Supplemental Material

CBE—Life Sciences Education

Batzli *et al.*

Chapter 6.
What can purple *Brassica rapa* plants tell us about genetics and the influence of environment?

Authors: Janet Batzli, Amber Smith, Seth McGee, Paul Williams

How is variation generated? What makes one individual different from another? How does the environment influence variation? How is variation inherited? How is understanding variation fundamental to understanding evolution? These are the fundamental questions that geneticists and evolutionary biologists study.

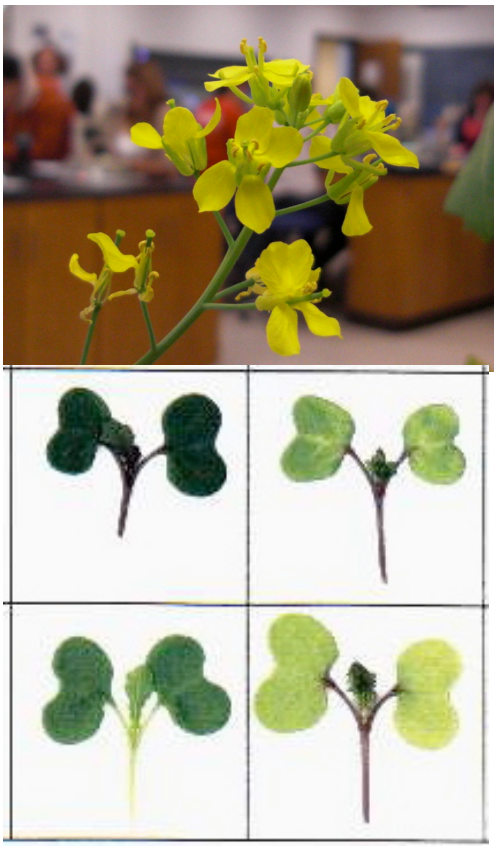


Fig. 1 *Brassica rapa* flowers (top), and variation in stem and seed leaves (cotyledon) color from greenish yellow to deep purple, dependent on expression of anthocyanin pigment (bottom).

We know that expression of traits or **phenotypes** (an observable characteristic, distinct morphology, behavior, biochemical makeup or physiology) is controlled by **genotype** (particular set of **alleles** that make up a **single gene** or **multiple genes**) and stimulated by a particular **environment**.

During this unit you will be investigating the relationships between genotype, phenotype, and the environment using rapid cycling *Brassica rapa* plants. The genus *Brassica* includes a remarkably variable group of plants from the mustard family that include cabbage, broccoli, cauliflower, Brussels sprouts, collard, kale, kohlrabi, turnip, bok choi, napa cabbage, rapini , rutabaga, mustard seed and canola oil seed. The phenotypic variation in *Brassica* is vast and has been mined in many geographic and cultural settings around the world. For at least a thousand years, humans selected and bred traits of value to human domestic economies. Traits such as growth rate, taste, nutritional content, seed-oil quality, storage and transportability, pest resistance, plant shape, size and color have all been bred into the many forms of *Brassica* crops. In addition, *B. rapa* has been bred to produce rapid cycling plants or "Fast Plants" that grow quickly from seed to flowering and are an excellent model system for studying genetics.

Dr. Paul Williams created Rapid Cycling Brassica Fast Plants while a professor at University of Wisconsin-Madison. In 1982, Dr. Williams established the Rapid Cycling *Brassica* Germplasm Collection and the Wisconsin Fast Plant® program providing well described seed stocks of *B. rapa* for investigating genetics. This semester in Biocore 302, we will be working with Paul to explore new aspects of genetics in *B. rapa*. For the phenotype that we examine in *B. rapa* you

will have the opportunity to ask novel questions about the inheritance of the trait and how the environment effects variation of the trait.

During this unit, you will examine the inheritance of anthocyanin (purple) plant pigment and investigate the influence of genetics and the environment on the expression of anthocyanin pigmentation phenotype. Your team will generate a testable question and hypothesis to examine 1.) the influence of genotype on expression of phenotype: if selection for high expression of anthocyanin pigmentation (25% most purple parents) leads to an increase in anthocyanin expression in the offspring, and 2.) the influence of environment on phenotype: if the expression of anthocyanin pigment is influenced by a particular environment of your choosing. Your group will design and carry out your experiment. You will prepare a research proposal and a scientific poster (both written individually) to communicate your research.

Learning Objectives:

By the end of this investigation, you will be able to:

1. Explain phenotypic variation as a function of variation in genotype and/or the environment (phenotypic plasticity)
2. Differentiate between discretely and continuously variable traits in terms of how these traits are inherited and expressed
3. Propose a hypothesis and carry out an experiment to provide evidence about the effects of artificial selection and environment on a phenotype
4. Use frequency histograms to analyze and interpret shifts in phenotypic variation due to both genetics and the environment
5. Construct a scientific poster to communicate your proposed research and final results of your research.

Terms you should understand

phenotype • genotype • allele • discrete trait • quantitative trait • Mendelian trait • inheritance • phenotypic variation • genetic variation • natural selection • artificial selection• genetic drift• phenotypic plasticity • gene expression

Why Plants?
Gregor Mendel studied genetics using peas—and now we are studying genetics using *B. rapa* Fast Plants. Why are plants useful for studying genetics?

Plants have a valuable attribute for the practical study of genetics called *seed dormancy*. Seed dormancy allows genetists, or even a common gardener "seed saver", to grow parents and offspring at the same time. How does this work? Say you have a packet of 100 seeds, you plant 10 seeds to form a population of parent plants. You then open pollinate (e.g. distribute and mix pollen among all 10 plants with a cotton swab or pollination stick) and produce a large volume of 'offspring' or first filial (F1) seed that matures on the parent plants. Finally you collect all

offspring seed in a packet and store until you want to plant. Later you subsample 10 offspring seeds and 10 parent seeds to grow at the same time. Since the seed remains alive but in a dormant state, it can be saved for years, and in some cases decades or even centuries, and then can be grown given appropriate environmental conditions. Seed dormancy allows genetists to compare distinct generations of genetically related populations (i.e. parents, grandparents, offspring etc.) at the same time in the same environment.

Another valuable attribute of plants is *pollen*. Pollen is a protected packet of plant sperm that can be transferred easily, by bee or human, from one individual or population of plants to another. Pollen can be transferred within a population of plants with the same genetic background (e.g. transfer pollen among all individuals within a parent population) or between populations with different genetic backgrounds (e.g. transfer pollen from an F1 population to a parent population). Using controlled pollination, genetists can produce large quantities of seed with a particular genetic heritage.

Since plants and animals share many of the same mechanisms for inheritance and gene expression, plants can tell us much about the inheritance and expression of genetic traits in animals. In addition to the essential connection that humans have to plants through agriculture, we can learn a great deal about basic human genetics by studying plants.

Why do individuals in populations differ?
Phenotypes and Variation in a Population

A **population** is defined as a localized group of individuals of the same species that are capable of interbreeding and producing fertile offspring. One of the fundamental attributes of individuals within a population is that they vary in observable and measureable characteristics, referred to as **phenotypes**. Since all individuals in a population have the same genes, why do they differ in phenotype?

Within each individual's set of genes, there are alternate or varied forms of genes called **alleles**. A phenotype, or observable trait, is influenced by a variable set of alleles at particular gene loci carried by an individual (its **genotype)** AND the **environment** that the individual experiences which influences the *expression of genes*. For example, some individuals in human populations are genetically predisposed (have a particular genotype) to express high blood cholesterol, but only when combined with a high fat diet (environment) would some individuals exhibit heart disease (phenotype).

Geneticists note this relationship by the equation**:**

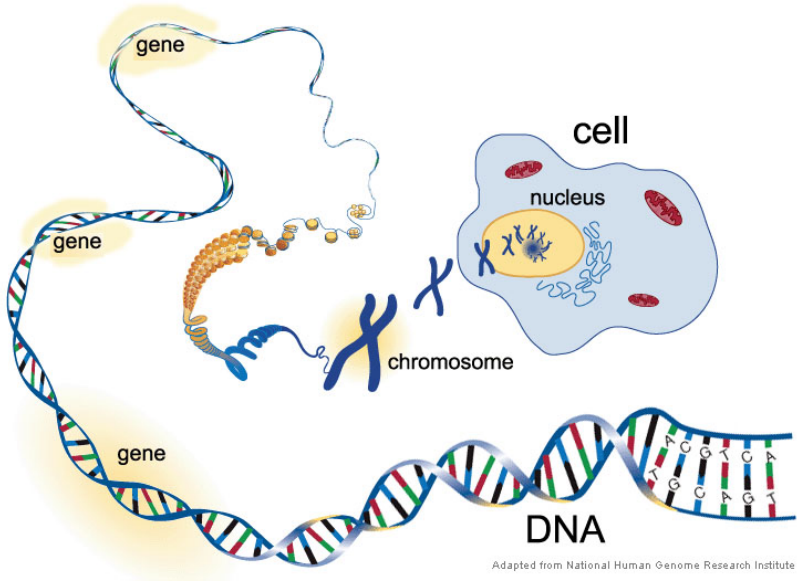
$$\text{Phenotype} = \text{Genotype} + \text{Environment}$$

$$P = G + E$$

It is common to describe a genotype using a set of letters, each variant denoting an allele. For example, if a gene has two alleles and one allele is dominant over the other, we can denote the

dominant form as 'F' and the recessive form as 'f'. Given that many organisms are **diploid** and carry two sets of genes (one from each parent), there are at least three possible allelic combinations for each gene: FF, Ff, ff. And that is the variation for just for <u>one</u> gene. Most phenotypic traits are conditioned by multiple genes and are referred to as multigenic traits. When you consider all of the tens of thousands of genes in an individual and allelic variation at each gene locus (gene location on a chromosome), added to chromosomal rearrangements, mutations, and environmental influences—that's a lot of variation!!

Big idea: All *B. rapa* have the <u>same genes</u>, just as all humans have the same genes. Genetic variation within a species is based on the variation in <u>alleles</u> that contribute to a unique <u>genotype.</u>



The cartoon image above illustrates a **gene** as a unique stretch of DNA located along a chromosome. Expression of a phenotypic trait such as anthocyanin pigmentation in *B. rapa* involves many (approximately 19!) unique genes that are located on chromosomes within the cell nucleus. The specific location of a gene along a chromosome is referred to as its' **gene locus**.

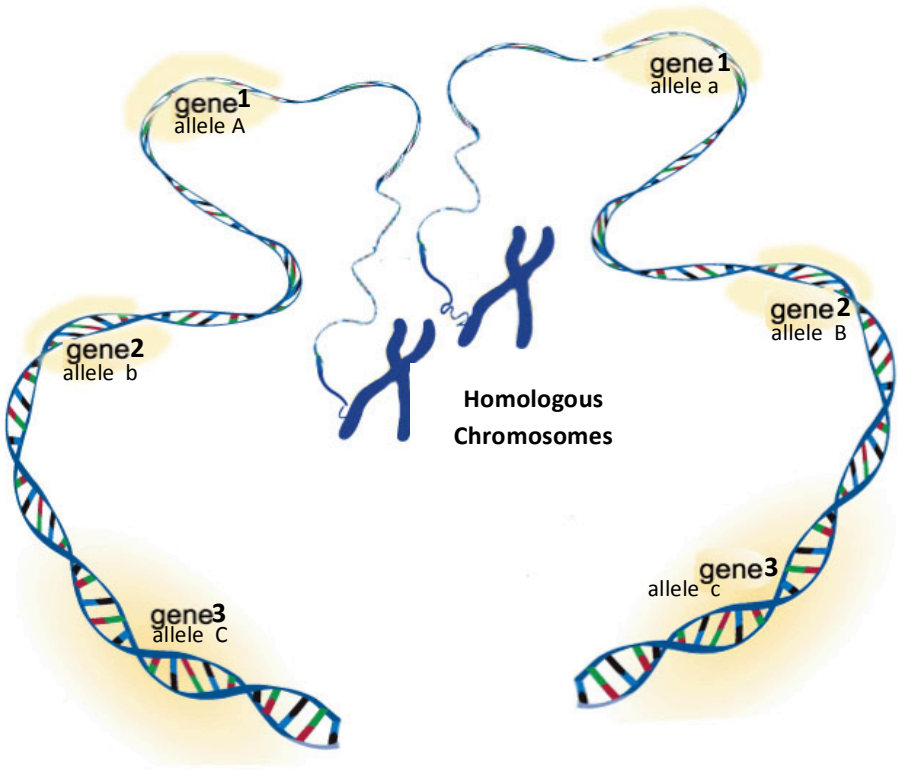


Figure 2. Cartoon illustrating genes as segments of DNA packaged into a chromosome and homologous pair of chromosomes with variant alleles at each gene locus. Image adapted from National Human Genome Research Institute Collection.

As a diploid species with two sets of chromosomes, *B. rapa* contains two copies of each gene- one inherited from a maternal parent and one from a paternal parent. Although there may be two copies of each gene in a diploid organism, each copy varies slightly from the other. These variant forms of the same gene are called **alleles**. In Figure 2, each of the three genes depicted have variant alleles (labeled A/a, b/B, and C/c). If gene 1, 2 and 3 all contributed to the expression of the same phenotype in an individual, the genotype for the trait would be AaBbCc.

Discrete Variation, Single Gene Mendelian Traits

When Gregor Mendel began analyzing peas, he saw variation in many traits—most of which he could identify and classify with discrete phenotypes e.g. either yellow or green, wrinkled or smooth, tall or dwarf. What Mendel saw and quantified as discrete traits was later understood to be inheritance of a **single gene** with two or more variant alleles at a single gene loci responsible for variation in a phenotype. Other examples of discrete variation are traits one can best describe qualitatively in words rather than in numbers. For instance, individuals might be rough vs smooth, sturdy vs flimsy, blue vs white etc. Generally, traits that can be described as contrasting, being one thing or another in words, are called ***discrete traits***. Discrete traits are the

result of variation in allelic make up at a single genetic locus and these traits show predictable Mendelian ratios or patterns of inheritance (e.g. offspring phenotypic ratios of 3:1 as shown in Figure 2). Given that Mendel was the first to describe the inheritance pattern of discrete traits, these traits are often referred to as **Mendelian traits** that show predictable **Mendelian inheritance.** Mendelian traits have fairly simple inheritance where Phenotype (P) generally equals Genotype (G) with little influence of Environment (E); although, E can still influence these traits in certain instances.

In rapid cycling *B. rapa* Fast Plants, there is a single gene referred to as anthocyaninless (*anl-*) that controls whether an individual plant can express anthocyanin purple pigment or not (expressed as green anthocyaninless trait). This single gene exhibits Mendelian inheritance as indicated in Figure 3. When *anl-* is present in a homozygous recessive state anthocyanin pigment production is shut down completely resulting in green plants.

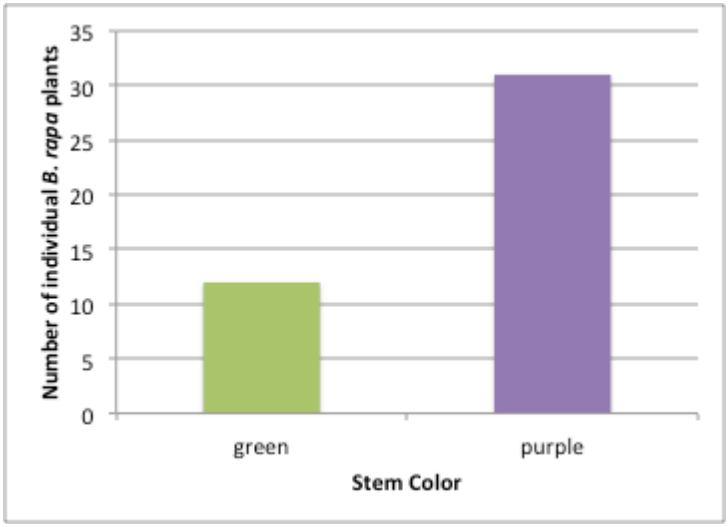


Figure 3. Frequency diagram of stem color in a rapid cycling *B. rapa* population of 43 plants. Stem color was evaluated as a discrete trait, either purple or green.

Geneticists that breed organisms to study complex, quantitative traits controlled by multiple genes often use **Mendelian traits** such as anthocyaninless to serve *as predictable markers* (a type of genetic *control).* Utilizing Mendelian traits as predictable markers in genetic crosses allows researchers to keep track of generations and to make sure that the crosses they are making are free from contamination or that population sizes are large enough to avoid excessive random loss of allelic variation. This is particularly important for plant breeders since crosses depend on the faithful transport of pollen from one set of plants to another. You will be using the anthocyaninless trait in your own experiment in much the same way.

Continuous Variation and Multi-genic Quantitative Trait

Although most famous for describing discrete traits, Mendel could have (and probably did at one time) measure some pea plant traits with continuous measures e.g. height, degree of

wrinkledness, degree of pigmentation. Most phenotypes within a population do not appear as discrete, but rather exhibit continuous variation. These phenotypes are quantified in numeric terms based on a standardized or commonly agreed upon measurement. Traits with continuous variation are termed ***quantitative traits*** and are influenced by **multiple genes AND the environment**. Quantitative traits typically involve many genes that are all influenced (to a greater or lesser extent) by the environment. With many genes involved and expression influenced by changes in the environment, there are typically many more "options" for expression of a trait rather than just two forms.

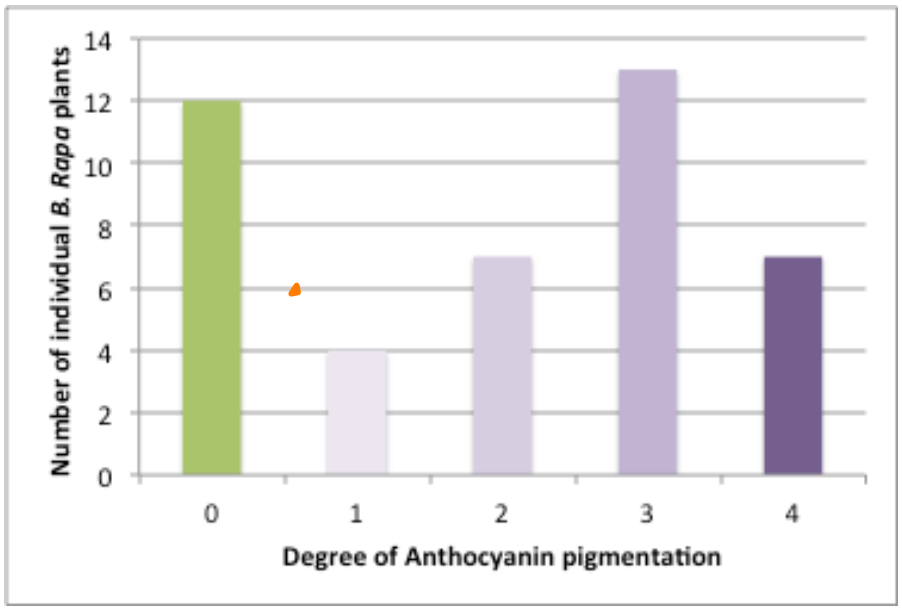


Figure 4. Distribution of the intensity or degree of anthocyanin pigmentation in the stem hypocotyls of a *B. rapa* population of 43 plants. Evaluated as a quantitative trait, intensity of purple was measured on a scale from 0-4; 0 being not purple (green) to 4 being completely purple.

Plants exhibit purple anthocyanin pigmentation along a continuum from low to high level

Interestingly, if one looks at a seemingly discrete trait like purple/green plants illustrated in Figure 3, careful observation of the same population may reveal variation in the degree of anthocyanin pigmentation as a phenotype and a quantitative trait (Figure 4.) Although individuals may share the same alleles at one gene locus they differ at other loci and, therefore, vary in the overall expression of the trait. As a result, the varied expression of all of these genes leads to great variation in the expression of continuous traits as compared to discrete traits.

Defining Genotype

For a discrete phenotypic trait generally conditioned by a single gene, genotype is typically thought of as the allelic combination (e.g. Aa) at that single locus.

For a quantitative trait, conditioned by multiple genes, a genotype is the combined set of allelic combinations at all gene loci associated with expression of the trait (e.g. AaBbCc). When illustrating a quantitative trait using a frequency distribution such as Figure 4 we can consider all individuals in the same bin "1", for instance, as having a unique cluster of alleles that results in a

recognizably distinct – light purple colored phenotype. By extension, we can consider individuals in bin "4" as having a cluster of alleles that results in a very dark purple phenotype. The suite of alleles represented in bin "1" are different than those in bin "4" and we can therefore, describe each bin – loosely- as representing a slightly different genotype.

Quantitative Traits- Influence of multiple genes and genotype on phenotypic variation

To begin thinking about quantitative traits- multiple genes, gene interaction, genotype and gene expression- consider that a quantitative trait such as intensity of anthocyanin pigmentation is the product of multiple genes being expressed in concert. Coordinated gene expression of these many genes results in many different proteins that control and regulate a complex biochemical pathway to a greater or lesser extent.

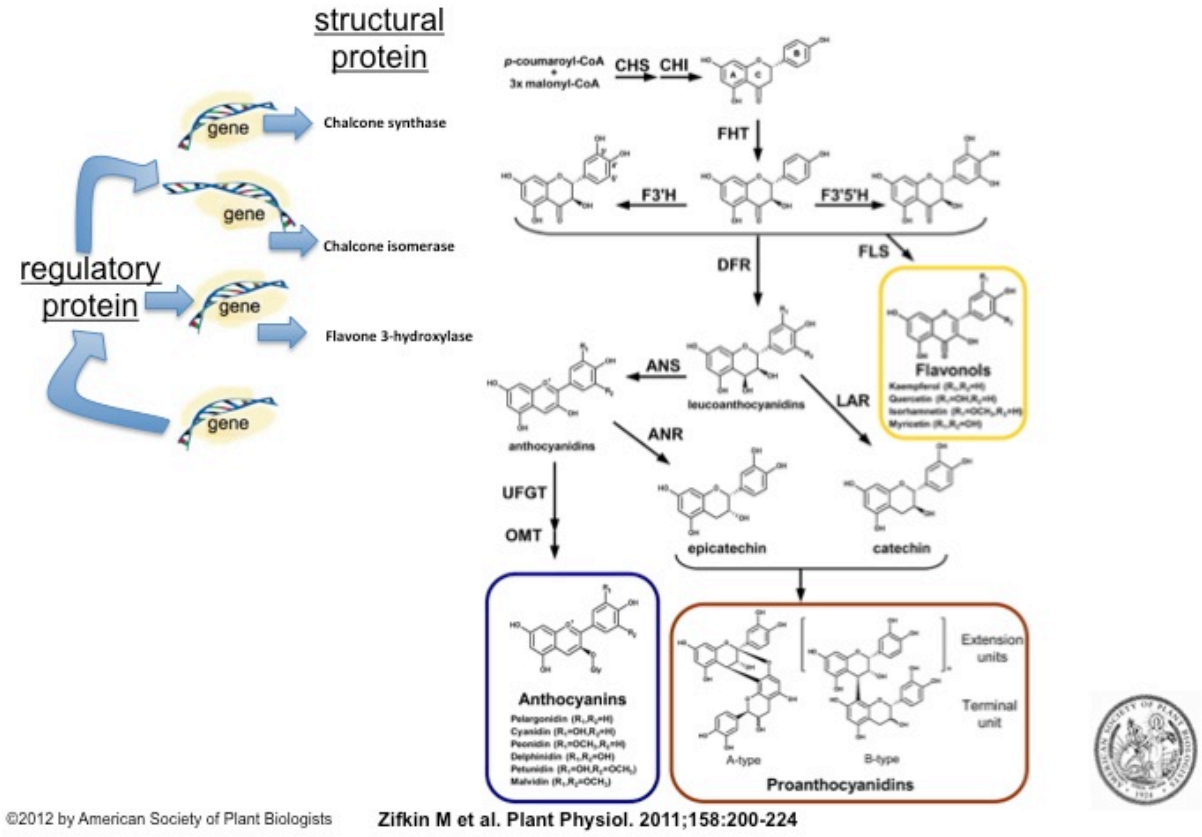


Figure 5. The biochemical pathway associated with synthesis of anthocyanin pigment involving many genes and associated structural and regulatory proteins. Image of anthocyanin biosynthetic pathway was adapted from Holton and Cornish 1995. The cartoon illustrates 3 specific genes that code for three structural proteins (CHS, CHI, and F3H) involved in the initiation of the anthocyanin biosynthetic pathway. There are many more genes that are involved in anthocyanin biosynthesis.

The biochemical pathway associated with synthesis of anthocyanin pigment involves upwards of 19 genes (Figure 5). Some genes encode for **structural proteins** involved directly as enzymes in catalyzing one step in the synthesis of anthocyanin pigments (cyanidin-O-rutinoside, pelargonidin-3-O-rutinoside, and delphinindin-O-rutinoside). Three major structural proteins

include chalcone synthase (CHS), chalcone isomerase (CHI) and flavone 3-hydroxylase (F3H). There are many other structural proteins involved in the biochemical pathway indicated in bold with labeled arrows in Figure 5. Other genes encode for **regulatory proteins** that regulate the expression of structural proteins. Regulatory proteins work like a rheostat or 'dimmer switch" controlling how much and to what extent a gene is expressed (Holton and Cornish 1995). In *B. rapa,* regulatory genes that encode regulatory proteins determine the amount of anthocyanin production, the timing of production in plant development, and the distribution of pigment.

Together, the multiple genes with variant alleles involved in anthocyanin pigmentation combine to create different and unique genotypes. These unique genotypes condition for the spectrum of anthocyanin pigmentation we observe. Figure 6 illustrates an F1 population that has in it a particular range of genotypes for anthocyanin pigmentation. In this illustration, we consider each level on the anthocyanin pigmentation scale (0.5-4) as a distinct and unique genotype consisting of a unique mixture of alleles. For instance, when an F1 population is grown in a standard control environment, individual plants in level '1' pigmentation have a set of alleles for expression of anthocyanin at low level which differs from a set of alleles in plants expressing anthocyanin at level '3' of pigmentation. With more sophisticated and refined observation, we can detected finer shades of putative allelic variation in a continuous quantitative trait.

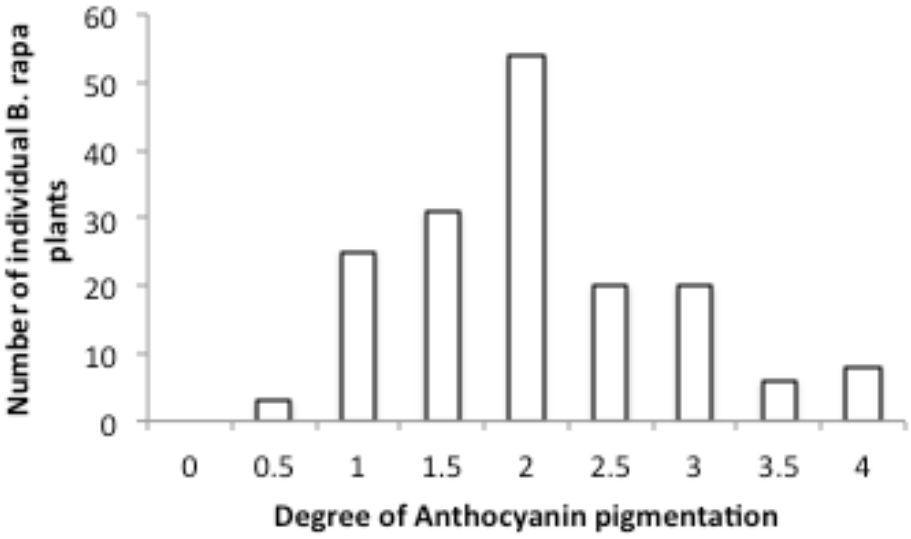


Figure 6. Hypothetical frequency distribution of anthocyanin pigmentation in F1 population (N=167).

Influence of environment on phenotypic variation

Some genes are always 'turned on' constitutively and make their protein products continuously in every cell in all tissues throughout the life of the organism. Most other genes including those involved in anthocyanin biosynthesis are stimulated to turn on, turn off, or modulate expression at varying rates in different tissues based on environmental queues. The capacity to modulate gene expression due to environmental stimuli is based on the unique complement of alleles held within the organism.

Figure 7. *Brassica rapa* seedlings that vary in degree of anthocyanin pigmentation in their stems.

Phenotypic plasticity is the capacity of individual genotypes (or genetically distinct populations) to produce different phenotypes (e.g. rate of development, size, shape, color, nutritional content, behavior) in different environments. Genotypes or genetically distinct populations differ in their degree of phenotypic plasticity or in this case, the possible range of pigmentation, based on their complement of alleles. Some individuals will be more sensitive to environmental stimuli than others based on their unique allelic make up at different gene loci.

Inheritance of Quantitative Traits

The most fundamental question we can ask about a quantitative trait such as degree of anthocyanin pigmentation (Figure 7) is whether the *phenotypic variation* in the trait is strongly heritable or whether phenotypic variation is primarily influenced by the individual's environment. All quantitative traits have a genetic basis, but *variation* from individual to individual in a population is not necessarily the result of different alleles but rather how/when those alleles are expressed in different environments. For instance, two plants with very similar genotypes for anthocyanin pigmentation could be growing in the sun and in the shade with the plant in the sun exhibiting level 4 pigmentation and the plant in the shade exhibiting level 1 pigmentation. If we came across these two plants without knowing their pedigree or heredity, we might deduce they are genetically unique when in actuality it was an example of phenotypic plasticity. To determine the relative influence of genotype on the variation in phenotype, we can do experiments where we attempt to separate the influence of genotype and environment using artificial selection.

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Using Artificial Selection to Detect the Influence of Genotype:

Conceptually, it may seem fairly simple to determine whether genotype influences the variation of a particular trait. If genes are involved, then offspring should appear more similar to their parents than to unrelated individuals—as long as all individuals are raised in a common environment. This is often easier said than done. For instance, when studying the heritability of human traits, we cannot control the environment where individuals are raised. However, we can test this concept using experimental organisms like *B. rapa*. By *selecting* and inter-mating individuals in a parent population that exhibit extremes of a trait—e.g. the 25% 'purplest' plants, we can compare the offspring of the selected population to that of an unselected control population, commonly referred to as a drift population. When you apply artificial selection to an F1 population to derive the most highly pigmented phenotypes in the next generation (F2selected) you may generate a shift in the frequency of genotypes from F1 to F2 population, as illustrated in Figure 8. In this instance, if the average intensity of anthocyanin pigmentation for the offspring of the selected population is greater than the parent and the drift population, then the expression of anthocyanin is strongly heritable. When measured in a common environment, this genetically based increase in phenotypic expression from parent to offspring population by means of artificial selection is referred to as a ***gain from selection*.**

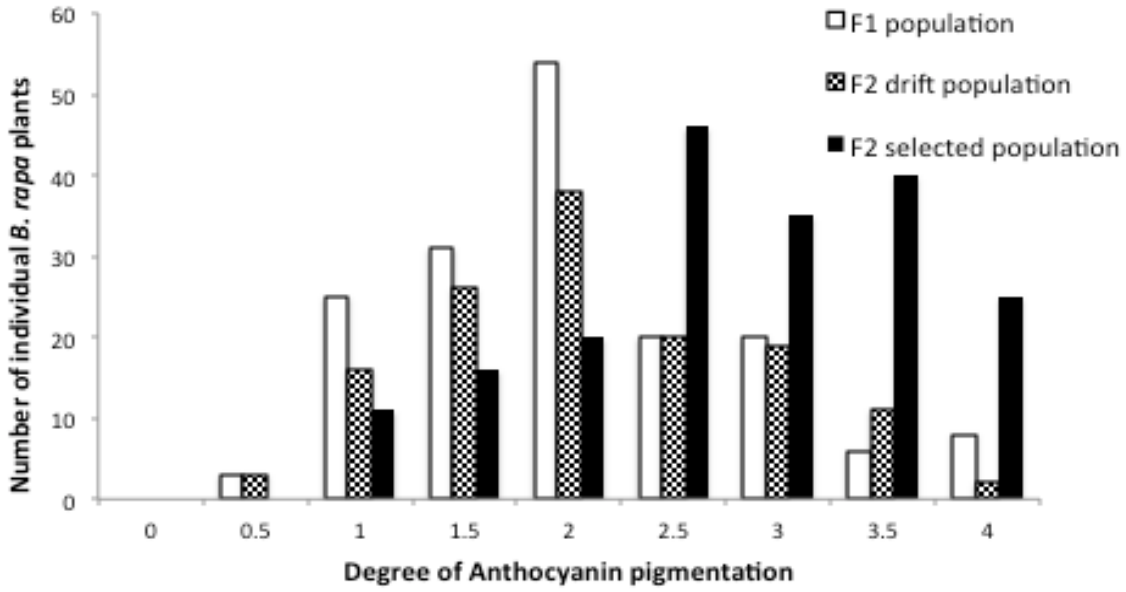

Figure 8. Hypothetical frequency distributions of F1, F2 drift and F2 selected populations. The F2 selected is derived from selection of the 25% most highly pigmented individuals in the F1 population. The F2 drift is derived from selection of 25% random selection of individuals in the F1 population.

To measure the influence of selecting a smaller sample from a large population, we could generate an F2 'drift' population from interbreeding a set (25%) of randomly chosen individuals

from the same parent population that produced the selected population. In Figure 8, why would you expect F2 drift population to appear more similar to F1 than to F2 selected population?

Using Predictable Genotypes to Detect Influence of Environment:

To determine the degree to which genotype and environment influence a phenotype, we can do experiments where populations with predictable genotypes are grown in variable environments such as low and high light (Figure 9).

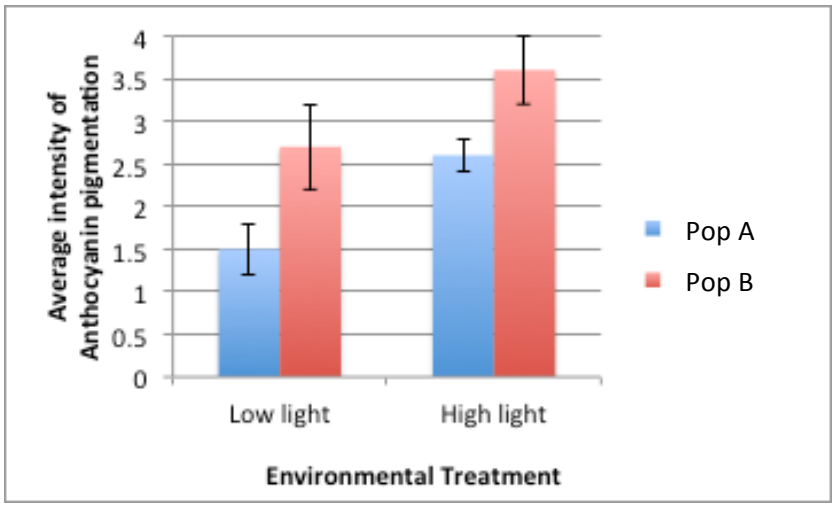

Figure 9. Hypothetical data for average intensity of anthocyanin pigmentation for two genetically distinct populations (Population A- unselected 'drift'; Population B- selected for high anthocyanin pigmentation) grown in low (40 µmol PAR m^{-2} s^{-1}) and high (200 µmol PAR m^{-2} s^{-1}) light levels. Bars are means (N=3) ± 1SE.

In this example, intensity of anthocyanin pigment for populations A and B are significantly different confirming that there is a strong genetic basis for the differences. Furthermore, if both A and B respond in the same direction and to an equivalent degree then we can conclude that the environment (high light) is acting more generally to regulate plant growth and metabolism independently of anthocyanin biosynthesis. These populations are exhibiting phenotypic plasticity to the environment where increased anthocyanin pigmentation is associated with increased growth in higher light due to higher rates of general metabolism. In short, if both genotypically distinct populations respond similarly to increasing light level—by increasing anthocyanin expression – then we might conclude that environment influences increased expression in a similar way for both populations.

Alternatively, if the populations respond differently (in the opposite direction) to increasing light level (Figure 10), then we can conclude that the phenotypic expression is based on a unique set of anthocyanin alleles at various gene loci that respond differently (to a greater or lesser degree) to light. Given this result, we would conclude that there is a genotype by environment interaction (G x E). In this situation, there are multiple genes controlling the expression of anthocyanin and that variant alleles at multiple loci are affected differently in the high light versus the low light conditions. This result suggests that the environmental cue of high light is acting directly to influence the multigene expression in the anthocyanin biosynthesis pathway.

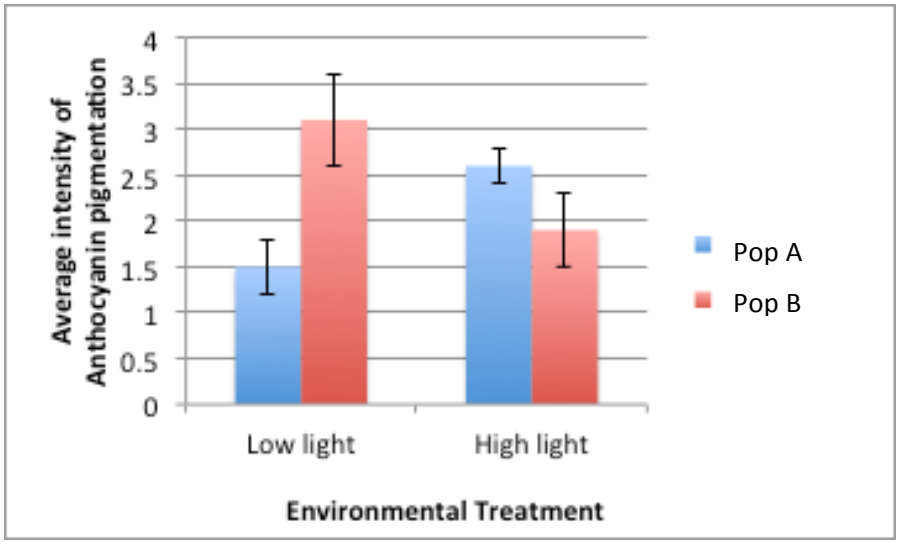

Figure 10. Hypothetical data for average intensity of anthocyanin pigmentation for two genetically distinct populations (F2drift- unselected 'drift'; F2 selected- selected for high anthocyanin pigmentation) grown in low (40 µmol PAR m^{-2} s^{-1}) and high (200 µmol PAR m^{-2} s^{-1}) light levels. Bars are means (N=3) ± 1SE.

Genotype by environment interaction is an additional level of complexity, where certain genes involved in a phenotype are alternatively expressed or regulated in different environments. The results in Figure 9 show no G x E, whereas Figure 10 shows statistically significant **G x E interaction.**

Phenotype = Genotype + Environment + *(Genotype by Environment Interaction)*

$$P = G + E + (G \times E)$$

P = **Phenotype** (expression of anthocyanin pigment indicated by intensity of purple)
G =**Genetics** (the potential of the individual to fully express a phenotype given its genetic makeup)
E = **Environment** (influence of factors, including nutrition, light quality/quantity, atmospheric conditions, soil texture, pathogens etc.. that affect an individual's capacity to express a phenotype)
(G X E) = **Genotype by Environment Interaction** (in general, how a given genotype responds to a particular set of environmental parameters to express a phenotype).

To summarize, when you complete your experiment, you will be able to gather evidence for

1. **The influence of G on the quantitative trait, intensity of anthocyanin pigmentation:** how phenotypes of offspring vary from their parents following artificial selection and cross fertilization of highly purple individuals in a single environment
2. **The influence of E and G x E:** how phenotypes of selected and unselected 'drift' populations vary in two different environments

Background Information on *Brassica rapa*, Anthocyanin, and Variation

The *Brassicaceae* family[1] is a very diverse family that includes plants such as cauliflower, bok choi, radish, mustard, and canola. Plants in the *Brassicaceae* family are identified as having flowers with four petals in the shape of a cross and 6 stamens, two of which are shorter. The genus *Brassica* contains important species used for food, condiments, animal fodder and feed, edible oils, industrial chemicals, biodiesel, and garden ornamentals.

One phenotype that is often studied in classrooms and research laboratories is the purple phenotype that results from **anthocyanin production**. Anthocyanin is a water-soluble pigment that gives red, purple and blue colors to fruit, flowers, stems and leaves. There are a number of chemical derivatives of anthocyanin, all from the flavonoid biosynthesis pathway and having the common structure shown in Figure 11. Refer back to Figure 5 to see how this chemical is derived through the entire biosynthetic pathway.

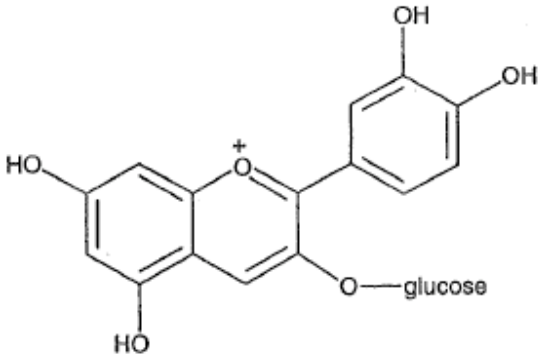

Figure 11. The structure of the most common anthocyanin leaf pigment, cyanindin-3-glucoside. Image from Lee and Gould, 2002.

The expression of anthocyanin pigmentation is studied because of its biological importance and value as a teaching tool. Some researchers have been published papers on the ecological significance of *Brassica* anthocyanin content. Klaper et al. (1996) investigated the effects of UV light exposure on relative production of anthocyanin pigment in *B. rapa* suggesting that the deeper colored pigment and higher anthocyanin concentration serves as protection from UV damaging radiation and that anthocyanin functions as a 'sunscreen' for the plant. Lee and Gould (2002) provide an excellent review of the history and ecological hypotheses for the functions of anthocyanin in plants including UV protection, leaf warming, herbivore and pathogen defense, pollinator attraction, drought resistance, antioxidant protection and others. There is now a growing interest in the genetics and function of anthocyanin, particularly due to increased attention on dietary antioxidants. Although we will be studying anthocyanin expression in leaves and stems *B. rapa*, keep in mind that this is the same pigment that gives apples their red color, the blue in blueberries, and the purple in petunias flowers. Anthocyanin supplies the brilliant colors in coleus leaves, and the red in red cabbage, *Brassica oleracea* (Figure 12).

[1] Modified from "Around the World with Brassicas". 1987. Wisconsin Fast Plants.

Figure 12. Anthocyanin pigment in leaves of ornamental coleus leaves (upper left), leaves of red cabbage *Brassica oleracea* (above) and blueberries (left).

http://journal.farmfreshliving.com/2011/01/23/health-benefits-of-blueberries.aspx

Anthocyanin-less (lack of anthocyanin pigment=green) is a phenotype that Wisconsin Fast Plants® has identified and used as a 'Mendelian marker'. The anthocyaninless gene is thought to encode regulatory proteins responsible for anthocyanin production and distribution (Burdzinski and Wendell 2007). As a marker, *anl*⁻ gene has been incorporated into many rapid cycling *B. rapa* seed stocks to track the effectiveness of plant crosses in combination with other traits undergoing artificial selection. Although purple and green plants are noted as discrete traits with predictable Mendelian inheritance, the extent to which plants are purple can be distinguished as a quantitative trait. You will be investigating anthocyanin expression as a quantitative trait.

The Experimental System- Rapid Cycling *Brassica rapa*

Rapid cycling *Brassica rapa* (Wisconsin Fast Plants®) were developed by plant breeder and pathologist, Dr. Paul Williams. Paul envisioned an "ideal" or model *Brassica* plant to suit his genetics research needs, which would have the following desirable phenotypes:

1. Minimum time from sowing to flowering
2. Rapid seed maturation
3. Absence of seed dormancy
4. Short plant height
5. High female fertility
6. Selection for early flowering

To breed a plant with these phenotypes Paul started with a diverse collection of diploid *B.rapa* plants and grew out a large population. Paul observed the phenotypes within this population and selected plants that had some or all of these characteristics. He then set aside a population of selected plants that were mass pollinated and later harvested seed. This seed was planted and the process of selecting phenotypes and mass pollination was repeated over and over until a 'Fast Plant' seed stock was generated from plants with all of the combined desired characteristics. This breeding scheme is called a *recurrent phenotypic mass selection* and it parallels the process of domestication of animals and plants through human guided artificial selection.

Your Research Team's Task: The Wisconsin Fast Plants Program Needs You!

The Wisconsin Fast Plants (WFP) program is continually investigating how previously described phenotypes vary and are inherited. The WFP program would like to invite you to investigate anthocyanin production in rapid cycling *B. rapa*. Your assignment is to gather evidence about the influence of genetic make up on the anthocyanin phenotype by investigating whether artificial selection can increase the intensity of anthocyanin pigment phenotype. The program is also interested in learning how the phenotypes of anthocyanin production vary in different environmental conditions, and if the variation in expression is highly influenced by the environment or not.

Experimental design for your investigation

Research teams of 4-5 students will make observations and gather initial data on parent populations (P1 and P2) and first filial (F1) populations in lab during the week of Oct. 21. Parent and F1 populations are described in detail in Appendix 1. The big question guiding your research will be:

Big Question:

Is expression of anthocyanin pigmentation in *B. rapa* more strongly influenced by genetic variation (Gv) or by the environmental variation (Ev)?

Or both (Gv x Ev)?

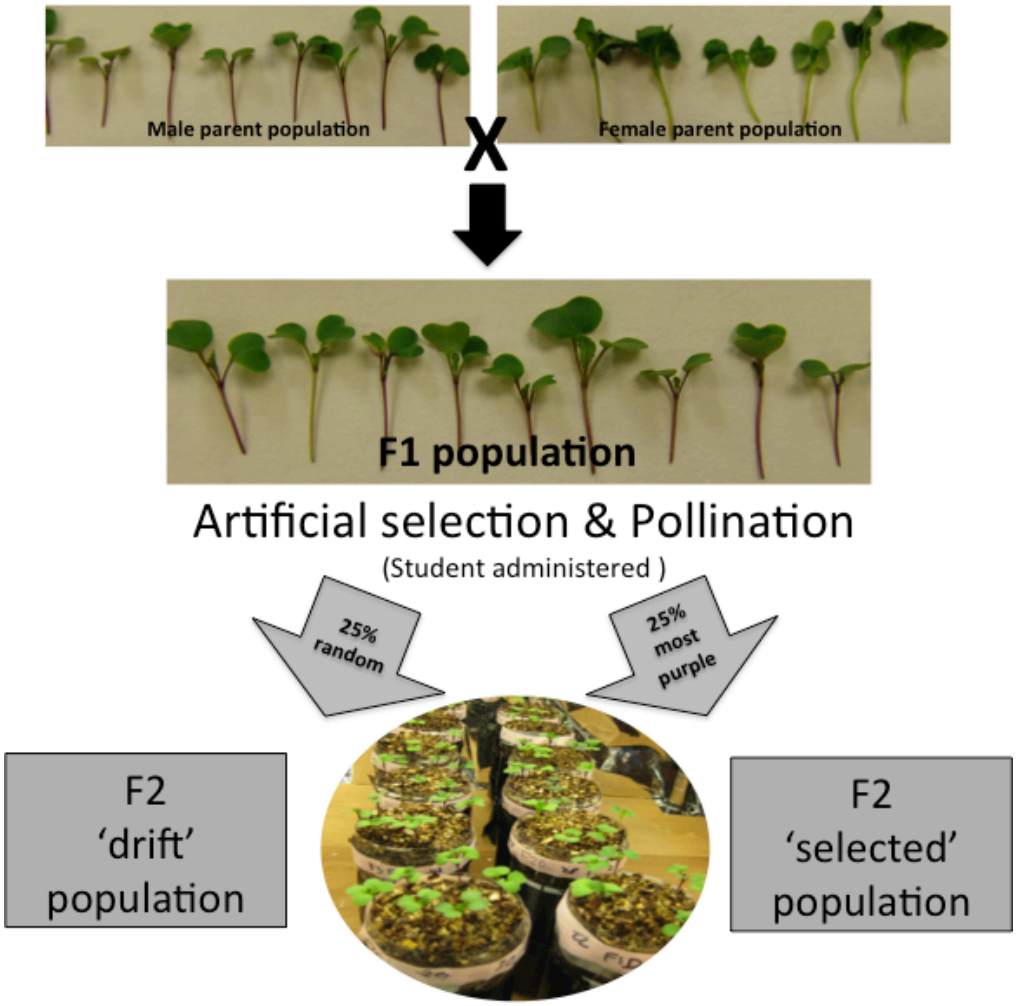

Figure 13. Flowchart illustrating breeding of male and female parent populations to produce F1 population, and derivation of F2 selected and F2 drift populations from interbreeding a subset of individuals from the F1 population.

Each team will generate a two-tiered hypotheses supported by a biological rationale that is grounded in observations and primary literature. Hypotheses should address:

1. *Influence of Genotype:* Your prediction on the **shift in intensity of anthocyanin pigmentation** (high or low) for a selected F2 population relative to unselected F2 drift and F1 populations, grown in optimal (control) laboratory environment
2. *Influence of Environment:* Your prediction about the change in phenotypic variation in **anthocyanin intensity** in the selected F2 population relative to the unselected F2 drift population as influenced by **environment** (your choice of environmental treatment).

Breeding Populations and Experimental Set up

Two parent populations (female P1 and male P2 populations) were intercrossed (open pollinated where pollen from all individuals in P2 population was transferred to all individuals in P1 population) to generate the first filial (F1) population we will be working with in this unit (Figure 11). More information about the background genetics of P1 and P2 populations is provided in Appendix 1. We can continue to produce F1 population seed each time we cross the specific P1 and P2 plant lines. During the first week of this lab unit, you will be observing overall phenotypes of P1, P2 and F1 populations, and making visual measurements of anthocyanin pigmentation using a color scale. Once the F1 population has been examined, you will interbreed a subset of plants, 25% of the population that is most purple, to produce F2 selected population. You will interbreed another 25% randomly to produce F2 drift population, and then make predictions about the expression of anthocyanin pigmentation in these populations when grown together in standard growing conditions (see growth conditions of control environment in

http://www.fastplants.org/grow.php

Control Environment

F1 F2 selected F2 un-selected drift

3 bottles/population x 6 plants/bottle =

18 plants / population x 3 populations=

54 plants total

Experimental Environment

F2 selected F2 un-selected drift

3 bottles/population x 6 plants/bottle =

18 plants/ population x 2 populations =

36 plants total

-

Appendix 3) as compared to an experimental environment of your choice.

Each group will have 15 plastic soda bottles to use as growing containers similar to the cartoon illustration below, together with seeds from F1, $F2_{selected}$ and $F2_{drift}$ populations to conduct investigations. The diagram below illustrates the general experimental design where your team will choose an experimental environment to compare with the 'optimal' control environment. The treatment environment should be one that you predict will influence phenotypic variation in anthocyanin pigmentation but will not kill the plants.

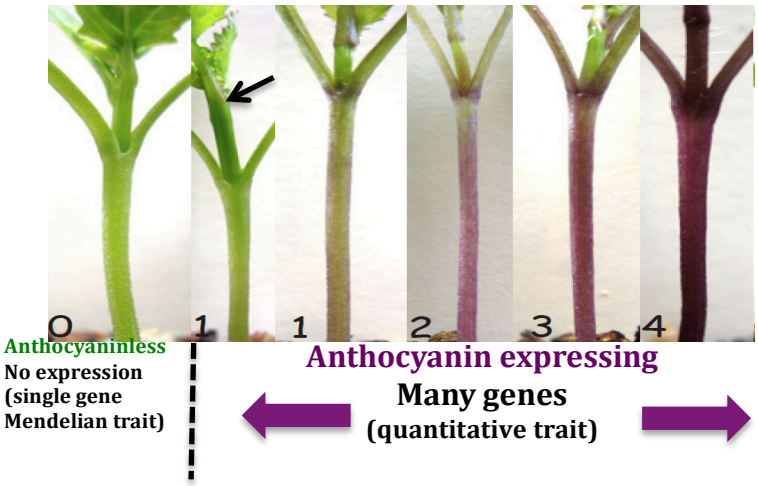

Figure 14. Stem color index for measuring intensity of anthocyanin pigmentation. Note the small arrow in first image for "1" indicating slight purple coloration at the stem node.

Determining the intensity of anthocyanin pigmentation

In your experiments, you will be visually inspecting plant color and ascribing intensity of color with a number consistent with the numbered scale below (Figure 12). Although the trait varies continuously as the color scale suggests we cannot easily distinguish smaller differences than what is indicated on the 4-point scale. Therefore, we will use a scale of 1-4 as a measure of the "intensity of anthocyanin pigmentation" phenotype in the hypocotyl region (stem below the seed leaves or cotyledons) as an indication of the extent of anthocyanin production. Plants scored as '0' on the scale are green and, therefore, indicate no expression of anthocyanin pigment production in the hypocotyl region or anywhere on the plant. We assume green plants are homozygous recessive for the anthocyaninless allele – used as a Mendelian trait marker to keep track of generations and effectiveness of crosses.

Schedule This lab will take 4 weeks (7 sessions) to complete

Week of Oct. 21:	Disc	Introduce P=G + E, the nature of variation and environmental influence
Week of Oct. 21:	Lab	• FastPlant/ Genetics Prelab due at beginning of lab • Introduce *B. rapa* and Fast Plants; anthocyanin production as phenotype; discrete and continuous variation; Mendelian traits and quantitative traits; and environmental influences on phenotypic variation • Do measurements of anthocyanin phenotype on P1, P2, F1 in standard growth environment • Identify range, 25% most purple. Calculate mean, standard deviation. • Make predictions about phenotypic variation in F2selected and F2drift populations supported by rationale. • Generate ideas for alternative environment to test influence on phenotype
Week Oct 28	Disc	Prep Time: Generate informal PowerPoint feedback presentation slides
Week of Oct 28:	Lab	• Present Fast Plant research proposal for feedback- your group will present your informal Powerpoint proposal for your project. Your classmates will ask you questions, provide feedback and any concerns can be addressed. • Introduction to scientific posters and poster template • Do selection and cross pollination: • Generate selected population: cross pollinate the 25% purplest F1 plants • Generate drift population: cross pollinate a randomly selected 25% subset of the F1 plants • *[Outside of class time]* Plant seed and set up growing environments; plant F1 and F2 seed stock in control (optimal) and experimental environments
Week of Nov 4:	Disc	Peer review Fast Plant research proposal mini-posters
Week of Nov 4:	Lab	• Introduce Fast Plant Data Analysis (worksheet) • Hand in Fast Plant research proposal mini-posters
Week of Nov 11:	Disc	Data collection and data analysis work time
Week of Nov 11:	Lab	Data collection and data analysis discussion Introduce Evolution and the Galapagos
Week of Nov 18:	Disc	Fast Plant final mini-posters due in discussion

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Appendix 1. **Fast Plant Seed Stocks**

The plants that are available for you to use in your investigations are described below.

Brassica rapa have flowers that are perfect, meaning each flower has male organs (stamen) and female organs (carpel). However, the plants are self-incompatible and thus pollen from one plant must be transferred to flowers of another plant for fertilization to occur. The F_1 population was produced by first growing up one population of P_1 plants (n=96) and one population of P_2 plants (n=96) and then intermating plants between the two populations. The P_1 population can be described as green and hairy while the plants in the P_2 population are purple and hairy (Figure 1). Controlled crosses were made to create the F_1 population that always used P_1 as the female and P_2 as the male.

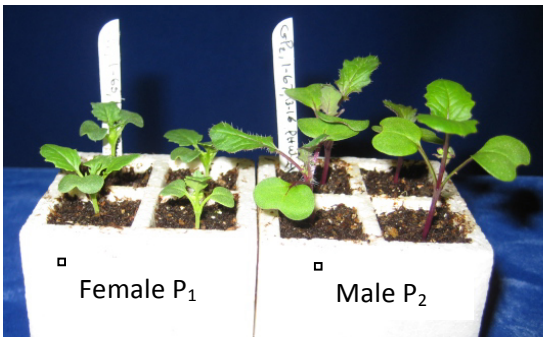


Figure 1. Parent *B. rapa* plants used as male and female to make the F_1 population.

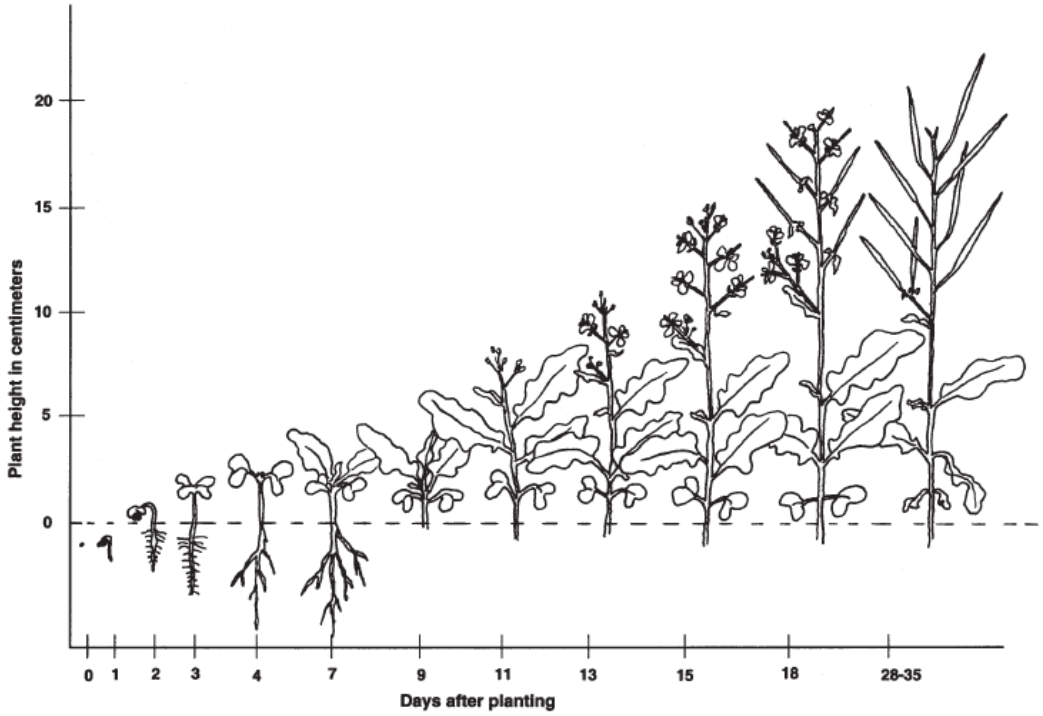
Female P_1, seed stock, 1-37, https://rcbc.wisc.edu/ has the recessive mutant gene, anthocyaninless, *anl*, and produces pure green seedlings, that lack any expression of purple (anthocyanin) color. Anthocyaninless, *anl* was specifically chosen for this investigation to illustrate an easily visualized phenotype and Mendelian marker with discrete variation.

Male P_2, seed stock 1-67, https://rcbc.wisc.edu/ has contrasting phenotypes for the *anl* phenotype exhibited by stock 1-37. Plants from stock 1-67 are wild type for anthocyanin production (*ANL*) meaning the plants produce purple pigments called anthocyanins in the stem and other plant parts including a brown seed coat color. Though stock 1-67 is mainly brown seeded, there is a low percentage of yellow seed in the stock due to the presence of other genes (not *anl*) conditioning yellow seed coat color. The intensity of purple anthocyanin pigment expression appears to be quantitatively inherited. The male parent, P2, seed stock, 1-67, has been developed to exhibit high, though somewhat variable, anthocyanin production as measured visually with 'intensity of purple' scale from 1-4.

F_1 and F_2 Populations The starting plant materials for your investigation are the F_1 plants produced by cross-pollinating genetically distinct populations of 1-37 (female P1) and 1-67 (male P2). These populations have the contrasting phenotypic Mendelian marker trait, green versus purple. These stocks are new to the Wisconsin Fast Plant program and have not been fully evaluated. $F2_{selected}$ and $F2_{drift}$ seed was produced by Biocore 302 – Fall 2011 class. Your class will be doing the same so you can produce F_2 seeds for Biocore 302 class – Fall 2013!

Appendix 2. Fast Plant Life Cycle

The life cycle of *B. rapa* described below is characteristic of the Fast Plant standard seed stock. The seed stock you will use in your investigations is genetically different than the standard stock, but the life cycle stages and growth conditions are the same.



Day -1-0: Quiescent Seed

A remarkable survival strategy that plants have evolved is the capacity to produce seed as a desiccated embryo and remain quiescent for long periods of time during which adverse conditions of the environment might exist. *Brassica* seed embryos will remain viable for more than 25 years if stored in a cool dry environment.

Day 0-2: Germination

Germination begins with the uptake of water, or imbibition, to re-start metabolic processes and begin growth and development. During this process hydrolytic enzymes are activated and begin to breakdown the stored energy of the seed to be used by the developing embryo. As the embryo develops it begins to elongate, pushing through the softened seed coat to expose the root and shoot. Other factors that influence germination include oxygen, temperature, and light.

Day 3-12 Growth and Development

Between days 3 and 12 the Fast Plants will go from a small seedling to a mature plant that is ready for reproduction. Many developmental processes are occurring as the cells expand, divide and differentiate into tissues and organs. Many phenotypes such as plant height, leaf number, stem width, leaf hairs, etc. can be observed and measured during this time.

Day 13-17 Flowering and Pollination

-

Approximately 2 weeks after planting, Fast Plants are sexually mature with flowers beginning to open. Open flowers are ready to receive pollen on their stigma. Fast Plants are self-incompatible meaning that pollen from the same plant will not fertilize an ovule on that plant. In order for fertilization to occur, pollen from one plant must be transferred to another plant. *Brassica* pollen is heavy and sticky and is not carried well on the wind. However, bees and other insects are excellent pollinators of *brassica* flowers.

Day 18-35 Fertilization, Seed Development, Senescence
Fertilization culminates in the creation of a diploid zygote and triploid endosperm. Following successful fertilization, flower organs involved in pollination; sepals, petals, nectaries, stamens and stigma wither and die, while the pistil comprising two fused ovaries (carpels) with their many fertilized ovules undergo rapid enlargement. Within the enlarging ovule the embryo and endosperm continue to develop along with the growth of the supporting fruit (pod) tissue. In the first week, the enlarged ovules contain largely endosperm containing the rich nutrients necessary for the developing embryo. As the embryo continues to develop, nutrients are transferred from the endosperm to the enlarging embryonic seed leaves, the cotyledons. Within the cotyledons, nutrients are first stored as starch and then as the seed begins to desiccate, the starch is converted to oil. As the embryo grows to fill the maternal tissue bounding the ovule, the endosperm is depleted, and the tissues of the ovule wall become the seed coat. As the fruit matures the parent plant begins to wither and die back in a process called senescence. The fruit will continue to dry out as the parent plant dies. Dry pods will ultimately dehisce or break open to spread the seed for the next generation.

Appendix 3. Growth Conditions

These are the conditions that Fast Plants are grown under in our Biocore lab and will be considered 'optimal' for the purposes of growing Fast Plants under controlled environment conditions. Keep in mind that Fast Plants are a product of artificial selection to grow well under these conditions. Wild type *B. rapa* growing in a natural environment likely differ in conditions for optimal growth.

Environmental component	**Optimum**	**Range**
Light (fluorescent)	≥ 200 µM PAR s^{-1}, m^{-2}, 24 hour	100 µM – 200 µM, 24 hours
Temperature	22-26° C	<20 °C growth slows, >30 °C sterility occurs
Gravity	Keep plants growing vertically	
Space	4 cm^2/ plant	
Growth medium Texture	Soil-less mix of 3 parts vermiculite: 1 part peat moss	SunGro®, Redi-earth® Mix
Growth medium volume	15 cc/ plant	
Water	Continuous via capillary wick to reservoir	
Air	Ambient, Relative humidity > 70%	
Nutrition	Based on the soil volume, texture, and watering system described above; plants receive complete fertilizer continuously containing 100 mg/L N, P, and K plus balanced quantities of other minor elements (B, Cu, Fe, Mn, Mo, Zn) http://www.jacksclassic.com/all_purpose.html	

Name:

Lab Section:

Fast Plant Genetics Pre-Lab

1. Rapid cycling *Brassica rapa* Fast Plants, turnips and Chinese cabbage are different domesticated varieties of plants within the same species - *Brassica rapa*. Based on your understanding of a biological species
   a.) Do Fast Plants, turnips and Chinese cabbage have the same *genes* and *alleles***?**
   b.) Explain why Fast Plants, turnips and Chinese cabbage are phenotypically distinct.

2. Describe anthocyanin pigmentation as a *discrete* AND *quantitative trait* in *Brassica rapa.*

3. Explain why quantitative traits such as intensity of anthocyanin pigmentation are influenced by environment to a greater extent than discrete traits.

True Story: Dr. Paul Williams of the Wisconsin Fast Plants program conducted a pilot study in summer 2011 to examine particular crosses of genetically distinct rapid cycling *Brassica rapa* Fast Plants that varied in the expression of anthocyanin (purple) pigmentation. Paul wanted to determine if intensity of anthocyanin pigmentation was highly heritable or whether variation he was observing had more to do with variable growth conditions. He started with parent plants, P1- green and P2-purple, which he cross-fertilized and gathered seed resulting in an F1 offspring population. He then planted the F1 seed, grew 36 plants to maturity, and scored them on a scale from 0= green to 1-3 for increasing purple pigmentation. He then selected nine F1 plants that had the deepest purple coloration and cross-pollinated those plants to produce an $F2_{selected}$ population of seed. As a comparison, he cross-pollinated a random set of nine F1 plants, with no consideration to intensity of purple coloration, and cross-pollinated them to produce an $F2_{drift}$ population of seed. Paul then used reserve seed of the F1 population, and grew F1 plants side by side with the $F2_{selected}$ and $F2_{drift}$ offspring plants. He grew 36 plants of each population under optimal environmental conditions in the Biocore lab and measured stem pigmentation using the standard scale described above.

Use the data Paul collected (Excel file in Learn@UW) to complete the following questions.

5. Draw two diagrams a.) illustrating the crosses that Paul did and b.) the experimental design from which the data in the Excel spreadsheet was generated.

6. Examine the $F2_{selected}$ and $F2_{drift}$ raw data as compared to the F1 data.

   a. Why are there zeros (green plants) in the F2 populations and not in the F1 population?
   b. What is the ratio of green to purple plants in the $F2_{selected}$ and $F2_{drift}$ populations?
   c. Why did Paul include P1 green plants in his initial cross if his objective is to study the variation in anthocyanin pigment expression?

7. Make 3 frequency histograms of raw data overlaid on one graph representing F1, $F2^{selected}$ and $F2^{drift}$ populations (exclude zeros from analysis and graph!). Use different colors to represent the different populations in your graph. Include appropriately labeled axes and a figure legend.

8. Compare and contrast the general trend and distribution for each population represented in the histograms. Explain differences in the distribution of F1, $F2_{selected}$ and $F2_{drift}$ populations based on underlying genetics.

9. Define or explain what a *genotype* is for a quantitative trait like anthocyanin pigment intensity.

10. Calculate descriptive statistics for intensity of anthocyanin pigmentation in F1, $F2_{selected}$ and $F2_{drift}$ populations. Only include plants that produced anthocyanin pigment (exclude zeros) when you calculate statistics for intensity of purple.

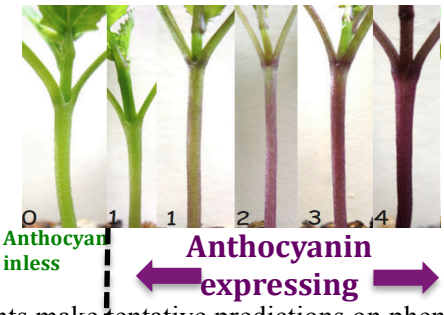
	Intensity of Anthocyanin Pigmentation in Populations		
	F1	**F2 selected**	**F1 drift**
Sample size (N)			
Mean			
s			
SEM			

11. Graph the mean value for each population with ± 1 standard error bars for F1, $F2_{selected}$ and $F2_{drift}$ populations. Include appropriately labeled axes and a figure legend.

12. Based on your analysis of population means, standard deviation, and SE, what can you conclude about the intensity of anthocyanin pigmentation as a result of artificial selection in this case?

13. Identify at least three (3) environmental factors that may influence the relative expression of anthocyanin pigment.

Four-week schedule of activities for inquiry-based curriculum beginning with the driving question "What determines phenotype (Pv=Gv+Ev)?"

	Activities
Week 1	• [Prelab homework] General questions associated with genetics terminology and problems requiring elementary analysis of data similar to analysis students will do during the unit. • Introduction of *Brassica rapa* Fast Plants and P1, P2, and F1 populations used in this unit • Practice using anthocyanin pigment color index  • Students make tentative predictions on phenotypic variation of F2 selected (F2s) and F2 drift (F2d) populations based on measurement of F1 population. Brainstorm environmental conditions that may influence the expression of anthocyanin pigmentation. • Describe final poster assignment and distribute expectations (Supplemental material 6) and experimental design worksheet (Supplemental material 5).
Week 2	• Do artificial selection and pollination on F1 populations of 25% most deeply pigmented plants to produce F2 selected populations and 25% random selection to produce F2 drift population for subsequent years' students. • Student teams propose research projects with PowerPoint presentation and gather feedback on experimental design and hypotheses predicting relative pigmentation intensity of F2selected, F2drift, and F1 populations exposed to control and experimental environmental conditions of their choice. • [Outside of class time] Student groups plant F2 selected, F2 drift and F1 populations in control and experimental conditions guided by online video instruction "Planting *Brassica rapa*" (http://www.youtube.com/watch?v=eEOCRz0j6iA).
Week 3	• Student teams gather preliminary data on pigment intensity of newly germinated seedlings based on color of cotyledons. • Classroom discussion of data and preliminary interpretation including analysis of anthocyaninless Mendelian trait (green v purple ratio) to check for contamination during pollination; analysis of frequency histograms of pigmentation intensity to estimate phenotypic variation of each population. • [Homework] Students' begin to draft scientific poster- individual assignment.
Week 4	• Student teams gather final pigment intensity data on 2-week old seedlings in F1, F2selected, F2drift populations growing in control and experiment environmental conditions. • Class discussion analyzing results of quantitative trait with emphasis on explaining the influence of genotype and environment on the anthocyanin pigmentation phenotype they observed. • Students complete final posters- individual assignment

Learning Objectives

1. Explain **phenotypic variation** as a function of variation in **genotype** and/or the **environment**

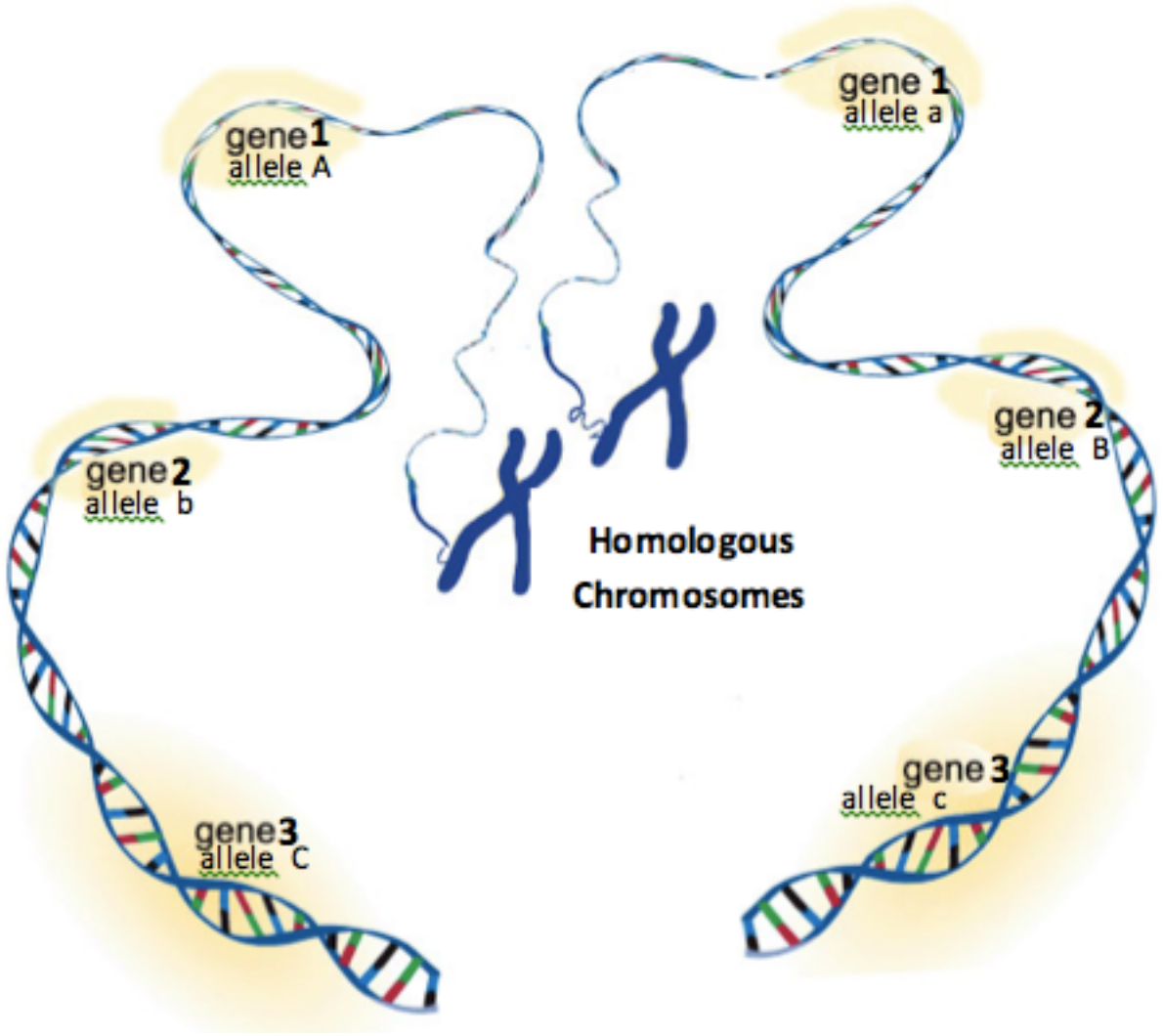
2. Differentiate between **discrete (Mendelian)** and **continuous (quantitative) traits** in terms of how these traits are inherited and expressed

3. Propose a hypothesis and **carry out an experiment** to provide evidence about the effects of artificial selection and environment on a phenotype

4. Construct a **scientific poster** to communicate your proposed research and final results of your research.

Which of the following have the same genes and same alleles?

A. Identical twins
B. Fraternal twins
C. Siblings – same mother & father
D. Half sibs- one parent in common
E. Individuals of the same species in an interbreeding population
F. Individuals of closely related but different species
G. Individuals of distantly related species



Batzli and McGee, UW Madison, Biocore

Photo from National Geographic January 2012 by Jodi Cobb www.jodicobb.com

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Brassica rapa
turnip

Image from
Ecolibrary.org

Brassica rapa
bok choy

Image from Rick and Karin Dina

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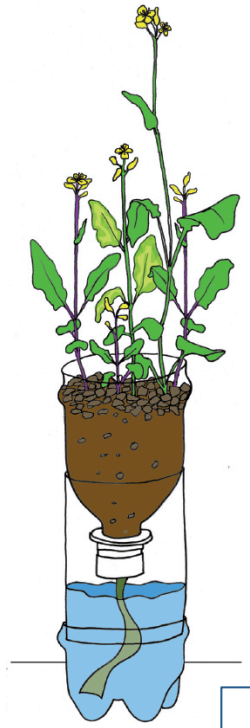
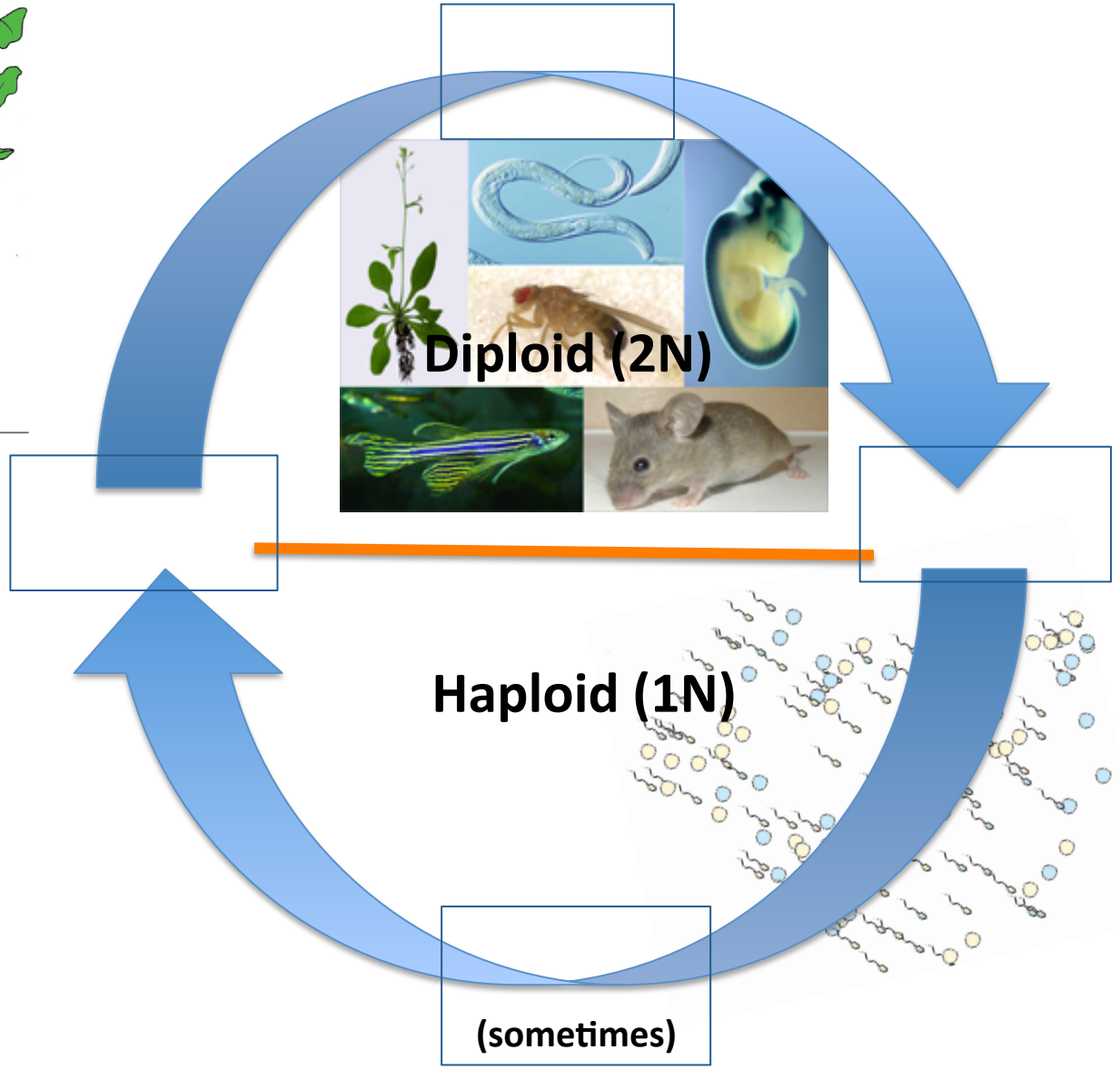
Generalized Life Cycle





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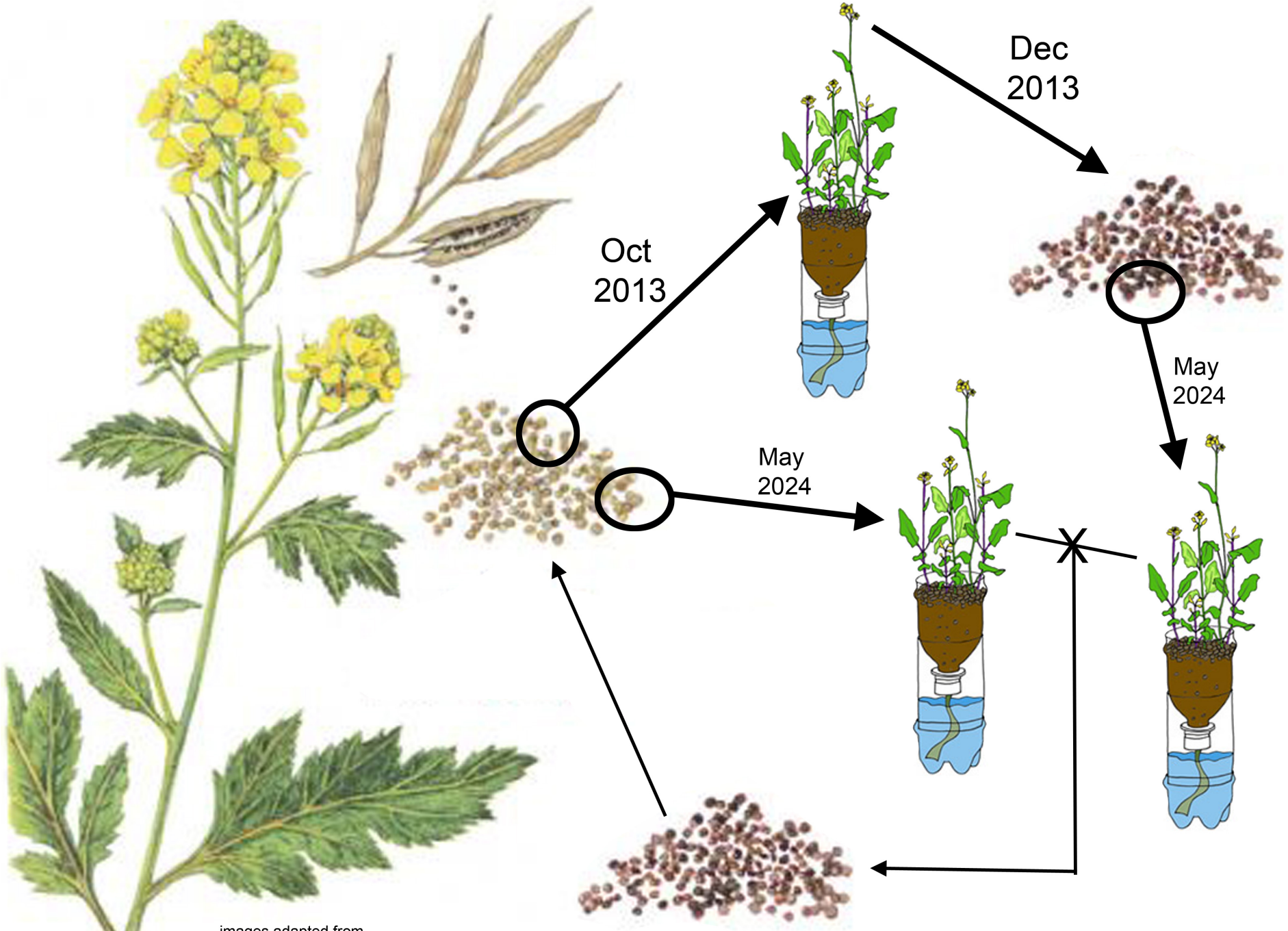

images adapted from
vegetablegardener.com and fastplants.org

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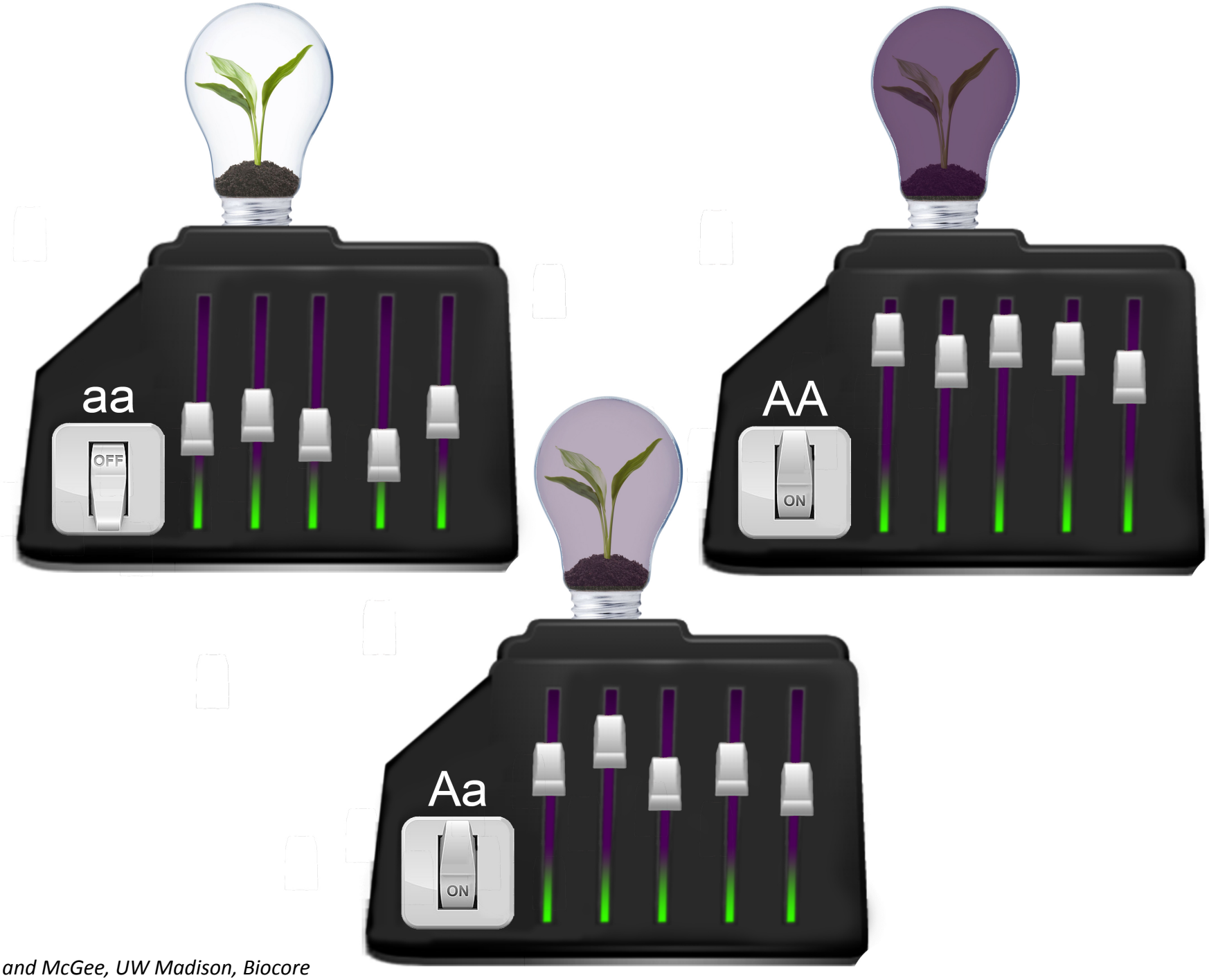

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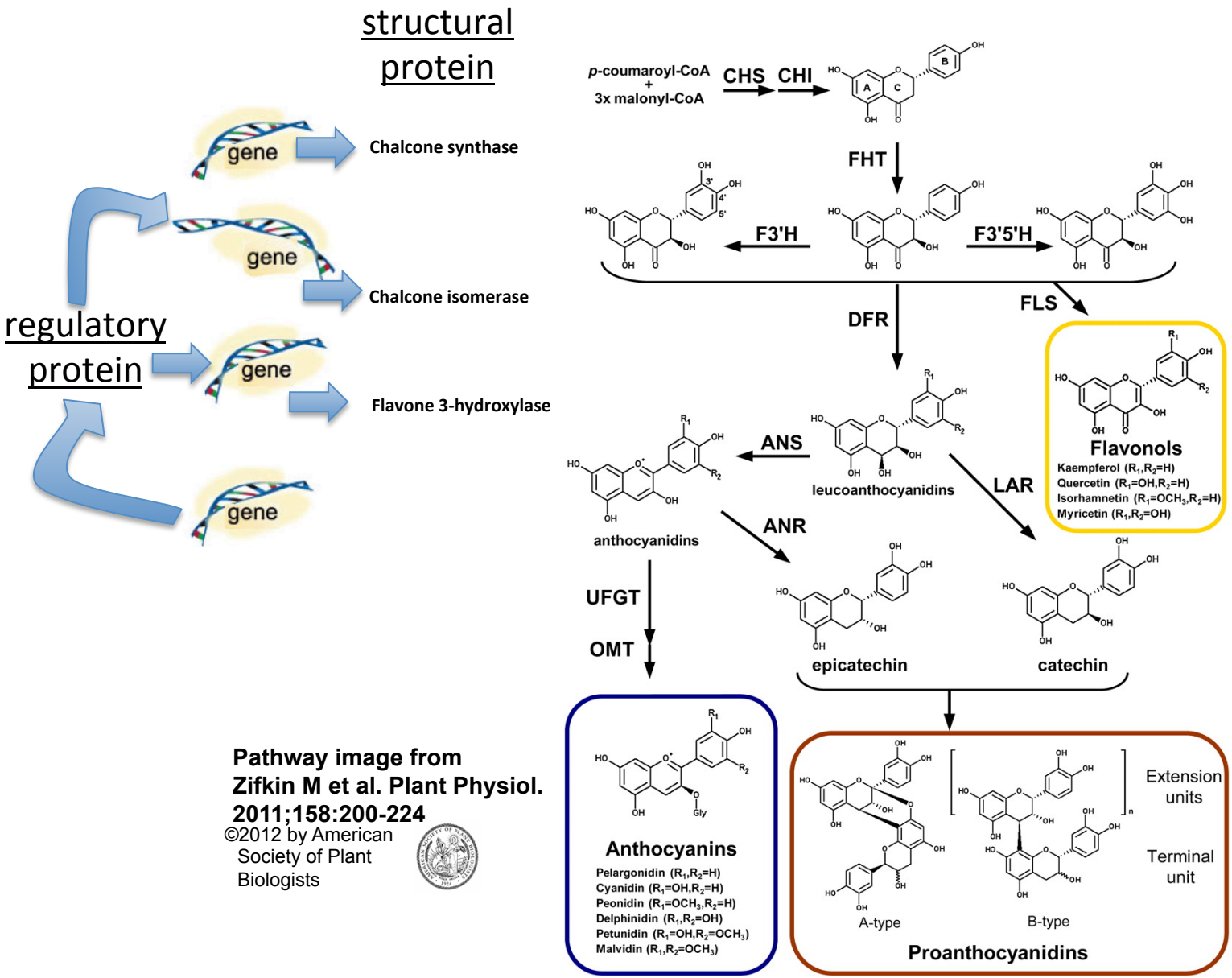
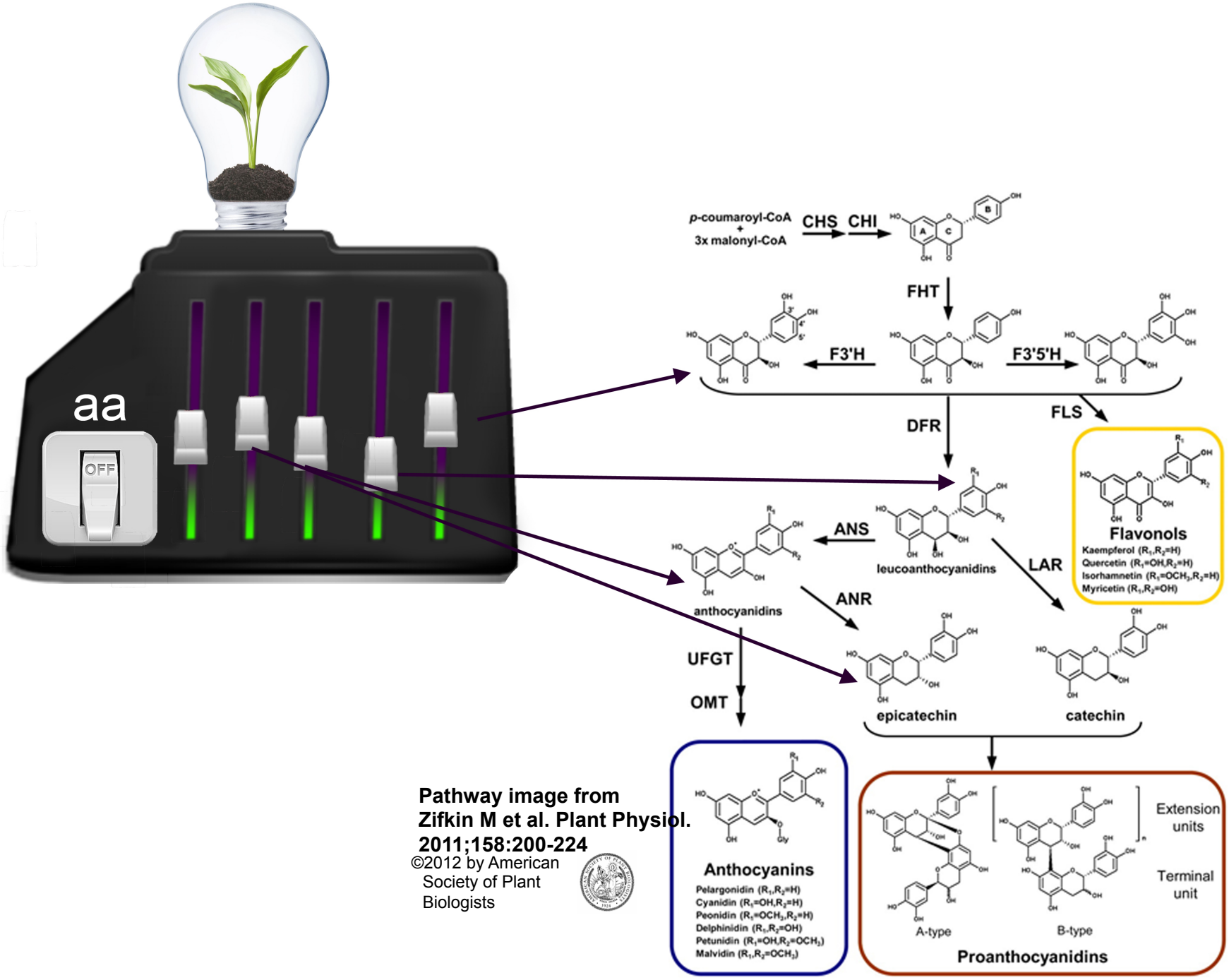
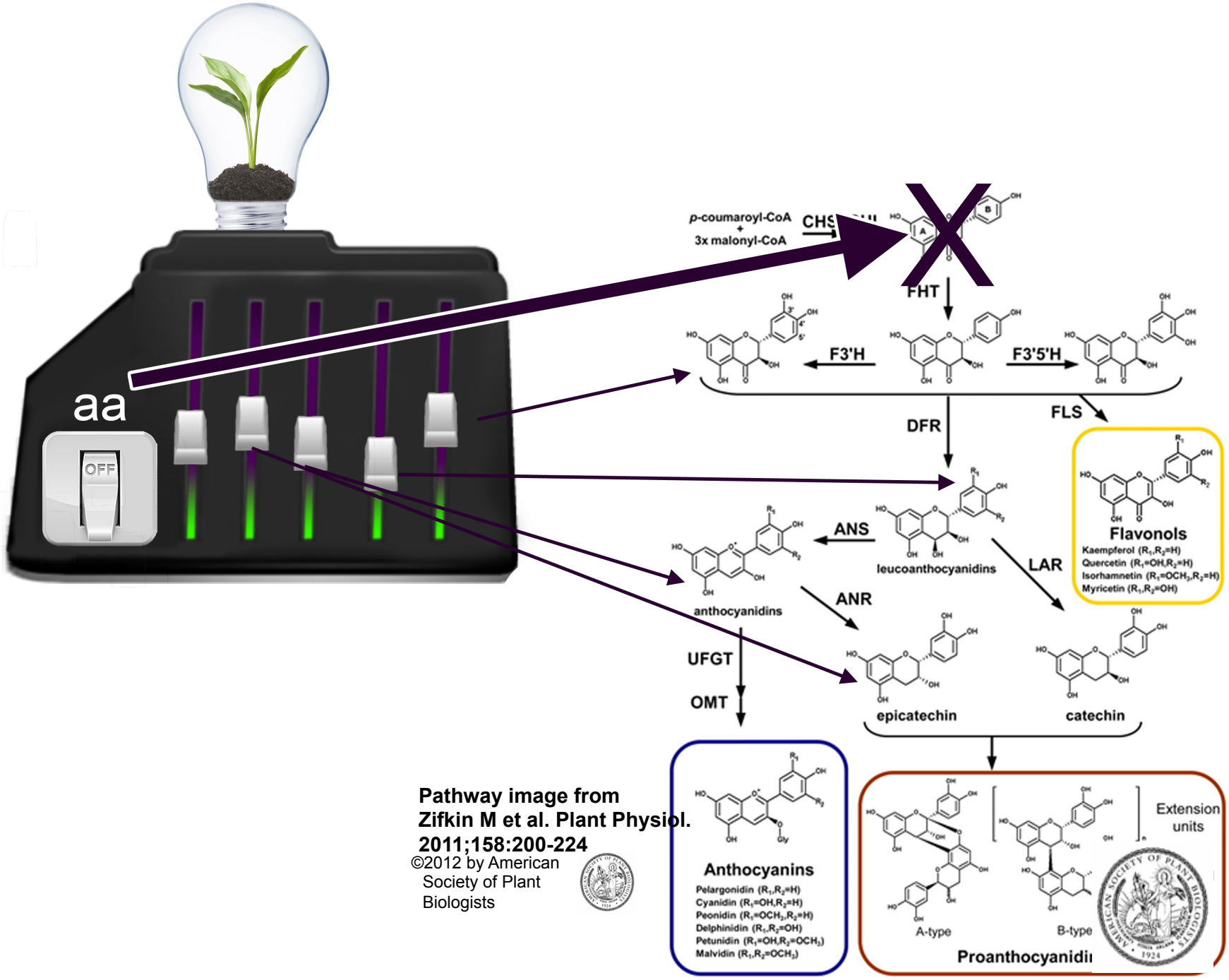


Pathway image from Zifkin M et al. Plant Physiol. 2011;158:200-224



Pathway image from Zifkin M et al. Plant Physiol. 2011;158:200-224

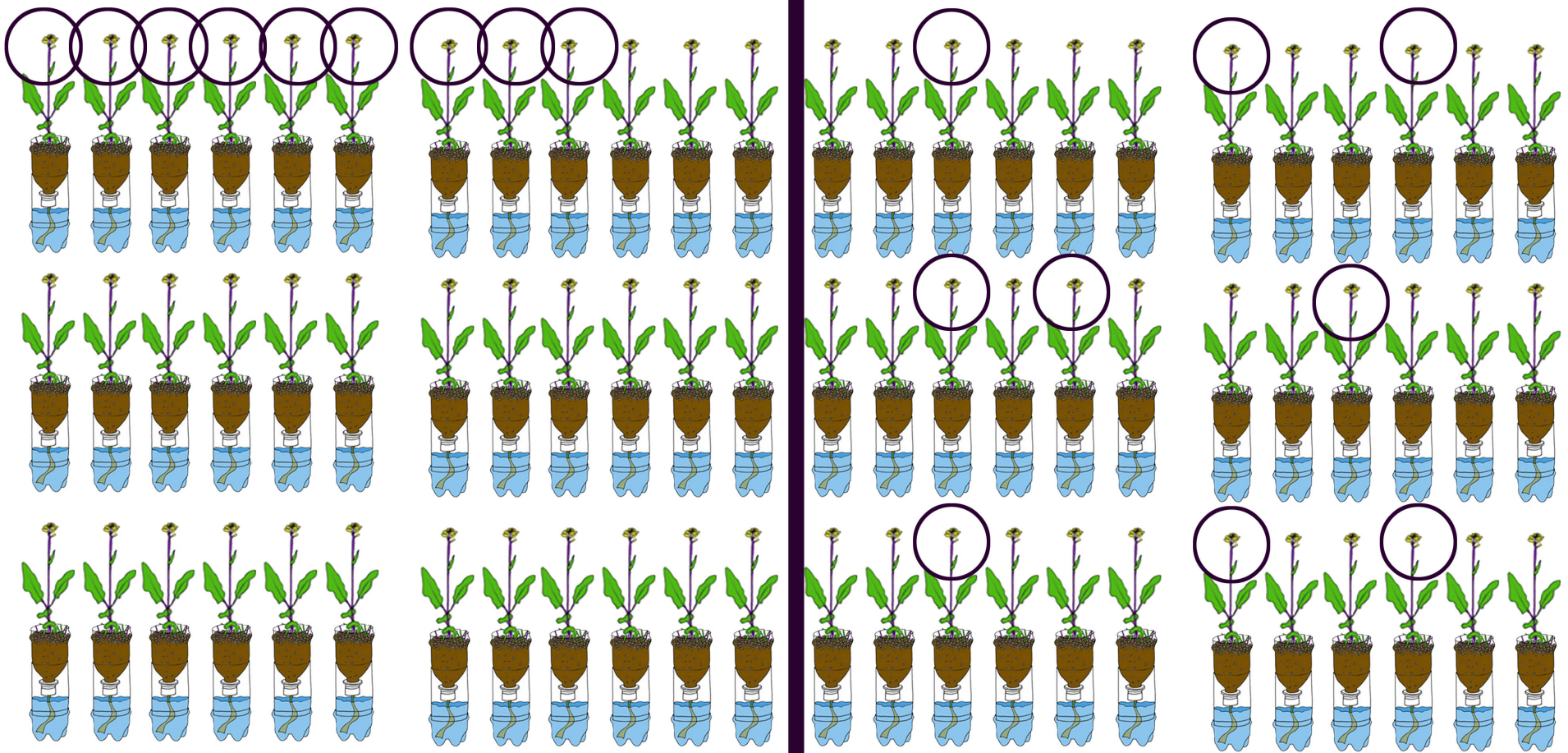


Pathway image from Zifkin M et al. Plant Physiol. 2011;158:200-224

F1

Selected
25% most purple

Drift
25% random



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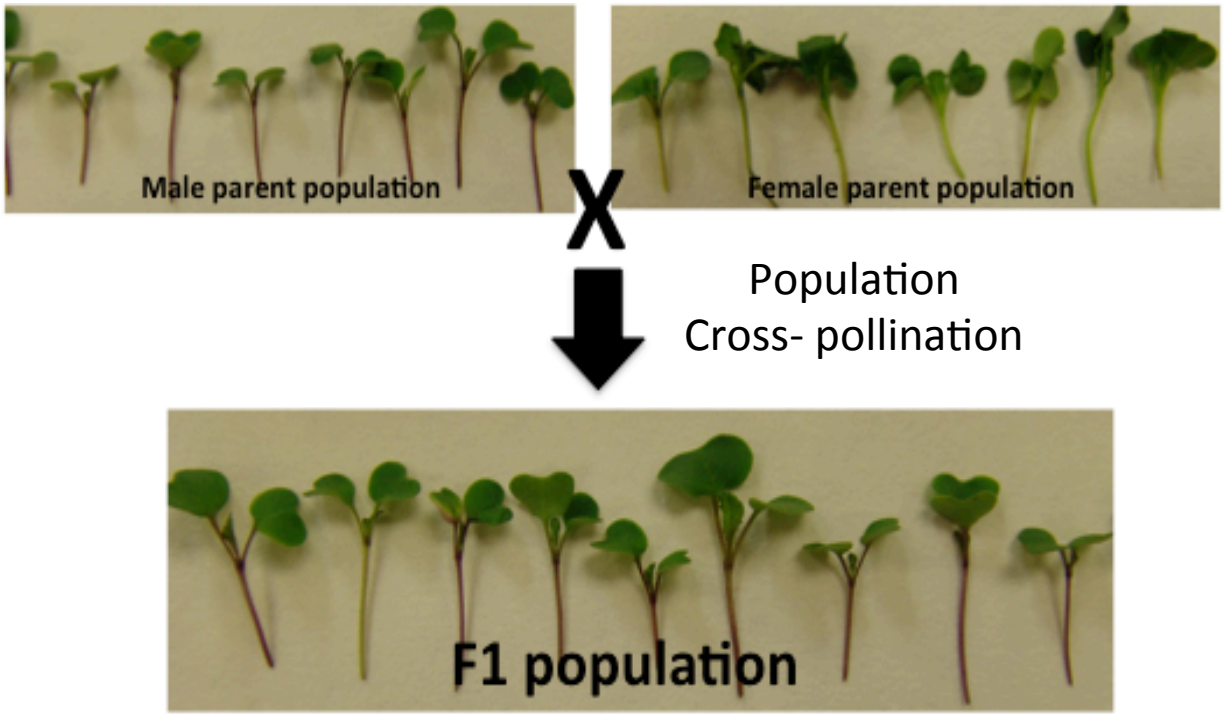

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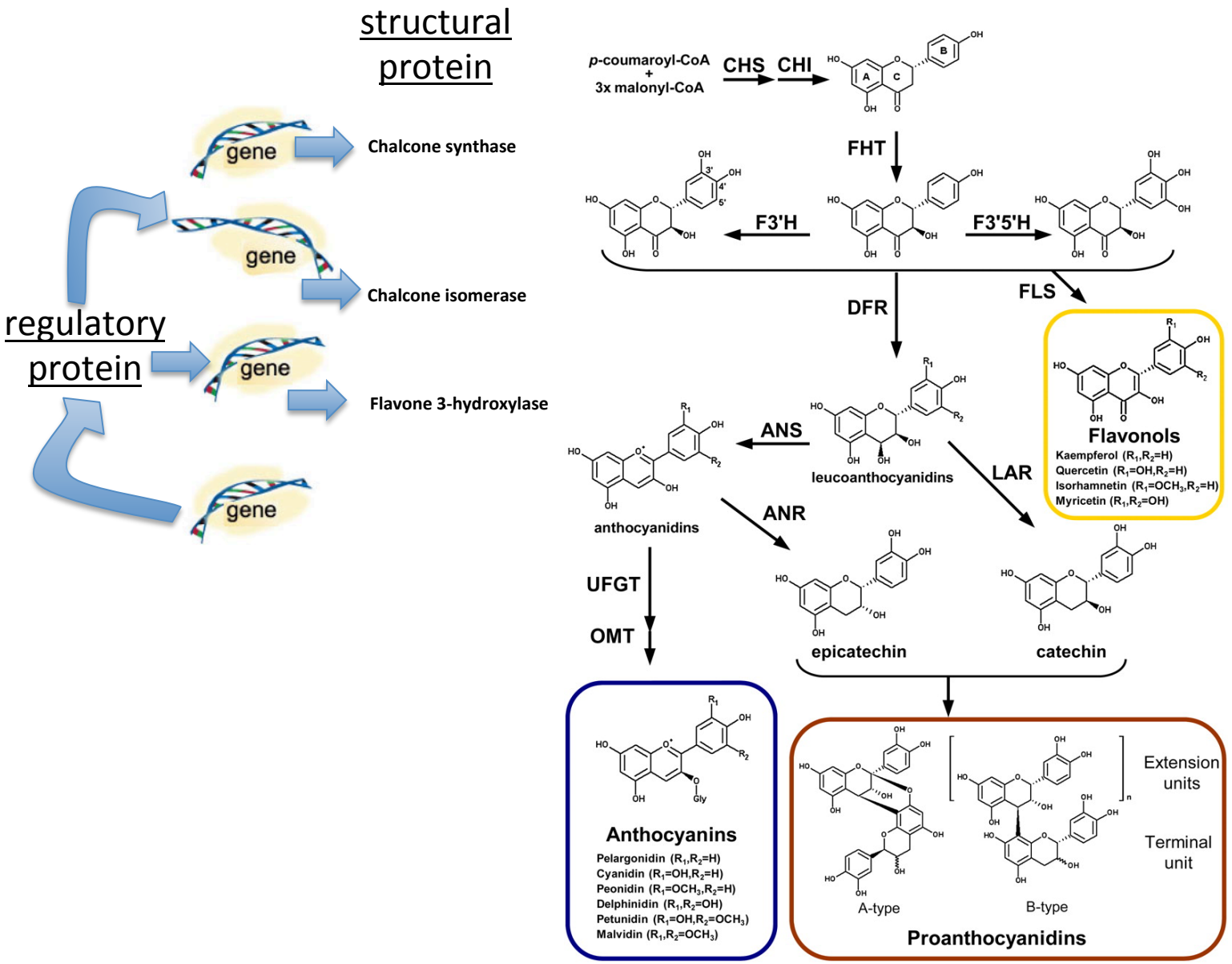
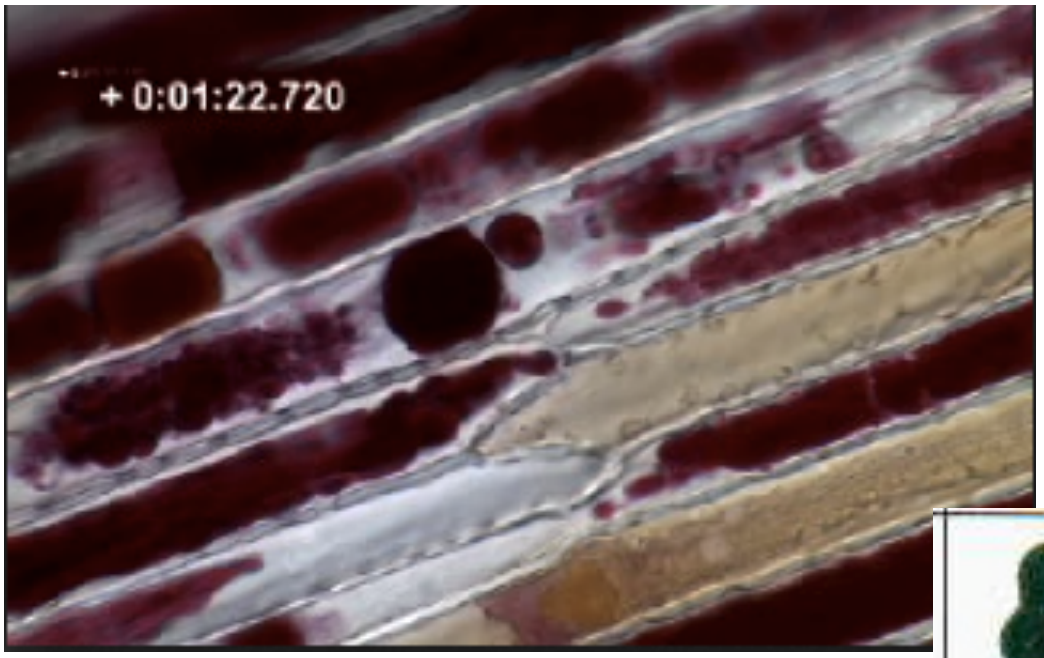
$$Phenotype_{variation} = Genotype_{variation} + ?$$

Discrete phenotype (Mendelian trait):

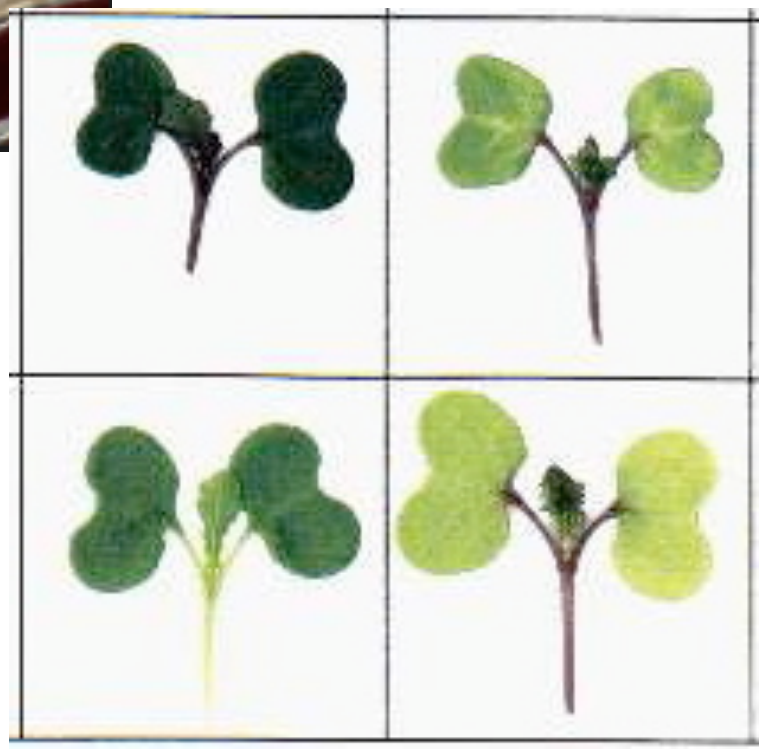
Continuous phenotype (Quantitative trait):



Zifkin M et al. Plant Physiol. 2011;158:200-224



Anthocyanin accumulation in plant cell



Video courtesy of Irani N, Grotewold E.
http://tinyurl.com/9f37y6c

Team members:

TA and Lab Section:

Experimental Design Worksheet

This worksheet is meant to help you prepare for your Fast Plant investigation and as a general outline for your feedback presentation during the week of Oct. 28.

1. State the testable question(s) that your group will investigate during the Fast Plant unit.

2. State the two-tiered testable hypotheses for your study.

3. List at least 3 topics/ concepts and at least 3 search terms per concept that you consulted when searching for background information on your question. We recommend going to general sources (e.g. textbook and lab manual) first to define key terms, and then diving into the library databases and primary literature.

4. Using bullet points, a diagram, and/or brief statements, describe your reasoning as to why you think each of your hypotheses is true (biological rationale). Make sure to provide reasoning for both of your hypotheses—tier 1 (genetics) and tier 2 (genetics and environment).

5. Create a diagram paired with descriptive text that explains the methods you propose to test your hypotheses. In your diagram and text, indicate what a sample is for your study, number of observations (replicates), how measurements are taken (measurement scale & where on plants), when & you take anthocyanin measurements?

6. Illustrate (histogram(s) for tier 1 and bar chart(s) for tier 2) your EXPECTED and ALTERNATIVE results using a set of 'hypothetical' data that corresponds to your hypotheses. Provide a full figure legend to accompany your graphic.

7. Describe your figures and how expected and alternative results differ from one another. Provide a plausible reason, based on the biology or some element of the system, why you may obtain alternative results. (What assumptions are you making that may not be in play? Could your biological rationale be flawed or not applicable in this case?)

8. Describe the implications of your investigation. How will your expected results inform the existing understanding of the system-- in light of assumptions you have made and the limitations you have? What are the implications of your alternative results?

9. State a list of questions you still have that have to do with your project. You may have questions about the biorationale (especially if you feel that you are making big assumptions), your methods, your independent or dependent variables and how to measure, confounding variables, or the plausibility of the expected results. Your questions will help focus the feedback during presentations.

10. Provide a full list of citations you referred to in the development of your project. Utilize proper citation format outlined in the WM.

Your results about variation in anthocyanin expression in *Brassica rapa* Fast Plants as influenced by variation in genotype and environment

Author and Collaborators

Introduction

Big idea is variation in phenotype resulting from $G_v+E_v+(G_vxE_v)$

1. What is phenotype composed of? What phenotype are you studying? What is phenotypic variation in this quantitative trait?
2. To what extent does this phenotype vary in *B. rapa?* What are rapid-cycling *B. rapa* Fast Plants? How can we use *B. rapa* to learn about genetics of quantitative traits?
3. What does the literature say about the heritability of anthocyanin in *B. rapa* Fast Plants? Influences of Gv and Ev, separately, and GxE interaction? How does artificial selection and drift account for differences in allele frequencies? Are there particular loci stimulated to express given particular E? Can you use a diagram to explain your rationale?
4. Why do you plan to use the F1 and $F2_s$ & $F2_d$ plants? What is the genetic origin of the F1 plants (appendix 1 lab manual)? What is the significance to investigation of G & E?

Hypotheses:

1) *Genotype/ Allelic:* Prediction for variation of intensity in anthocyanin for in $F2_s$ & $F2_d$ and F1 grown under optimal conditions

2) *Environment & Genotype:* Would you expect to see the same or different relationship between $F2_s$ & $F2_d$ phenotypes grown in alternative environment as compared with optimal (control) conditions ?

Big Clear Results

1. Be sure to label axes well and include figure legends for histograms and bar charts
2. If illustrating means in bar charts, include error bars and make it explicit whether bars represent standard deviation or standard error.
3. Include brief text explaining graphs and emphasizing particular attributes of graphs that will be further discussed in the implications section.

Methods

1. Clear diagram of experimental design with treatment would be helpful- with labels or additional explanations as necessary.
2. What about Mendelian trait? Why included/ how used?
3. Data analysis- what statistics will be generated and what will each statistic tell you?
4. Make it clear how you are defining replicates.

Discussion and Conclusion

1. How do expected results inform our understanding about about how phenotypic variation is influenced by variation in G as well as variation in E in the context of the $F2_s$, $F2_d$, F1 populations and your treatment environment?
2. What are the implications of your predictions if you observe a gain in selection or not?
3. What are the implications if there is a difference in response of $F2_s$ and $F2_d$ to the control and experimental environment (GxE)? What do results tell us about gene expression of a multigenic trait, biosynthesis or biodegradation of anthocyanin?
4. Why might you get alternative results? State reasoning with biological explanation.
5. Can your observations of P1, P2, F1 plants from week 1 lab be useful in helping you explain expected / alternative results?

Literature Cited

- You need to support your ideas with literature and observations
- Make sure you look in lab manual reference section; numeric citation format is fine for posters
- Lee and Gould 2002; Holton & Cornish 2007 are good review papers to help you begin your search.

Note: be sure to refer to WM p. 48 for preparing posters and p. 58 for proposal poster rubric

A. pre
B. post
allele
allele frequency
chromosome
crossover
disease
DNA
dominant
evolution
gene
GxE interaction
genetic drift
genotype
heredity
heritability
inheritance
meiosis
Mendel
mitosis
mutation
phenotype
phenotypic plasticity
Punnett square
quantitative
quantitative trait
recombination
reproduction
selection
trait
variation
high co-occurrence
low co-occurrence
high occurrence
low occurrence