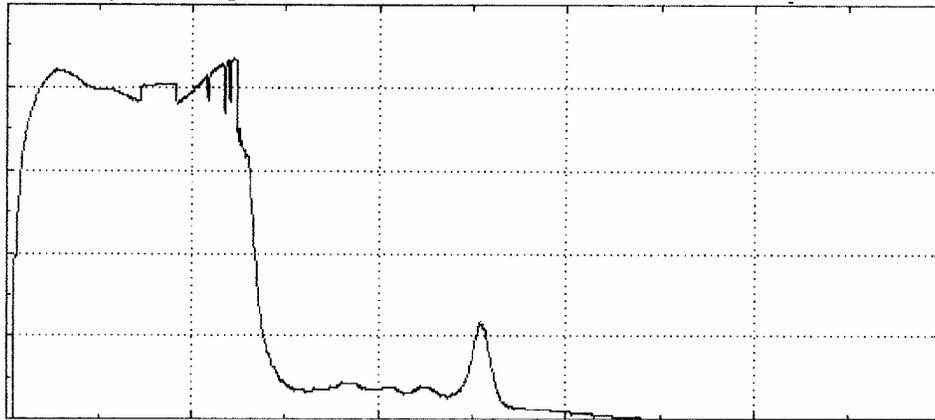
Optional protein electrophoresis of crude whole plant preparations on a large polyacrylamide gel will yield 10 or more bands visible with Coomassie Blue staining. Differences between the plant varieties in the pattern and intensity of protein bands are typically very slight. Obvious (expected) genotypic differences between plant varieties are not clearly seen. This result indicates that either the expected protein differences are difficult to detect or the differences cannot be detected in the gel system I have been using (see comments on electrophoresis in Appendix B).

Figure 13.5. Methanol extracted anthocyanin analysis. Absorbance as a function of wavelength for a range of wavelengths (190nm - 1100nm) is shown for methanol extracts from the three varieties of *Brassica rapa*. The absorbance and wavelength axes are the same scale in each graph.

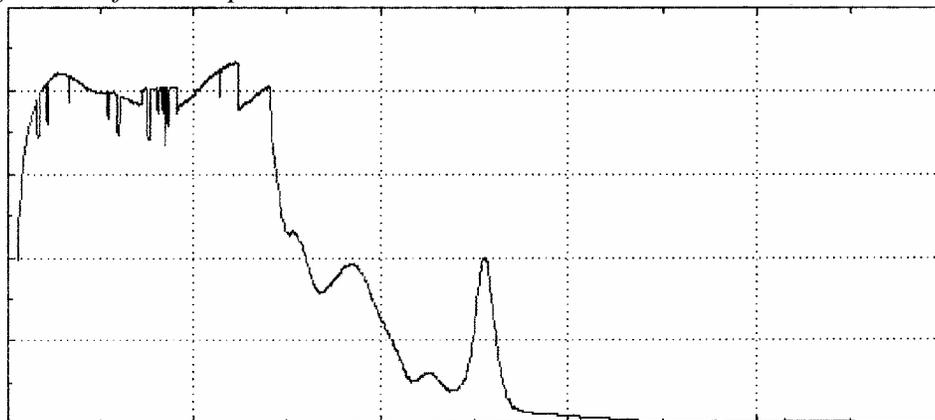
Scan of extract from TA plants



Scan of extract from TC plants



Scan of extract from SA plants



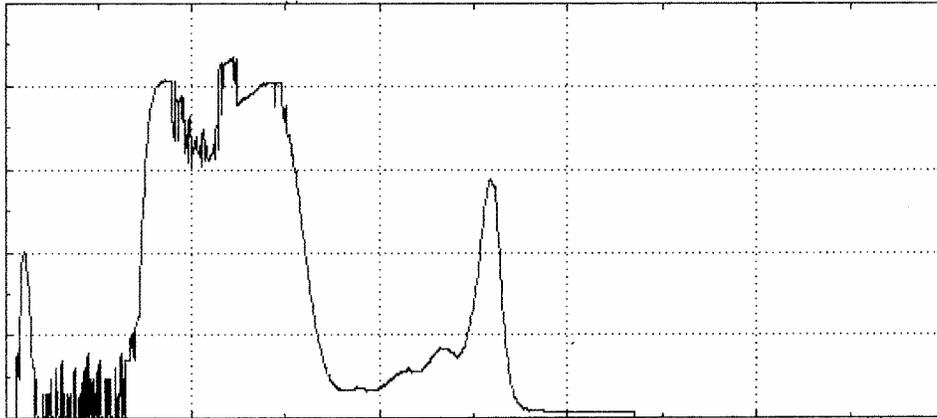
190nm

Wavelength (nm)

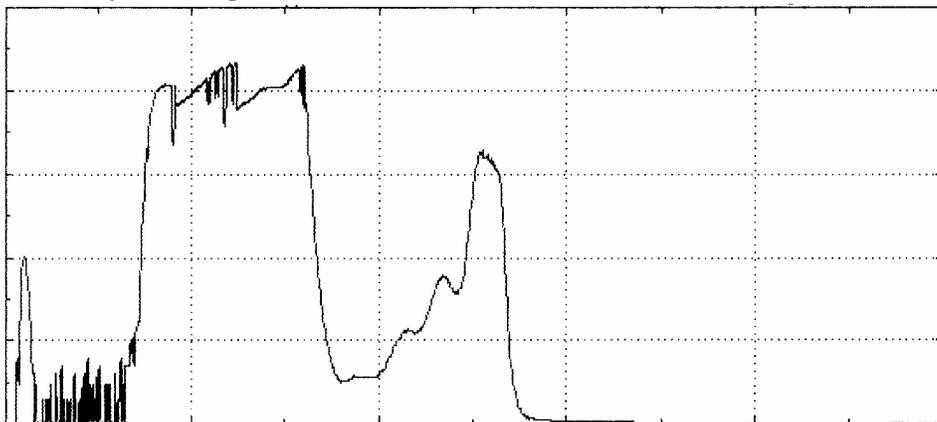
1100nm

Figure 13.6. Acetone extracted chlorophyll analysis. Absorbance as a function of wavelength for a range of wavelengths (190nm - 1100nm) is shown for acetone extracts from the three varieties of *Brassica rapa*. The absorbance and wavelength axes are the same scale in each graph.

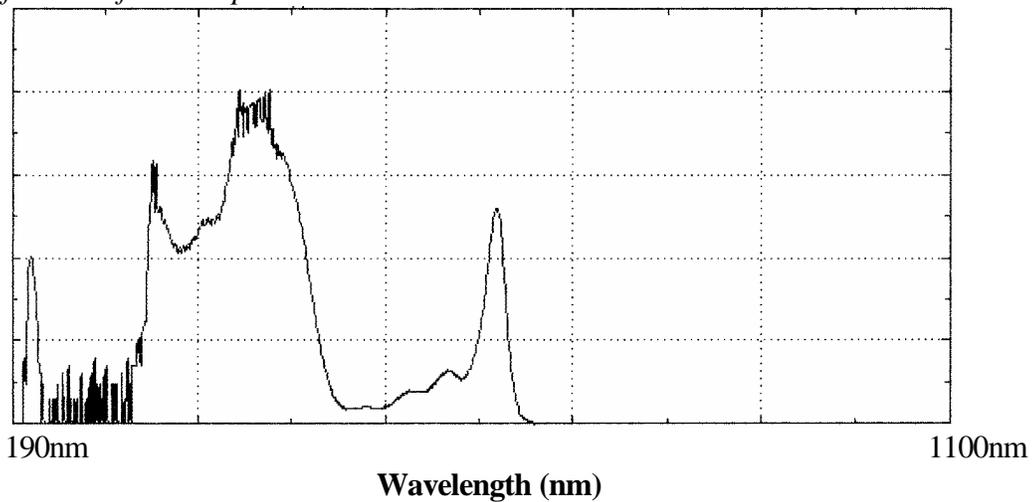
Scan of extract from TA plants



Scan of extract from TC plants



Scan of extract from SA plants



APPENDIX B

Preparation Guide and Sources of Materials

I am assuming that students will be working in groups of two or three and that there will be a maximum of ten groups per laboratory session. The quantities described below are those needed for ten student groups.

Plants should be started in each of the four treatment conditions 2 or 3 weeks prior to the time of data collection.

Three varieties of Wisconsin Fast Plants are grown in polystyrene quads using a self-watering system. A full supply list for all the Wisconsin Fast Plant materials that are available from Carolina Biological Supply Co. is given below. Each quad contains four cells in which a paper wick is inserted prior to filling with soil. A standard seed starter soil mix works fine or a prepared soil can be purchased from Carolina Biological. Two seeds are sown in each quad to ensure that at least one germinates. After one week of growth, extra seedlings are removed with scissors so each quad will have only one plant per cell (four plants per quad). All plants in a given quad will be the same variety. The Fast Plant varieties are: Basic Brassica (TA), Anthocyaninless (TC), and Rosette (SA).

There are three plant varieties and five treatment levels, and each group of students will need one quad of plants from each variety and treatment combination, a total of 15 quads per group (Table 4). The protein prep quads (for plants to be used for protein electrophoresis) are grown under the same conditions as the Full-Light treatment.

Table 13.4. Quad Assignments to Treatments and Varieties.

Treatment						
Variety	15 C	25 C	Full-Light	Half-Light	Protein Prep	Totals
TA	1 Quad	1	1	1	1	5
TC	1	1	1	1	1	5
SA	1	1	1	1	1	5
Totals	3	3	3	3	3	15 Quads

15 Quad per group \times 10 groups = 150 quads needed

Each quad gets 4 wicks \times 15 quads per group = 60 wicks/group

60 wicks /group \times 10 groups = 600 wicks total

Each quad cell is sown with two seeds and each quad contains 4 cells so each quad is sown with 8 seeds and each group needs: 8 seeds/quad \times 5 quads/variety = 40 seeds/group of each variety.

40 seeds/group of each variety \times 10 groups = 400 seeds of each of the three Wisconsin Fast Plant varieties needed.

The following table shows the total number of quads to be prepared and placed in each of the treatments (Table 5):

Table 13.5. Total Quad Assignments to Treatments and Varieties for a Class of Twenty Students.

Treatment						
Variety	15 C	25 C	Full-Light	Half-Light	Protein Prep	Totals
TA	10 Quads	10	10	10	10	50
TC	10	10	10	10	10	50
SA	10	10	10	10	10	50
Totals	30	30	30	30	30	150 Quads

The quads with seeds sown should go immediately into each treatment condition so germination occurs under specific environmental conditions.

15° C (cold room or incubator) and 25° C (room temperature or incubator) should have the same light conditions, photosynthetically active radiation (PAR) of approximately 150 $\mu\text{E}/\text{m}^2/\text{sec}$ at the level of the quads. Avoid window light addition to the 25° C treatment by locating the quads away from windows or make a black plastic film enclosure to eliminate extraneous light. A bank of six 40W fluorescent tubes set at a height of 15cm above the tops of quads should provide adequate illumination.

Full-Light (PAR of approximately 150 $\mu\text{E}/\text{m}^2/\text{sec}$) and Half-Light (PAR of approximately 75 $\mu\text{E}/\text{m}^2/\text{sec}$) should have the same temperature conditions, approximately 25° C or room temperature. A bank of six 40W fluorescent tubes set at a height of 15cm above the tops of quads should be adequate for the Full-Light treatment. Use only three fluorescent tubes in the same type fixture for the Half-Light treatment. It is important to eliminate sources of extraneous light so the Half-Light treatment is really half that of the Full-Light treatment. The Protein Prep treatment is identical to the Full-Light treatment, and is an optional part of this study.

All quads must be kept moist by maintaining them in water filled trays, or on wicking pads so the soil is always moist.

In the pigment extract preparation, a large number of syringes and syringe filters are used to remove debris from the extract. An adequately clean extract may be prepared by performing two sequential centrifugations. This would eliminate the need for the syringes and filters.

Wisconsin Fast Plant Supplies

Carolina Biological Supply (800) 334-5551
pp 150-151 in 1996 Catalog

Quads #K3-15-8960 sold in units of 16 quads
Need 150 Quads (10 units)

Soil #K3-15-8966 2 cu ft bag

Wicks #K3-15-8978 sold in units of 70 wicks
Need 600 Wicks (9 units)

Seeds

Basic Brassica (TA) (Need 400 seeds)
#K3-15-8805 200 seeds
Anthocyaninless (TC) (Need 400 seeds)
#K3-15-8813 200 seeds
Rosette (SA) (Need 400 seeds)
#K3-15-8816 200 seeds

Protein Electrophoresis

Detailed protocols for conducting protein electrophoresis and staining gels are provided with most electrophoresis chambers. Pre-cast polyacrylamide gels for vertical protein electrophoresis can be purchased from a number of sources. I have had excellent result with gels from Jule Biotechnology, 25 Science Park, New Haven, Connecticut (203) 786-5490. Jule Biotechnology also distributes a detailed protocol manual (free) for protein electrophoresis titled: Operator's Manual Jule Gels. Pre-

cast gels must be purchased to fit the specific electrophoresis chamber you are using. Generally, a large gel will yield better separation than a mini-gel. I have had better results with denaturing gels than with non-denaturing gels.

Statistical Analysis

The statistical analysis and graphics program that I have used in this study, and highly recommend, is StatView 4.5. This program is available for both Macintosh and PC Windows computers. The program can be purchased directly from the manufacturer (ask about educational institution discounts): Abacus Concepts, 1918 Bonita Ave., Berkeley, California 94704-1014, 1-800-666-7828.