NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

I'm Looking Over a White-Striped Clover: A Case of Natural Selection

by
Susan Evarts, Department of Biology, University of St. Thomas
Alison Krufka, Department of Biological Sciences, Rowan University
Luke Holbrook, Department of Biological Sciences, Rowan University
Chester Wilson, Department of Biology, University of St. Thomas

Learning Objectives

- Understand the process of natural selection and the importance of environment-specific adaptations.
- Acquire an understanding of the concepts of *variation, natural selection, fitness, selection pressure, evolution,* and *adaptation*.
- Use these terms in a paragraph to describe the frequency of the two types of white clover.
- Predict the distribution of cyanogenic clover in given microhabitats.
- Gain experience with the scientific method and be able to propose hypotheses and justifications to explain the frequency of the two types of white clover.
- Design experiments to test hypotheses and describe data that would support these hypotheses.
- Understand and synthesize information from figures and tables.
- Be able to quantify the strength of selection and the relative fitness of the different clover forms in different habitats.
- Understand what values for strength of selection and relative fitness mean in terms of how selection is acting on these plants.

Part I – "I'm Looking Over. . ."

White clover *(Trifolium repens)*, a small perennial plant, is found throughout the world, and has two forms. One variant has entirely green leaves *(plain)* and the other has green leaves with a prominent white stripe *(striped)*.

Both variants of white clover (plain and striped) are found along the coast of Long Island, New York. Most of Long Island is only a few feet above sea level. A series of low grass-covered hills separated by shallow depressions covers the area behind the oceanfront dunes. The shallow depressions reach to the water table, so they tend to be permanently moist year round and do not freeze in winter. Water drains away quickly from the low hills, which tend to dry out many times over the year and freeze in the winter. The habitat in the shallow depressions is more hospitable to molluscs (snails and slugs) that feed on clover. One type of clover is more common in shallow depressions while the other type is more likely to be found on low hills.

At the end of the case, we will come back to New York and ask you to predict which type of white clover is most abundant in each microhabitat. But first, let's consider the abundance of these two types of clover on a larger scale.

Figure 1 shows the relative frequency of white clover variants in Minnesota and North Carolina. Table 1 provides additional information on Minnesota and North Carolina.

Figure 1. Relative frequency of plain and white-striped clover in two different habitats.

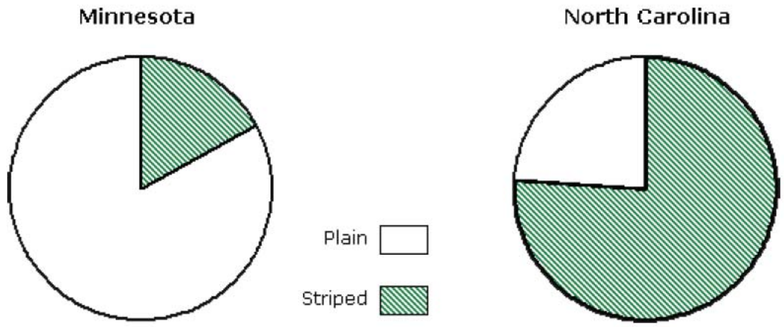

Table 1. Physical and ecological factors of typical habitats in Minnesota and North Carolina

	Minnesota	*North Carolina*
Latitude	43–49° N	34–36° N
Mean elevation	0.365 km	0.213 km
Ave. monthly temp. range	–19.4° to 28.6° C	–2.6° to 31.3° C
High temperature	45.6° C	43.3° C
Low temperature	–51° C	–37° C
Mean # days with high above 32° C*	14	38
Mean # of days with low below 0° C*	154	75
Ave. yearly precipitation	66–76 cm	107–117 cm
Presence of herbivores (molluscs such as snails, slugs)	smaller population, not present in winter	larger, more active population, present all year
Data from Netstate.com and National Oceanic and Atmospheric Administration. *Data for capitol cities (St. Paul , MN, and Raleigh, NC).		

Exercise 1

A habitat is defined as the place and conditions under which an organism lives. This includes physical factors such as temperature, soil type, availability of nutrients, and availability of moisture as well as biological factors such as presence of herbivores, competitors for nutrients, and pathogens. Using the information in Table 1, briefly summarize the habitat features for white clover in each state.

Part II – Unlucky Clover

Some variants of white clover produce cyanide (CN), which is a powerful poison. Two gene products are required to produce active cyanide. One gene encodes an inactive cyanide-sugar complex that is stored in the plant cell's cytoplasm. The other gene encodes an enzyme that cleaves the sugar to activate the cyanide. This enzyme is stored in the cell wall. In general, striped clover contains cyanide; plain clover does not.

In consistent freezing temperatures, plant cell membranes (surrounding organelles and the cell itself) can burst. This is why the parts of plants above ground die back in colder climates. Root cells, however, are less likely to burst because they are underground and often store sugars, which protect the cell from freezing (just like antifreeze). This allows perennial plants to survive and grow again in the spring. Like the damage caused by freezing, herbivores can also damage plant cells. In the process of eating a leaf, herbivores destroy the membranes and organelles of the cells that make up the leaf.

Exercise 2

a. Why do you think the two gene products are stored in different parts of the cell?

b. Suggest at least two ways these products might come together to make active CN in nature.

c. Suggest a reason that clover may produce cyanide. That is, what advantage does a plant gain by producing cyanide? Also suggest a possible disadvantage of producing cyanide. Or might there be no advantage?

d. It takes energy for an organism to produce a particular structure such as a stripe on a clover leaf that is otherwise plain. Why might cyanide-producing clover produce striped leaves?

e. To explore this idea a little further, consider the following results of the hypothetical experiments shown in Table 2. In each situation, snails that have been taken from a wild habitat where both types of clover are present were put in a Petri dish containing varying types of clover. How would you interpret each result? Fill in the table with your interpretations.

Table 2. Interpretations of results from hypothetical experiments using snails and clover.

Clover presented to snails	*Snail response*	*Interpretation*
plain leaves	eaten	
striped leaves and plain leaves	plain leaves eaten	
striped leaves	not eaten	
plain leaves painted with white stripe	not eaten	

Exercise 3

To understand why cyanide producing/striped clover is found at a higher frequency in North Carolina than in Minnesota, you must consider the "fitness" of each variant in the different habitats available in the two states. Fitness is determined by the ability of an organism to survive, grow, and reproduce in a particular habitat. You have probably heard the term "survival of the fittest," but if an organism is not able to also grow and reproduce, it will not be able to pass any of its alleles (genetic information) on to its offspring. An organism that has high fitness does well in its habitat and passes those favorable alleles onto its offspring when it reproduces.

Go back and review the habitats you described in Exercise 1 and think about the factors that would be important for plant fitness. Then list the ecological differences between North Carolina and Minnesota that might affect the fitness of each variant. In other words which factors might increase plant growth, survival, and reproduction in each habitat, and which factors might inhibit them?

We can express fitness and selection with numbers that tell us not only whether one organism has a higher fitness than another, but also the degree to which that organism is more likely to survive and reproduce than another organism. It is not always possible to make an absolute measure of fitness, but often we can measure relative fitness. For each type of organism, we take some measure related to fitness, such as the percentage of individuals of that type surviving to the next generation. We then divide the measures for each type by the highest value. Thus, the organisms with the highest fitness will have a relative fitness of 1.0, whereas other organisms will have some value less than 1.0, but no lower than zero.

Here is an example of how we can calculate relative fitness from a classic study by Bernard Kettlewell (Kettlewell 1955, 1956) on peppered moths *(Biston betularia)*. The moths come in two forms (essentially dark and light), and Kettlewell studied them in two types of environments, polluted forests with trees with dark bark, and unpolluted forests with trees with light bark. He hypothesized that the color of the moth would determine how easily predators could spot it, such that dark moths should have a higher fitness in polluted forests, and light moths should have higher fitness in unpolluted forests.

To test this hypothesis, he marked moths of both types and released them into both environments. Sometime later, he collected moths from both environments and determined how many of the marked moths he recovered. Here are his results (Table 3).

Table 3. Survival rates of dark and light moths in unpolluted and polluted forests.

	Unpolluted			*Polluted*		
	Released	*Recaptured*	*% Recaptured*	*Released*	*Recaptured*	*% Recaptured*
Dark	406	19	4.7	447	123	27.5
Light	393	54	13.7	137	18	13.1

How do you explain the fact that Kettlewell did not recover every marked moth? Based on the data, which moths had a higher fitness in the unpolluted forest? What about in the polluted forest?

Now we can calculate the relative fitness of each type of moth in each environment. We can treat the percent recaptured as a reflection of survival. In the unpolluted forest, the light moths are fittest, so we divide each percentage recaptured by the percentage of light moths recaptured, or:

Relative fitness of light moths = 13.7/13.7 = 1.0

Relative fitness of dark moths = 4.7/13.7 = 0.34

Calculate *relative fitness* for dark and light moths in the polluted forest.

One thing you might have noticed is that the difference between relative fitness of the fittest moths and the least fit moths is not the same for the unpolluted and polluted forests. This difference is called the strength of selection, or the selection coefficient(s). We can calculate this value for each environment. Here's the calculation for the unpolluted forest:

Unpolluted forest: $s = 1.0 - 0.34 = .66$

Calculate s for the polluted forest.

Since the unpolluted forest has a higher value for s, selection is stronger in the unpolluted forest than in the polluted forest. Describe what this means in terms of survival and reproduction of the moths in each environment.

Part III – Investigating Clover Distribution

Now that you have considered the different habitats in which the white clover is found and the factors affecting fitness in clover, you will develop hypotheses to explain the observed distribution of plain and striped clover. A hypothesis is a tentative answer to a well-framed question. This means that one has developed an explanation of an event based on preliminary data, observations, and perhaps the work of other scientists. Scientists use observations and data to develop and justify their hypotheses. A hypothesis is presented as a statement, not a question, and must be both *testable* (there must be some way to test if it is valid) and *falsifiable* (it must be possible to show that an incorrect hypothesis is false).

Exercise 4

Based on the data presented above and the differences in habitat between Minnesota and North Carolina, propose a hypothesis to explain each of the following: a) the higher frequency of plain clover in Minnesota, and b) the higher frequency of cyanide producing/striped clover in North Carolina. Justify the reasoning leading to each of your hypotheses. Be specific in terms of which variables (conditions) affect the frequency of each type of clover in each habitat. Remember to write your hypotheses as statements, not as questions.

Exercise 5

Are your hypotheses the same for the different habitats? Explain why individuals or populations from the same species may show different traits in different habitats. Use the term "selection pressure" in your explanation. Selection pressure refers to the influence a particular factor has on the ability of an organism to survive and reproduce.

Exercise 6

Once a scientist has formed a hypothesis, the next step is to test it with observations or experiments. Experiments should test only one variable at a time, and keep as many other factors as possible constant (which doesn't mean "unchanging," but only that they are the same for all experimental groups). Design experiments to test at least one hypothesis for each habitat.

Exercise 7

For each of the experiments you proposed in Exercise 6, describe data that would support your hypothesis and data that would falsify your hypothesis.

Exercise 8

Is there any reason you might expect selection to be stronger in one environment than the other?

Here are results of a hypothetical experiment looking at survival of the different types of clover at different locations (Table 4). Do these results support or refute your hypothesis?

Table 4. Survival rates of plain and striped clover in Minnesota and North Carolina (based on a hypothetical experiment)

	Survival	
Variety	*Minnesota*	*North Carolina*
Plain	59%	27%
Striped	13%	72%

Calculate the relative fitness of each type of clover at each location. Calculate the strength of selection *(s)* at each location.

♣

Part IV – What Did You Learn?

You have already thought about and used several concepts from evolutionary biology that aid in our understanding of how organisms adapt to their habitats. Now let's formally define them.

Variation: Differences among individuals of a species; different forms of the same trait.

Natural Selection: Differential survival and reproduction of individuals bearing different forms of the same trait.

Evolution: Genetic change in a population over time.

Adaptation: The evolution of a trait that increases the likelihood of survival and reproduction of an organism in a particular environment.

Exercise 9

a. What are examples of variation in the clover?

b. Refer back to Figure 1 showing the relative frequency of plain and striped clover in Minnesota and North Carolina. Explain why there is variation in the frequency of each type of white clover between each of these areas.

c. Adaptation in the white clover means that over time there is an increase in the frequency of particular traits that would help individuals in that population of white clover survive and reproduce in that particular habitat. What are examples of possible adaptations in the clover? Remember, adaptations are specific to a particular habitat.

d. Comparing the white clover populations in Minnesota and North Carolina, what would you need as evidence that evolution has occurred?

e. Several factors may exert selection pressure on different traits in white clover in each habitat. What factor would you propose is exerting the strongest selection pressure on the production or nonproduction of CN in white clover in Minnesota? In North Carolina?

Part V – Checking Your Understanding

Exercise 10

Based on your understanding of the clover case and the definitions provided above, which of the following statements are true? Explain why each of the correct statements is true or correct each of the false statements to produce a true statement.

a. Natural selection can fully be explained by the phrase "survival of the fittest."

b. Variation is necessary for natural selection to occur.

c. Adaptation is defined with respect to local environmental conditions (e.g., heat, cold, rainfall, competitors, herbivores).

d. Natural selection acts on populations, not individuals.

Exercise 11

a. Predict which variant of white clover would be most frequent in each of the microhabitats on Long Island (refer to Part I).

b. Write a paragraph that describes the distribution of clover in the microclimates of Long Island using the terms *variation, adaptation, natural selection,* and *evolution.* Be sure to fully describe each of these terms in your detailed paragraph.

♣