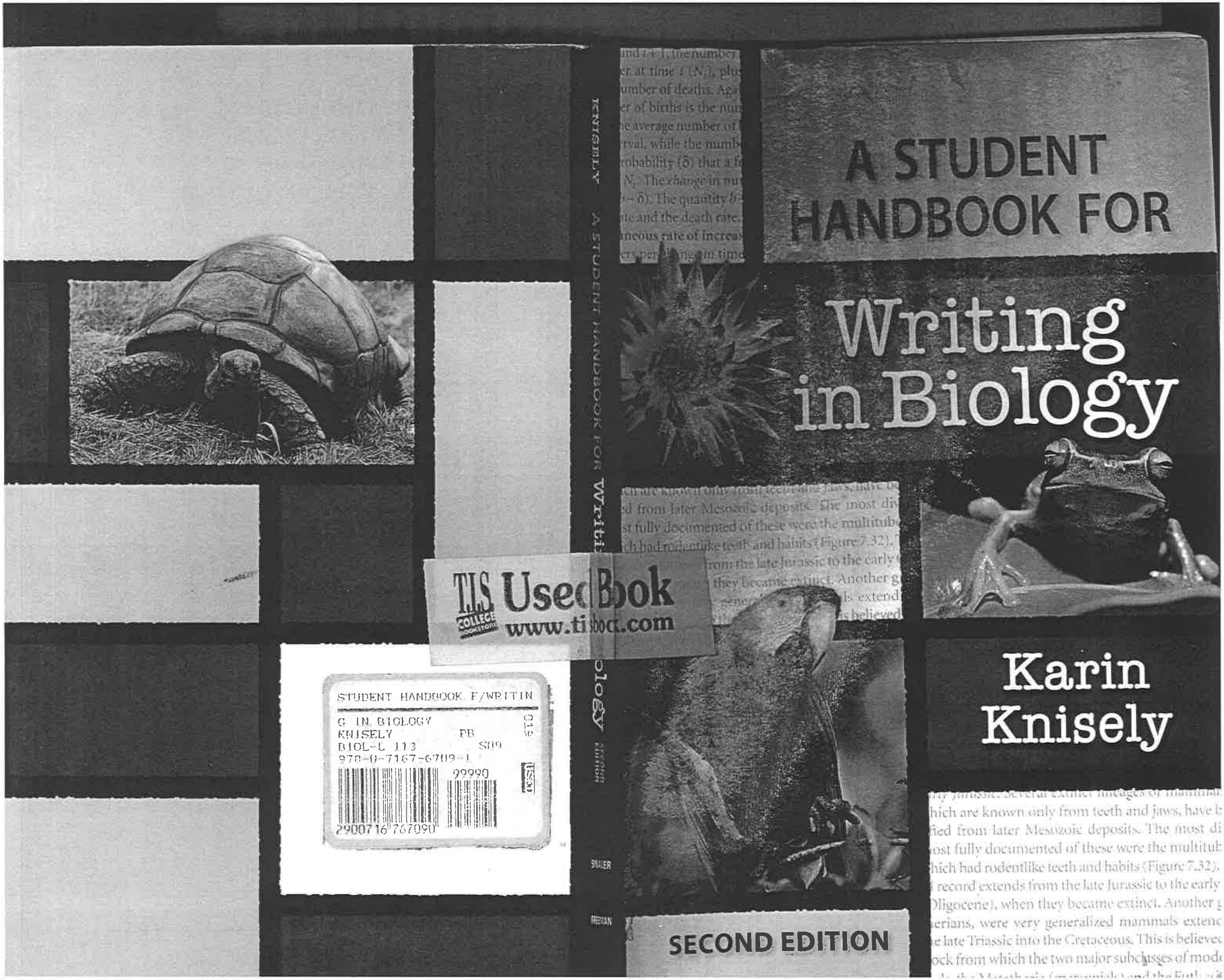
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The Scientific Method

Trying to understand natural phenomena is human nature. We are curious about why things happen the way they do, and we expect to be able to understand these events through careful observation and measurement. This is known as the **scientific method**, and it is the foundation of all knowledge in the biological sciences.

An Introduction to the Scientific Method

The scientific method involves a number of steps:

- Asking questions
- Looking for sources that might help answer the questions
- Developing possible explanations (hypotheses)
- Designing an experiment to test a hypothesis
- Predicting what the outcome of an experiment will be if the hypothesis is correct
- Collecting data
- Organizing data to help interpret the results
- Developing possible explanations for the experimental results
- Revising original hypotheses to take into account new findings
- Designing new experiments to test the new hypotheses (or other experiments to provide further support for old hypotheses)
- Sharing findings with other scientists

Most scientists do not rigidly adhere to this sequence of steps, but it provides a useful starting point for how to conduct a scientific investigation.

Ask a Question

As a biology student, you are probably naturally curious about your environment. You wonder about the hows and whys of things you observe. To apply the scientific method to your questions, however, the phenomena of interest must be sufficiently well defined. The parameters that describe the phenomena must be measurable and controllable. For example, let's say that you made the following observation:

> Dwarf pea plants contain a lower concentration of the hormone gibberellic acid than wild-type pea plants of normal height.

You might state the question in the following terms:

> Does gibberellic acid regulate plant height?

This is a question that can be answered using the scientific method, because the parameters can be controlled and measured. On the other hand, the following question could not be answered easily with the scientific method:

> Will the addition of gibberellic acid increase a plant's sense of well-being?

In this example, "a sense of well-being" is not something that can be measured or controlled.

Look for Answers to Your Question

There is a good chance that other people have already asked the same question. That means that there is a good chance that you may be able to find the answer to your question, if you know where to look. Your textbook, journal articles, and the Internet (see Harnack and Kleppinger, 2001 for how to determine a website's reliability) are all good places to begin finding answers. Curiously, attempts to answer the original question often result in new questions, and unexpected findings lead to new directions in research. By reading other people's work, you may think of a more interesting question, define your question more clearly, or modify your question in some other way.

Turn Your Question into a Hypothesis

As a result of your literature search or conversations with experts, you may now have a tentative answer to your original (or modified) question. Now it is time to develop a hypothesis. A **hypothesis** is a possible

explanation for something you have observed. **You must have information before you can propose a hypothesis!** Without information, your hypothesis is nothing more than an uneducated guess. That is why you must look for possible answers before you can turn your question into a hypothesis.

A useful hypothesis is one that can be tested and either supported or negated. A hypothesis can never be proven right, but the evidence gained from your observations and/or measurements can provide support for the hypothesis. Thus, when scientists write papers, they never say, "The results prove that… ." Instead, they write, "The results suggest that…" or "The results provide support for… ."

You might transform your question "Does gibberellic acid regulate plant height?" into the following hypothesis:

> The addition of gibberellic acid to dwarf plants will allow them to grow to the height of normal, wild-type plants.

Design an Experiment to Test Your Hypothesis

It doesn't matter whether your hypothesis is ultimately negated. A hypothesis is simply a starting point for designing an experiment to test your explanation. Designing an experiment requires experience, creativity, and a sense for what is practical. Experience may be gained from reading methods published by others and modifying them to suit your purpose. Creativity involves brainstorming (possibly with others) to view the subject from many different perspectives. A sense for what is practical is required, because your experimental design will be limited by considerations such as availability of equipment and supplies, cost, time, and so on.

When you design an experiment, pay attention to the following points.

Define the variables. There are three kinds of variables: dependent variables, the independent variable, and controlled variables. **Dependent variables** are variables such as growth, number of seeds, number of body segments, number of offspring, and so on that you can measure or observe. These variables may be altered by the experimental conditions.

The *one* variable that a scientist manipulates in a given experiment is called the **independent variable**. In a different experiment, a different independent variable can be manipulated. It is important to have *only one* independent variable in a given experiment, because otherwise you don't know which factor is affecting the dependent variable(s).

There are many variables that could possibly affect the outcome of an experiment. Thus, it is important to vary only one (the independent variable) and to keep all others constant. The ones that remain constant are called the **controlled variables**. If, for example, you decide to investigate the effect of gibberellic acid on plant growth, variables such as temperature, humidity, age of the plants, day length, amount of fertilizer, watering regime, and so on would all have to be kept constant. These are the controlled variables.

Design the procedure. Determine how you will carry out the experiment. The procedure can be based on articles in scientific journals, suggestions from colleagues, intuition or experience, or simply the desire to try out a new idea. The procedure must include the following components.

- **Control treatment.** This is the condition that serves as a reference for the test treatments. Experiments may have both positive and negative control treatments, as illustrated by the following example:

  Hypothesis: The addition of gibberellic acid to dwarf plants will allow them to grow to the height of normal, wild-type plants.

  Negative control: Dwarf plants not given gibberellic acid.

  Positive control: Normal plants not given gibberellic acid.
- **Level of treatment for independent variable.** What concentration of gibberellic acid should be tested? We don't want to choose a concentration that is too low, otherwise we might not see any effect. On the other hand, the concentration should not be so high that it is toxic to the plant. The level of treatment is usually based on previous research (journal articles) or preliminary experiments. The level can even be a range that is appropriate for the biological system or organism to be tested.
- **Replication**. A single result is not statistically valid. The experiment must be repeated several times to see whether similar results are found. The results from several trials may be averaged and analyzed using statistical tests.

Make Predictions about the Outcome of Your Experiment

Before you carry out your experiment, you must have some idea of what results to expect if your hypothesis is correct. Making predictions

involves **deductive reasoning**, whereby you use general knowledge or experience to predict the outcome of a specific experiment. For the previous example, you might predict the following results:

- **Test treatment:** Dwarf plants should grow taller when they are treated with gibberellic acid.
- **Negative control:** Dwarf plants treated with plain water should be short.
- **Postive control:** Wild-type plants treated with plain water should reach their normal, tall height.

If the outcome of the experiments matches your predictions, then your hypothesis is supported. If not, then your hypothesis is negated, and you may wish to repeat the experiment, double-check your experimental set-up, and/or come up with a different hypothesis.

Predictions are an important tool, because they give you a sense of direction. But there is a danger here, too: Do not let your predictions affect your objectivity. **Do not make your results fit your predictions**—instead, modify your hypothesis to fit your results.

What is learned from a negated hypothesis can be just as valuable as what is learned from a "successful" experiment. The subsequent modifications of the hypothesis and the associated experiments help researchers gain assurance that their explanation of a particular phenomenon is valid.

Collect Data

Once you have planned and set up your experiment, you are ready to make observations or measurements about your test subject. Consistency is very important in this regard. If, for example, you are measuring plant height, you must always measure according to the same criteria. If you measure from the rim of the pot to the shoot tip the first time, you can't measure from the soil to the shoot tip the next time without introducing error into the results. Sometimes it is necessary to modify during the course of your experiment *how* you collect data and *what kinds of data* are important. These modifications may also be included when you design your next experiment.

You should realize that even some of the most elementary questions in biology have taken hundreds of scientists many years to answer. One approach to the problem may seem promising at first, but as data are collected, problems with the method or other complications may become apparent. Although the scientific method is indeed methodical, it also requires imagination and creativity. Successful scientists are not discouraged when their initial hypotheses are discredited. Instead, they are already revising their hypotheses in light of recent discoveries and planning their next experiment. You will not usually get instant gratification from applying the scientific method to a question, but you are sure to be rewarded with unexpected findings, increased patience, and a greater appreciation for the complexity of biological phenomena.

How to handle variability and unexpected results. Variability is a fact of life. If you toss a coin 10 times, you may get 5 heads and 5 tails the first time, 4 heads and 6 tails the second time, and 8 heads and 2 tails the third time. Even though you used the same coin and tossed it the same way, you got different results in different trials.

This same kind of variability is likely to be present in experimental data. Even when you apply the same treatment to different individuals of the same species of plant, the individual plants may respond differently. How do you know if the different results are due to variability among individuals, the imprecision in making measurements, or some other factor?

This is where statistical analysis comes in. Statistics can help you determine how far you can trust the results when you are sampling a subset of a population in which individuals differ. Statistics can help you decide if the different measurements you obtained for replicates are significant or not.

Some common statistical tests and their uses are given in Table 1.1. Consult a good statistics text (see the Bibliography: Samuels and Witner, 1999; Moore, 2000; Utts and Heckard, 2002) for details on the different tests.

What if your results cannot be explained by variability? Although you would like to give yourself the benefit of the doubt, human error is a possibility, especially in introductory biology laboratory exercises. Human error includes failure to follow the procedure, failure to use the equipment properly, failure to prepare solutions correctly, variability when multiple lab partners measure the same thing, and simple arithmetic errors. If you suspect that human error may have influenced your results, it is important to acknowledge its contribution. If you had time to repeat the experiment, you would first try to eliminate sources of human error rather than revise your original hypothesis.

TABLE 1.1 Common statistical tests and their uses

TEST	APPLICATION
Chi square	To compare how closely the observed or measured data compare to the expected results (e.g., for crosses in genetics)
t-test	To compare the means of two groups
ANOVA	To compare the means of three or more groups

Organize the Data

Raw data must be summarized and organized before you can begin any interpretation. Two ways to organize data are in tables and figures. Tables have rows and columns and are useful for displaying several dependent variables at the same time or when you want to emphasize the numbers themselves rather than the trend shown by the numbers. Figures are graphs, pictures, diagrams, gel photos, X-ray images, and microscope images—any visual that is not a table.

Line graphs and bar graphs are commonly used in data analysis. **Line graphs** show the effect of the independent variable (the one the scientist manipulates) on a selected dependent variable (the one that changes in response to the independent variable). By convention, the independent variable is plotted on the *x*-axis, and the dependent variable is plotted on the *y*-axis. Let's say that for 3 weeks, you treated dwarf and normal plant groups as described under "Predictions" (page 5). If you choose to summarize your results in a line graph, plot time on the *x*-axis, because time is the variable you controlled. Plot stem height on the *y*-axis, because this is the variable you expect to change over time, depending on the treatment.

Bar graphs allow you to compare individual sets of data when the data are *discontinuous*. In the previous example, if you wanted to compare the final heights of the plants in the different treatment groups, each bar would represent the height of one group of plants at the end of the 3-week period. The data are discontinuous because different treatment groups are being compared.

The best way to display the data depends on the point you are trying to make. For example, if you want to show that the rate of growth is faster in plants treated with gibberellic acid than that in untreated plants, then a line graph would work well. If you want to emphasize that the end effect of treating plants with gibberellic acid is a taller plant, then a bar graph would be suitable. If you want to compare the actual heights of your plants with those in the literature, then a table would be useful. Also keep in mind that a "picture is worth a thousand words," especially when you are comparing physical differences like height, color, and overall appearance of the organisms. Photographs may call your attention to variables that you didn't measure, but that you might want to consider in future experiments. Photographs are also very effective in poster presentations.

The process of summarizing and organizing data initially involves trial and error, especially when you have a lot of data, the results are variable or unexpected, or you do not have a clear understanding of the purpose of the experiment. These difficulties are a normal part of the learning curve; your organizational ability will improve with practice and experience. See the section on "Organizing Your Data" in Chapter 4 as a starting point.

Regardless of how you choose to display the data, you must be honest. Do not exaggerate axes or trends to support your hypothesis when, in truth, the data do not support it. Show variability when necessary. Use statistical methods to reduce huge amounts of data, and be prepared to explain your reasoning.

Keep in mind that there may be *no difference* between the control and the experimental treatments. If there was no difference, say so, and then try to develop possible explanations for these results.

Try to Explain the Results

Once you have organized the data, you are ready to develop possible explanations for the results. You already found sources on the topic when you developed your hypothesis. Return to this material to try to explain your results. Do your results agree with the findings of other researchers? Do you agree with their explanations? If your results do not agree, try to determine why not. Were different methods, organisms, or conditions employed? What were some possible sources of error?

Revise Original Hypotheses to Take New Findings into Account

If the data support the hypothesis, then you should suggest additional experiments to strengthen the hypothesis. If the data do not support the hypothesis, then you should look for "human error" factors first. If these kinds of factors can be ruled out, suggest modifications to the hypothesis. You may also describe ways to test the new hypothesis. Ideally, scientists will thoroughly investigate a question until they are satisfied that they can explain the phenomenon of interest.

Share Findings with Other Scientists

The final phase of the scientific method is communicating your results to other scientists, either at scientific meetings or through a publication

in a journal. When you submit a paper to refereed journals, it is read critically by other scientists in your field, and your methods, results, and conclusions are scrutinized. If any errors are discovered, they are corrected before your results are communicated to the scientific community at large.

Poster sessions are an excellent way to share preliminary findings with your colleagues. The emphasis in poster presentations is on the methods and the results. The informal atmosphere promotes the exchange of ideas among scientists with common interests. See Chapter 7 on how to prepare a poster.

Oral presentations are different from both journal articles and poster sessions, because the speaker's delivery plays a critical role in the success of the communication. See Chapter 8 for tips on preparing and delivering an effective oral presentation.