Teacher's guide

Clover Lab – Cyanogenesis Assay Pre-Lab: Activity A

http://pages.wustl.edu/cloverproject

Essential Questions

- What adaptations have evolved in plants to provide protection from herbivory?
- What is the genetic basis of the protective adaptations?
- What factors might cause the genes for those adaptations to vary within a species?

What *is* Clover Cyanogenesis?

White clover (*Trifolium repens*) is a forage plant, native to Eurasia, which has spread throughout the world. It is very tasty to many herbivores including, insects, aphids, mollusks, and large mammals (horses love it!).

1. **Together with your lab partners, brainstorm, then list, different types of protective defenses that plants have evolved to prevent herbivory (being eaten by herbivores).**

   Thorns, chemicals, taste, thick skin, etc.

2. **Based on the appearance of clover, which of the protective adaptations from your list above do you think clover would use to avoid being eaten by herbivores?**

   Chemical, since none of the other defenses are evident.

The Cellular Biology

White clover produces a poisonous compound called **hydrogen cyanide**. Cyanide is a very fast acting poison that inhibits enzymes in the mitochondrial electron transport chain, and hence blocks electron transport. Without the electron transport chain, most organisms cannot produce ATP. The inability to produce ATP quickly leads to death. Small organisms that eat cyanide producing clover either die from eating too much or leave the plant alone in favor of other acyanogenic (non-cyanide producing) clover or other herbaceous plants.

The ability to produce cyanide in clover is the direct result of two genes that control the production of two compounds required for cyanogenesis, as well as the absence or presence of herbivores in the area. One gene codes for a cyanogenic glucosides (sugar compounds with a cyanide containing group attached), and the other gene codes for an

enzyme that is required to cleave the cyanide from the sugar so that the cyanide can be released.

3. **Do you think cyanide will kill the plant cells? Why or why not?**
   Yes

4. **Why would having two genes to control the release of the poisonous cyanide be advantageous to the plant?**
   Two genes to control the production of cyanide would provide the plant with more control over the production of the toxic chemical. The two gene products could be sequestered in different parts of the cell to prevent the cyanide from killing the cell.

5. **Recall what you learned about plant cell structure. How might a clover plant keep the two compounds separate within the cell?**
   One of the chemicals could be sequestered in the central vacuole. The enzyme is actually contained in the cell walls.

6. **How would grazing on clover cause the release of the cyanide?**
   Mechanical crushing of the cells would cause the two chemicals to come in contact with each other and produce cyanide, thus hopefully discouraging continued herbivory damage.

The Genes

In order to produce cyanide, a plant needs a copy of the dominant allele for gene controlling production of the cyanide containing sugar **and** a dominant allele for the enzyme to release the cyanide from the sugar. In clover, the cyanide-sugar compound is called **linamarin/lotaustralin** and the enzyme is **linamarase**. For simplicity purposes, we will use the letter **C** for the cyanide-sugar compound and **E** for the enzyme.

7. **Which of the following genetic combinations would result in the production of cyanide?**

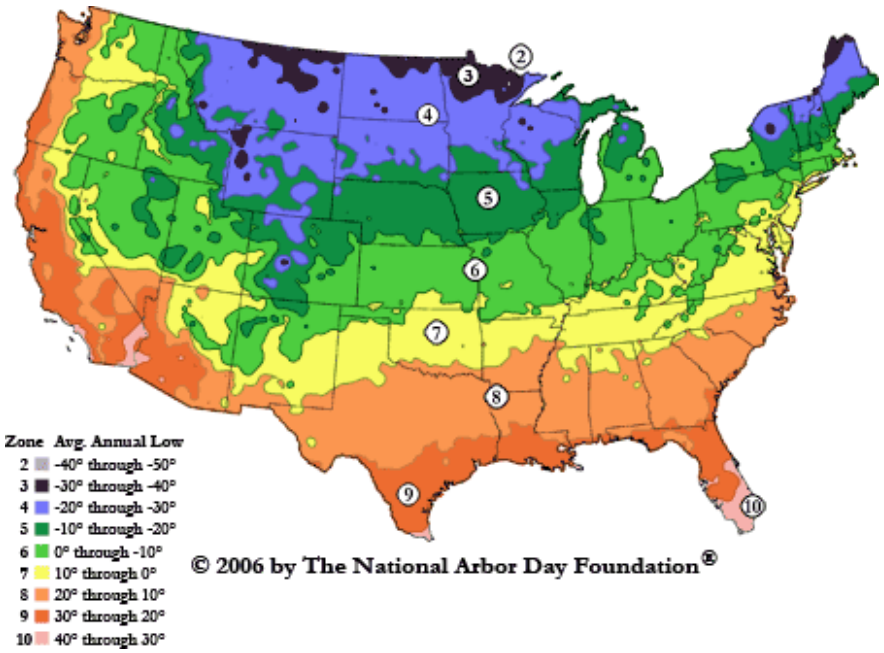
Genotype	Cyanide-sugar Production (y/n)	Enzyme Production (y/n)	Cyanide Produced (y/n)
CCEE	*Y*	*Y*	*Y*
CCee	*Y*	*N*	*N*
CcEe	*Y*	*Y*	*Y*
ccEE	*N*	*Y*	*N*
ccEe	*N*	*Y*	*N*
ccee	*N*	*N*	*N*
Ccee	*Y*	*N*	*N*
CCEe	*Y*	*Y*	*Y*

The Environment

Cyanogenic clover plants are found more often in warmer climates. The frequency of cyanogenesis **decreases** as one moves further north into cold climates as well as at higher elevations.

8. **Why would it be a disadvantage to a plant to produce cyanide in climates where the temperature regularly falls below freezing?**
   Cold temperatures that cause water to freeze and cells to rupture would result in the sugar-cyanide compounds and linamarase coming in contact with each other and poisoning the cells. Also, the higher metabolic cost of being cyanogenic would perhaps place the cyanide producers at a survival disadvantage in less than favorable climatic conditions, especially if there are fewer herbivores in colder climates and less need for protection.

9. **Using the climate map below, make a prediction about the percentage of your clover plants you expect to be cyanogenic (produce cyanide). Use the data below as a guide for your prediction.**
   86% in New Orleans, LA 15% in St. Louis, MO 10% in Wassau, WI Answers will vary. Colder climates would be expected to have less cyanide- producing clover than warmer climates.



Location	New Orleans, LA	St. Louis, MO	Wassau, WI
Predicted % of Cyanogenesic Clover Plants	*86%*	*15%*	*10%*

The Metabolism

Biosynthesis is the production of biological compounds by organisms. Clover plants, in order to be cyanogenic, must synthesize both compounds – the cyanide-sugar and the enzyme. Synthesis is an energy consuming process.

10. **Based on the fact that synthesis requires energy, do you think it would be an advantage to a clover plant to produce the linamarase enzyme if the cyanide containing sugar is not present or to produce the sugar if the enzyme is not present?**
    No. It is energetically wasteful to invest in biosynthesis of one compound if the other is not available. Such metabolic waste would most likely be selected against.

11. **Based on your answer, make a prediction about the relative frequency of the following genotypes:** *Results will vary.*

Genotype	Predicted Relative Frequency
C_ee	
ccE_	
C_E_	
ccee	

12. **Cyanogenic glucosides can be used as a way of storing energy and so can serve a function besides cyanogenesis. Linamarase, on the other hand, is only useful in cyanogenesis and is energetically especially expensive to produce. Based on this information, would you expect to find more C_ee plants or more ccE_ plants in nature? Explain why?**

    More C_ee plants. C_ee plants can use cyanogenic glucosides even though they don't have linamarase. In contrast, ccE_ plants carry the energetic burden of making linamarase, but without the benefit of being cyanogenic.

Reviewing Mendelian Genetics and Natural Selection

First, define the following terms in your own words.

Heredity	*Transmission of traits from parents to offspring.*
Gene	*A unit of heredity; also, a region of DNA that is transcribed as a unit.*
Allele	*Either of 2 or more alternative forms of a gene.*
Genotype	*The combination of alleles for a given gene; the genetic make-up of an organism.*
Phenotype	*The physical appearance or function of an organism as determined by their genotype as it is expressed in the particular environment in which that organism lives.*
Natural Selection	*Differential survival and reproduction of some individuals over others in a population based on their phenotypic differences.*
Evolution	*Changes in species over multiple generations.*
Adaptation	*A trait that is favored by natural selection because it increases an organism's ability to survive or reproduce in its environment.*

Applying Mendel's Concepts

13. **There are 2 alleles of the gene that controls the ability to produce the cyanide containing sugar. In order to express this trait, the clover must have a dominant allele at the C gene. Using upper-case or lower-case letters for dominant and recessive alleles, write the allele combinations that could be found in a clover plant. Underline those that would produce the cyanogenic glucoside.**
    CC *Cc* *cc*

14. **There are 2 alleles of the E gene which controls the production of the enzyme required to break down the cyanogenic glucosides and release cyanide. In order to express this enzyme, the clover must have a dominant allele. Using upper-case and lower-case letters, write the allele combinations that could be found in a clover plant. Underline those that would produce the cyanide-releasing enzyme.**
    EE *Ee* *ee*

15. **Cross a clover plant that is homozygous dominant for both the cyanogenic glucoside and linamarase enzyme trait with a clover plant that is recessive for the cyanogenic glucosides and enzyme trait. What genotype and phenotype ratios could we expect in the offspring?**

    All of the offspring will be heterozygotes at both genes: Cc Ee
    So all of the offspring will be cyanogenic.

Applying Darwin's Theory of Natural Selection

Natural selection occurs in populations of living things as a result of genetic variation and an overproduction of offspring within the population. This results in a struggle for existence, which leads to differential survival and reproduction within the population. Over time, this process can result in big changes in a population.

16. **Based on your understanding of how natural selection and heredity works, explain how cyanogenesis in clover (and other plants) might have evolved.**

    In environments where herbivores are abundant, plants that are cyanogenic would be better able to survive and reproduce, so the frequency of cyanogenic plants would increase. If there are some environments where herbivores are less common, the costs of making linamarin and linamarase could outweigh the benefits; under those conditions, plants that are not cyanogenic would be the ones that survive and reproduce best.