### Introduction

The Scientific Method simply refers to the manner in which scientists approach any given scientific problem. Regardless of the scientific field, be it astronomy to zoology, all scientists employ the scientific method as they go about their research. The scientific method is really quite simple. It requires making initial observations and using these observations to form the basis of a hypothesis, which is an educated guess about what is going on. The hypothesis is then tested to determine its accuracy at explaining the situation. Your test may support your hypothesis; but if not, you may modify it based on the results of the testing procedure (see Fig 1.20; p. 17 in your text).

In this exercise, you will apply the steps of the scientific method to a very simple investigation. You and your group will make observations and hypothesize about the number of beads of a certain color in a jar containing a mixture of colored beads. You will test your hypothesis by randomly sampling the beads in the jar, recording the data (information), and analyzing these data through a simple statistical analysis.

## **Materials**

Jar containing 200 plastic beads

### **Procedure** - Students work in groups of four

#### **Step 1: Observation and Formulation of a Working Hypothesis**

Observe the container of beads on your lab bench. Each one contains EXACTLY two hundred beads. Each color is found in a multiple of 25 beads (25, 50, 75...etc.). Your lab instructor will assign your group a specific color to investigate. Using your OBSERVATIONS of the beads in the container, formulate your HYPOTHESIS as to the ratio of your assigned color of beads to the rest of the beads in the container.

An example of such a hypothesis might be: One out of every four beads is purple.

As far as this hypothesis is concerned, there are just two kinds of beads in the container: PURPLE and NOT PURPLE. Since all colors of beads exist in multiples of 25, it would be impossible to have say, a one to ten ratio (20 out of 200). Scientists often have a small amount of data at the beginning of their investigation that allows them to eliminate some hypotheses. Your knowledge of the number of beads in the container (200) and that they are in multiples of 25 thus allows you to exclude certain hypotheses.

Record your container number and your group's hypothesis on your worksheet.

#### **Step 2: Testing the Hypothesis**

The obvious way to test your hypothesis is to empty the container and count all of the beads of various colors. However, there are many circumstances where such an approach is not practical or even possible. For example, if you want to determine the ratio of male to female bald eagles in the state of Idaho, it would be impossible to locate and determine the sex of EVERY bald eagle in the state. However, if you take a random sampling of eagles, you can predict, with great accuracy, the sex ratio of these birds. The

key words here are RANDOM and SAMPLING. This is the approach you will use in testing your hypothesis today.

#### Sampling Method - Each person in the group completes five samples

- 1. Shake the container thoroughly (cover the hole on top).
- 2. Shake out exactly ten beads through the hole in the lid. If you accidentally get an extra bead, close your eyes and return a randomly selected bead to the container.
- 3. Count the number of beads of your target color in your sample. Record this number on your data sheet along with the number of non-target beads.
- 4. Return the beads to the container (this is called sampling with replacement).
- 5. Repeat Steps 1-4 four more times for a total of five samples.

Complete the worksheet up to the point of the Table for Chi-square analysis. Your TA will assist with this section.

#### The Chi-Square Test

The Chi-Square test can be used to determine if there are problems with your sampling method or your hypothesis or if some other factor is affecting the outcome. We will use a much-simplified version of the test and not concern ourselves with how it works, but rather with how to complete the test and interpret the results.

The easiest way to perform the test is to set up a series of columns made up of the different variables used in the test. The first column contains the OBSERVED value and is labeled O. The next column contains our EXPECTED values and is labeled E. The third column contains the sum of the observed value minus the expected value (O – E). The fourth column is simply the value in the third column squared (O-E)<sup>2</sup>. The fifth column contains the values obtained from dividing the value in the fourth column by the value in the second column (O-E)<sup>2</sup>/E.

We will use our example hypothesis that one out of every four beads is purple. Since we took a total sample of 200 beads from the container, we would expect that 50 out of the 200 beads in our sample would be purple. Likewise, 150 beads in our sample would not be purple. We will assume that our sampling procedure turned up 56 purple beads and 144 non-purple beads. When completed, the Chi-square table would look like this:

	(0)	(E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
Purple:	56	50	6	36	.72
Not Purple	144	150	-6	36	.24

Adding the values from the fifth column we come up with a sum of 0.96 (0.72+0.24) for our Chi-square value. In order to be able to use this figure, we also need to determine the DEGREES OF FREEDOM associated with our work. As a rule of thumb, the degrees of freedom are equal to the number of categories in a problem minus one. Since we only considered two categories of beads, purple and not purple, our degrees of freedom (df) for the problem will be equal to (2-1) or 1. Using this value and the sum of our Chi-square values, we can now use the Chi-square table to get some meaningful information.

Degrees			Probability		
of					
Freedom					
(df)	0.9	0.5	0.1	0.05	0.01
1	0.02	0.46(1)	2.71	3.84	6.64
2	0.21	1.39	4.60	5.99	9.21
3	0.58	2.37	6.25	7.82	11.34

Look at the partially reproduced Chi-square value table below:

Now, look on the first row (this row corresponds to one degree of freedom). Move across the row until you find our chi-square value of 0.96. As you can see, 0.96 does not appear on the chart. If it did, it would be located between number 0.46 and 2.71, (second and third columns).

If you look above the numbers in this first row you will find another set of numbers. These numbers correspond to probabilities. Essentially, these probabilities refer to the chance that the observed values we got from our experiment differed from our expected values by mere chance alone. Statistically, we accept that any probability greater than 0.05 is "good." That means that our observed and our expected values do not significantly differ from each other.

Since 0.96 falls between 0.5 and 0.1, our value is in the "safe" range. This indicates that our original prediction about the numbers of heads and tails was accurate. Had our value been greater than 3.84 (a probability value of less than 0.05), we would have had to reject our initial hypothesis and go "back to the drawing board" to form a new hypothesis based on our new data. This new hypothesis would then have to be **tested**, and perhaps **modified**. **This constant testing and modifying of hypotheses is a key element in the scientific method**.

Test your group's hypothesis about the beads in your container using the Chi-square analysis. Does your initial hypothesis need to be modified? Why or why not?

When you have finished with the group's data, test your hypothesis on the five samples that you collected on your own. Which sample, yours or the whole group's, is more accurate. Why do you think this is so?

#### Things to think about... Potential Problems with Sampling

One of the most common problems with sampling is inadequate sample size. For example, imagine an aquarium filled with 500 fish. All the fish are the same size; five are red; the rest are blue. Mathematically speaking, one percent (1%) of the fish are red, 99% are blue. Now imagine dipping a net into the tank and scooping up the first fish that comes by. Would you agree that the fish in your net is PROBABLY blue? If you repeated this ten more times, would you still agree that each time you would most likely end up with a blue fish in your net? However, does the fact that you only netted blue fish mean that there are only blue fish in the tank? Of course not. You can see a few red fish in the aquarium among the blue ones. You simply haven't caught a red fish yet; rare ones can be missed with too small of a sample size.

Now imagine the walls of the aquarium are opaque. You have been told that there are lots of fish in the tank, but nothing about their coloration. Suppose you dip your net into the tank twenty times, each time bringing up a blue fish. If you were asked the color of the fish in the tank, you might form the hypothesis

that they are PROBABLY blue. Does this mean that there are no red fish in the tank? No, just that your SAMPLE of fish from the tank is too small to enable you to make an accurate prediction. This demonstrates just one of the things that can go wrong with making a prediction based on a small sample of a larger population.

Another source of sampling error is failure to obtain a RANDOM sample. An example of this would be if you could see the fish in the tank and deliberately chased and captured the red fish while ignoring the blue fish. If you were to do this five times, each time netting a red fish, it might look as if the tank contained only red fish when in truth, only a small fraction of the fish were red.

#### Questions to think about...

1. You take one of the dice from your home casino kit and roll it 300 times. If you want to analyze your results with the chi-square test, how many degrees of freedom would you use?

2. Suppose you were only interested in the number of threes you rolled. How many would you expect to have gotten during your 300 rolls? How many degrees of freedom would be involved in this particular problem?

3. You buy a trick coin in a magic shop that is supposed to give more tails than heads. You toss the coin 100 times and get 55 tails. Should you ask for your money back? Why or why not?

Worksheet (to hand In)	Name	
Jar #		
Color assigned:		
Hypothesis:		

**Data Table:** Fill in the number of beads from your sample. Remember that the target color is the color you are after. All other beads fall into the "non-target color" category.

			Sample #			
	1	2	3	4	5	
Target Color:						-
Non-target Color:						

Total the number of beads of your target color in your five samples: \_\_\_\_\_. Out of the 50 beads you sampled, what percentage was the target color? \_\_\_\_\_%

Obtain a group total for the target color from all 20 samples: \_\_\_\_\_. Out of the 200 beads sampled, what percentage was the target color? \_\_\_\_\_%

How closely do your results correspond to the expected outcome based on your hypothesis?

How does the number of samples affect your conclusions?

#### Table for the Chi-square analysis:

Your data:	(0)	(E)	(O-E)	$(O-E)^2$	(O-E) <sup>2</sup> /E
Target Color:					
Non-target color:					
				Total:	
Group data:	(0)	(E)	(O-E)	$(O-E)^2$	(O-E) <sup>2</sup> /E
<b>Group data:</b> Target Color:	(0)	(E)	(O-E)	(O-E) <sup>2</sup>	(O-E) <sup>2</sup> /E
<b>Group data:</b> Target Color: Non-target color:	(0)	(E)	(O-E)	(O-E) <sup>2</sup>	(O-E) <sup>2</sup> /E

Conclusion:

Define the following terms:

- A. Hypothesis:
- B. Sample:
- C. Non-random sample:

# **Exercise 2: Natural Selection Simulation -Evolution of the Peppered Moth**

# Introduction

The case of the Peppered Moth in Manchester, England is an excellent and well- documented example of Darwin's theory of Natural Selection. It is also a clear example of how human beings can affect the process of evolution.

In 1954, an ecologist, H. B. D. Kettlewell set out to determine what had caused the drastic change in moth populations in England. There were two color forms of pepper moths: light and dark. Until approximately 1850, the most common form of Peppered moth in the Manchester area was light in color. The dark form of Peppered moth was considered rare and sought by collectors. After 1850, the number of dark moths greatly increased, while the light form of the moth became increasingly rare. Why?

Kettlewell hypothesized that increased air pollution from factories (i.e., soot) associated with the Industrial Revolution had gradually blackened the bark of trees on which Peppered moths rested during the day. As a result, the light-colored moths became more visible and more likely to be eaten by birds (their major predators), while the dark moths became better camouflaged and less vulnerable to predation. This human change in the environment, Kettlewell thought, led to a decrease in the number of light-colored moths and an increase in number of dark moths.

To test his hypothesis, Kettlewell reared populations of equally mixed dark and light moths. He released one group of moths into a relatively unpolluted area and the other group into a heavily polluted area. Later, he collected moths from each area and found a higher proportion of light moths in the unpolluted area and a higher proportion of dark moths in the polluted area. These results supported Kettlewell's hypothesis.

This exercise has been designed to duplicate Kettlewell's original study as best as possible given the constraints of a classroom situation. You, a predator, will try to "eat" moths positioned on backgrounds that simulate bark from light, medium and dark trees. The light-colored background represents trees in an unpolluted area; the dark background represents a polluted area; the intermediate background is intended to serve as a transitional stage as pollution was gradually increasing. This middle stage is included to demonstrate Natural Selection is a gradual process and not an immediate change.

# Objectives

Using artificial means, students will investigate the case study of the peppered moth, collect and analyze data on population trends, graphically represent the findings, and answer a series of questions.

# Materials

3 bark simulation trays: light, medium, dark 15 light-colored "moths"; 15 dark-colored "moths" timer or clock with second hand

# **Procedure -** Work in groups of three or four people. Part 1: Light Background - Pre-Industrial Revolution

- 1. Choose one person to be the moth predator. While this person looks away, place 15 white moths and 15 black moths in random locations on the paper tray with the lightest background. The moths should not overlap.
- 2. From a position one arm's length away from the tray, the moth predator removes, **one at a time**, as many moths as possible **within four seconds** and **then looks away again**.
- 3. Replace each black moth removed with a white moth. Replace each white moth removed with a black moth (the total number in the tray should still be 30).
- 4. Record data for Trial 2 in Table 1.
- 5. Repeat steps 2-4 three more times.
- 6. Calculate percentages for each trial. Complete graph #1 using information recorded in Table 1.

# Part 2: Medium Background - Early Industrial Revolution

- 1. Choose a different person to be the moth predator. While this person looks away, place 15 white moths and 15 black moths in random locations on the intermediate colored tray.
- 2. Conduct four trials as described in steps 2 and 3 above. Record data in Table 2 after each trial.
- 3. Calculate percentages for each trial and complete graph #2.

## Part 3: Dark Background - Industrial Revolution - Present

1. Choose yet another moth predator. Conduct four trials as described in Part 1 but using the dark colored tray. Record data after each trial in Table 3, calculate percentages, and complete graph #3.

## Questions to think about:

- How does this experiment demonstrate Natural Selection and the "Survival of the fittest" concept?
- Based on your studies, what could have originally caused the existence of two forms of the same species of moth?
- Based on your observations, what would happen to the moth populations if the pollution created by the Industrial Revolution were to be reversed?
- This investigation of the peppered moth is clearly an artificial situation. Describe how you think the natural situation might differ from this simulation.
- Now, building on what you have learned in class, name two other ways in which humans have influenced evolution. Can we speed evolution up? How?