



Student guide

Name:

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).**
  
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?**

### 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?**
  
4. **Why would having two genes to control the release of the poisonous cyanide be advantageous to the plant?**
  
5. **Recall what you learned about plant cell structure. How might a clover plant keep the two compounds separate within the cell?**
  
6. **How would grazing on clover cause the release of the cyanide?**

### 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.

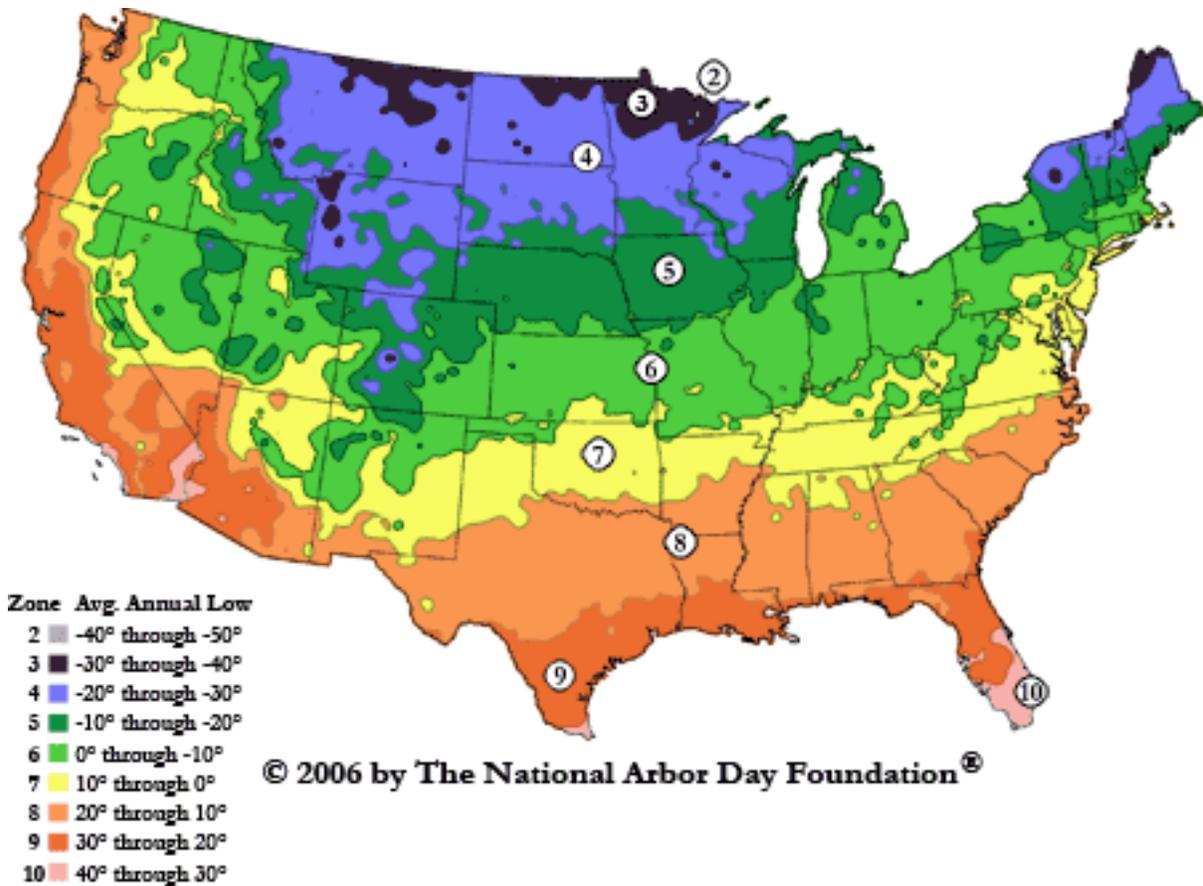
7. **Which of the following genetic combinations would result in the production of cyanide?**

Genotype	Cyanide-sugar Production (y/n)	Enzyme Production (y/n)	Cyanide Produced (y/n)
CCEE			

## 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?
  
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.



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

## 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?**

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

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?**

## Reviewing Mendelian Genetics and Natural Selection

First, define the following terms in your own words.

<b>Heredity</b>	
<b>Gene</b>	
<b>Allele</b>	
<b>Genotype</b>	
<b>Phenotype</b>	
<b>Natural Selection</b>	
<b>Evolution</b>	
<b>Adaptation</b>	

### 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.

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.

- 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?**

### **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.**