Designing a Controlled Experiment

Science is a way of examining and finding order in the natural world. It is a dynamic process of asking questions and then seeking answers. Observations lead us to formulate questions and, with our limited knowledge, we may offer tentative explanation or make educated guesses about the answers to our questions.

Scientists call a tentative explanation a **hypothesis**. Experimentation follows, providing information that may support or refute a hypothesis. From data, often reinforced by statistics, conclusions can be made about what it is we wish to know.

**The Scientific Method**

The scientific approach is a powerful method for understanding the natural world because it is based on observation of how the world works. However, not just any observation will do: the observations must be systematic and objective. The scientific method of inquiry is an important part of everything we do in our daily lives; we simply do not recognize the steps because we are so used to them. Scientific inquiry involves the steps outlined below.

**Problem:** You want to find out whether a combination of anti-cholesterol drugs X and Y is more effective in reducing high cholesterol levels than either of the drugs given separately. You might proceed as follows:

**Step 1:** Make observations that lead to the formulation of a question.

You observe that drugs X and Y, used independently, lower LDL levels in the blood. You question whether drug X plus drug Y, given in combination, would be even more effective.

**Step 2:** The question leads to a tentative explanation or educated guess – a hypothesis.

Prior knowledge or research or even intuition can contribute to the formulation of a hypothesis. The hypothesis must be tested in away that allows it to be proven false. We can never prove that a hypothesis is true, but we can support the hypothesis if repeated experiments do not falsify it.

You formulate the following hypothesis: If individuals with high cholesterol levels are treated with a combination of drugs X and Y, their cholesterol levels will be lowered more than for similar groups treated with drug X alone or drug Y alone.

For statistical reasons, you will also devise a **null hypothesis**, or prediction of what would happen if the experiment treatment has no effect.

The null hypothesis is that cholesterol will be lowered by the same amount with all three drug treatments.

**Step 3:** Make predictions about the results you would expect if the hypothesis were correct. In this way, scientists begin to formulate an experimental design. Hypotheses are often stated in the form of predictions.

You predict that treating individuals with drugs X and Y in combination will be more effective than treatment with either drug alone. As part of your experiment design, you know that you will need to compare at least three experimental groups, and you may begin to plan how to identify participants for your study.
Step 4: Clearly define the experiment’s independent, dependent, and standardized variables.

The independent variable is the factor that is being manipulated in the current experiment.

*The independent variable is the type of drug treatment.*

The dependent variable is the aspect of the system that is showing some response to the manipulation of the independent variable.

*The dependent variable could be any of the many aspects of an individual’s condition that defines the difference between life-threatening high cholesterol levels and lower levels typical of healthy individuals.*

The standardized variables, or constants, are all the variables that are held constant between the treatments.

*The way the drugs or drug mixture are administered, the frequency with which the subjects are checked, and the average ages and general health characteristics of the individuals assigned to the treatments are all standardized variables.*

Step 5: Define the experimental treatments.

A treatment group is a test group of individuals that are subjected to the same levels of the independent variable.

*The groups that get drug X alone is one treatment, the group that gets drug Y alone is another treatment, and the group that gets both drug X and Y is the third treatment.*

Step 6: Select materials and identify experimental methods and methods of data collection and analysis, as part of a well-planned experimental design. These are incorporated into a procedure that tests whether the predicted results occur.

You identify a large group of individuals who have high cholesterol levels but are otherwise healthy. You randomly assign them to groups that will get drug X alone, drug Y alone, and drugs X and Y.

The experiment should also have a fourth control group.

*In the control groups, individuals with high cholesterol get no drug treatment but are given a placebo and are held to the same standardized variables.*

Step 7: Perform experiments and collect data

Step 8: Analyze data from the experiments that test the hypothesis. This leads to a conclusion. Experiments can only offer evidence that either supports, or fails to support, hypothesis.
Step 9: Repeat the process, using a more refined question about the system.

Assume that the combination of drugs was more effective than either drug taken individually. Next, you might ask if the best results are obtained when the two drugs are given in equal or in unequal amounts. Or, you might ask if a combination of drugs X, Y, and Z is more effective than a combination of just X and Y.

The usual result of an experiment is more questions.

Let us go into each of these steps in more detail….

Step 1. Making Observations and formulating a hypothesis

Observations often lead to devising a question about the observed system. A hypothesis is a question, often stated in the form of an educated guess or possible answer (tentative explanation) to a question. Hypotheses lead to predictions – indeed, hypotheses are often stated as predictions. Two additional criteria must be met by a hypothesis:

1. We must have a hypothesis that can be falsified (refuted or proven false). If there is no possibility of proving that a hypothesis in false, then it cannot be tested. Why? Because we can never truly ‘prove’ that a hypothesis is ‘true’. We can only add to the body of evidence that supports a hypothesis.

2. The hypothesis must focus on a limited, specific, well-defined problem.

Formal testing of a hypothesis requires that we distinguish between two alternative possibilities:

1. The variable being manipulated has an effect.
2. The variable being manipulated has no effect.

The second possibility (no effect) is often referred to as the null hypothesis because it states the alternative possibility – that no effect occurs.

1. Write a hypothesis for each of the following:

   a. Guinea pigs are kept at different temperatures for 6 weeks. Percent weight gain is recorded.

   b. Batches of seeds are soaked in salt solutions of different concentrations and the number of seeds that germinate is counted for each batch.

Step 2. Experimental Design

To test a hypothesis, you have to design an experiment. The design process often begins when you make predictions from your hypothesis. Next, you must identify your experimental variables and treatments. You must also determine how to control all other factors that might influence your results. Finally, you should consider how you want to collect and analyze data.

Step 2a. Identifying Variables
Developing a good experimental design requires the experimenter to define what factors will be varied or held constant during the test. The independent variable can be anything the experimenter decides to vary – density, temperature, light intensity, altitude, concentration, to name a few. Probably the most commonly used independent variable is time: the experimenter takes measurements once a day, every 2 hours, every 2 minutes, or whatever time period is appropriate for the experiment.

The dependent variable is the aspect of the system that is showing response to the variations of the independent variable. It is what is measured at different ‘settings’ or levels of the independent variable. An experiment may have several dependent variables – all of the things that are affected and can be measured as you manipulate the independent variable. In any experiment, however, you should always strive to have only one independent variable.

For instance, an investigator wants to study how the maximum yield can be obtained from a peanut field. One possible dependent variable is the height of the corn plants.

2. **Name some other aspects of corn growth that can be measured.**

3. **Name some factors that might affect the number of ears of corn produced by corn plants. One of these would be chosen as the independent variable.**

4. **Why is the scientist limited to one independent variable per experiment?**

5. **Identify the dependent and independent variables in the following examples:**
   a. The diversity of algal species is calculated for a coastal area before and after and oil spill
      
      Dependent variable
      
      Independent variable

   b. Light absorption by a pigment is measured for red, blue, green, and yellow light
      
      Dependent variable
      
      Independent variable

A third type of variable is the standardized variable (or constants). Standardized variables are factors that are kept equal in all treatments, so that any changes in the dependent variable can be attributed to the changes the investigator made in the independent variable. Since an experiment should have only one independent variable, all other things that could vary during the experiment must be kept constant or be controlled.
For example, if the scientist has chosen the amount of fertilizer as the independent variable, he want to be sure that there are no differences in the type of fertilizer used. He would use the same formulation and same brand of fertilizer throughout the experiment.

6. **What other variables would have to be standardized in this experiment?**

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**Defining experimental treatments**

A treatment group (or test group) is a group of individuals that are subjected to the same levels of independent variable(s). But how can the experimenter assure that the outcome is in fact due to the manipulation of the independent variable? *This is usually done by running a control for the experiment.* When comparing two treatments, one treatment groups may serve as the control treatment group and the other as the experimental treatment group, exposed to the independent variable.

In the fertilizer example, the investigator must be sure that the corn doesn’t grow just as well with no fertilizer at all. The control would be a treatment in which no fertilizer is applied. An experiment on the effect of temperature on guinea pigs, however, cannot have a 'no temperature' treatment. Instead, the scientist will use a standard temperature as the control and will compare weight gain at other temperatures to weight gain at the standard temperature.

7. **Tell what an appropriate control treatment would be for each of the following examples.**

   a. An investigator studies the amount of alcohol producing by yeast when it is incubated with different types of sugar.

      Control treatment:

   b. The effect of light intensity on photosynthesis is measured by collecting oxygen produced by a plant.

      Control treatment:

   c. A solution is made up to simulate stomach acid at pH 2. Maalox antacid is addend to the solution in small amounts, and the pH is measured after each addition.

      Control treatment:

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**Step 2b. Determining Replication**

Another essential aspect of experimental design is replication. Replicating the experiment means that the scientist repeats the experiment numerous times using exactly the same conditions to see if the results are consistent. Being able to replicate a result increases our confidence in it. However, we shouldn’t expect to get exactly the same answer each time, because a certain amount of variation is normal in biological systems. Replication the experiment lets us see how much variation there is and obtain an average result from different trials.
A concept related to replication is **sample size**. It is risky to draw conclusions based upon too few samples.

For instance, suppose a scientist is testing the effects of fertilizer on peanut production. He plants four peanut plants and applies a different amount of fertilizer to each plant. Two of the plants die.

8. *Can he conclude that the amounts of fertilizer used on those plants were lethal? What other factors might have affected the results?*

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**Step 2c. Writing the Method**

After formulating a hypothesis and selecting the independent and dependent variables, the investigator must find a method to measure the dependent variable; otherwise, there is no experiment. Methods are learned by reading articles published by other scientists and by talking to other scientists who are knowledgeable in the field. For example, a scientist who is testing the effect of fertilizer on peanuts would read about peanut growth and various factors that affect it. He would learn the accepted methods for evaluating peanut yield. He would also read about different types of fertilizers and their composition, their uses on different soil types, and methods of application. The scientist might also get in touch with other scientists who study peanuts and fertilizers where other scientists present results of investigations they have completed.

**Step 3. Forming a conclusion**

A distinctive quality of scientific thinking is that a hypothesis can never be considered to be ‘proven’ as a result of experiments or observations. Scientists accept ideas, theories, and hypotheses when they cannot show them to be false. They accept the null hypothesis unless they have evidence to falsify it.

Scientists often employ statistics to interpret experimental results and to determine whether the results meet a minimal level of acceptance or should be rejected. This allows us to come to a conclusion about a hypothesis. We might then suggest other hypotheses and test them again the null.

The experimental method, designed to be replicated or repeated by others, is the process that precedes the scientific acceptance of a hypothesis. Such acceptance is always provisional. We cannot prove that a hypothesis is true because we can never be certain that we examined all the evidence or considered all the possible alternative hypothesis.

Testing hypotheses leads to more questions. In this way, evidence accrues in support of a hypothesis, which may, after years of testing, gain the status of a theory. A theory is a generalization (based on many observations and experiments) that forms the basis for further studies.

**4. What is a Theory?**

A theory is an explanation for natural events that is based on a large number of observations. It’s important to realize that theories EXPLAIN what we observe and make PREDICTIONS about the results of experiments. For instance, the Germ Theory explains why we get sick and how we get infections. In short, it uses observation of viruses and bacteria as well as data from those who all get ill to explain a theory on what causes the illness.

You may say, Duh? Isn't that an absolute fact? We know there are germs and they make us sick. To a degree, some of what we know now (with increased technology) is more refined than what it was 100 years ago. Did you know that some doctors were laughed at for washing their hands? It was a
ridiculous notion hundreds of years ago to believe that there were invisible things in the air that would make us sick. Now, we accept it as common knowledge. However, the germ theory continues to be refined as we expand our knowledge on what actual components and parts of a bacteria or virus make us sick and why. Theories are changeable and expandable, and most importantly, theories are falsifiable.

In order to be a valid scientific theory, there must be some way that an observation or experiment could prove to be false. For example, Einstein’s theory of Relativity made PREDICTIONS about the results of experiments. These experiments could have produced results that contradicted Einstein, so the theory was (and still is) falsifiable.

In summary, there are three important points to a scientific theory:

- Theories must explain a wide range of observations
- Theories must be falsifiable
- Theories can be changed if new evidence presents itself.
**QUESTIONS FOR REVIEW**

1. A team of scientists is testing a new drug, XYZ, on AIDS patients. They expect patients to develop fewer AIDS-related illnesses when given the drug, but they do not expect XYZ to cure AIDS.
   
   a. What hypothesis are the scientists testing?
   
   b. What is the independent variable?
   
   c. What is the dependent variable?
   
   d. What control treatment would be used?
   
   e. What variable should the researchers standardize?

2. A group of students decides to investigate the loss of chlorophyll in autumn leaves. They collect green leaves and leaves that have turned color from sugar maple, beech, and aspen trees. Each leaf is subjected to an analysis to determine how much chlorophyll is present.
   
   a. What is a reasonable hypothesis for these students?
   
   b. What is the independent variable?
   
   c. What is the dependent variable?
   
   d. What would you advise the students about replication for this experiment?

3. A scientist wants to study mating behavior in crickets. She hypothesizes that males that win the most male-versus-male contests mate with the most females. She observes the crickets to obtain data. For each male, she counts the number of male-male fights he wins and the number of females he mates with.
   
   a. What is the independent variable?
   
   b. What is the dependent variable?
   
   c. What constitutes replication in the experiment?