

## Single-celled Organisms and Symbiotic Relationships

**Lesson Objectives:** Students will be able to do the following:

- Describe an experimental method used by scientists
- Compare and contrast two studies involving the same symbiotic relationship
- Identify at least two technologies used in these particular studies

**Key Concepts:** experimental design, enzyme, antibody, transmission electron microscopy, autoradiography

### Methods Used by Scientists



Scientists study the natural world. They look for answers to questions they might have about something they have observed in nature.

In scientific research, a process is used to answer these questions. This process is not always the same, but it generally contains these steps: observation, questioning, using the results of previous scientific research, developing a testable prediction, creating and carrying out an experiment, gathering results, and drawing conclusions. From observation and research scientists begin to develop a **conceptual model**. This is a model in your head based on connected units of information or a concept. Then it is time to make a testable prediction based on the model. These predictions are testable **hypotheses** that help scientists evaluate their model. Researchers then design and carry out experiments to test their hypotheses. The experimental design depends on the type of

question being asked. Usually researchers try to design experiments with a **control**, which means that they can make comparisons between the experimental group and nonexperimental group. Sometimes experiments indicate that the scientist's hypothesis was falsified. This means that the hypothesis could not be shown to be true in that particular instance. In this case, researchers refine their question and continue to conduct other experiments. Sometimes important scientific breakthroughs are discovered accidentally. In other cases practical applications may result from studying the natural world. These practical applications of scientific discovery are called **technology**.



Let's take a closer look at two specific examples of how scientists work and design research experiments.

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## Research Design 1

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These research designs represent the steps that two scientists used to find answers to their questions. The thinking sections give a more detailed view of the process.

### The Carpenter Research Team's Experiment

**Observation:**

Some **dinoflagellates** have **cyanobacteria** living inside them.

**Thinking:**

This observation made the team curious. They began to ask questions.

**Broad Questions:**

Why would these two organisms live together?

Does this relationship benefit either partner? Is it a mutualism?

**Thinking:**

The research team knew that some other cyanobacteria could both do **photosynthesis** and fix nitrogen. They knew that the sugars and nitrogen produced by these processes were used by organisms. They also knew that some symbioses involving cyanobacteria were **mutualisms** that involved the cyanobacterium providing nitrogen to the **host**. They wondered if the same thing could be happening in this situation. So they came up with the following question.

**Specific Question:**

Does the cyanobacteria provide sugars from photosynthesis and nitrogen from nitrogen fixation to the dinoflagellate?

**Thinking:**

The research team knew that special molecules called **enzymes** are required for carbon fixation (part of photosynthesis) and nitrogen fixation. Each type of enzyme is specific for a target molecule. When the enzyme finds its target molecule, it will bind to it and create a new complex. The research team used this information to come up with their hypotheses.

**Hypotheses:**

1. If the cyanobacteria are fixing carbon (carrying on photosynthesis), then they will have the key enzyme that fixes carbon (**Rubisco**) in their cells.
2. If the cyanobacteria are fixing nitrogen, they will have the key enzyme for nitrogen fixation (**nitrogenase**), in their cells.
3. In addition, if the dinoflagellate depends on the cyanobacterium for both carbon and nitrogen, then the Rubisco and nitrogenase enzymes will only occur in the cyanobacterium and not in the dinoflagellate.

**Thinking:**

Now the team had to come up with a plan to test their hypotheses. They drew on their knowledge about the way substances react with one another to develop their design. They knew that they could make special reagents called **antibodies** that would recognize the Rubisco and nitrogenase enzymes. If the enzymes were present in the sample, the antibodies would connect with their target molecule. The antibody and its enzyme would stick together creating complexes. A tracer could be added to the sample that would connect to the antibody complex and allow it to be seen using a transmission electron microscope. Scientists would also be able to see where the complexes were localized within the cells. If the enzymes were not present, the antibodies could easily be washed out of the cells after the experiment. They would not be seen in the sample viewed by electron microscopy.

**Plan:**

Antibodies specific for rubisco and nitrogenase would be made. These antibodies would be introduced to the sample. The antibodies would attach to the target enzymes present in the sample. A second reagent would be created with gold beads attached to it. This second complex would act as a tracer. When it attached to the original enzyme-antibody complex, the gold's reflective properties would allow it to be seen with a transmission electron microscope. If the enzymes were not present, the complexes would not be seen using the microscope.

**Procedure:**

The samples were collected from the seawater. They were exposed to the antibodies. They were viewed using a transmission electron microscope.

**Results:**

1. Enzymes for carbon fixation (Rubisco) were only found in the cyanobacteria.
2. Enzymes for nitrogen fixation (nitrogenase) were not found in either organism.

**Conclusions:**

1. Hypothesis one was supported. The cyanobacteria were capable of photosynthesis because they had the Rubisco enzyme; the dinoflagellate probably was not capable of photosynthesis since it did not have the enzyme Rubisco.
2. Hypothesis two is falsified. Neither organism had the enzyme nitrogenase. This means that the cyanobacteria are probably not providing the products of nitrogen fixation to the dinoflagellate.

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## Research Design 2

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Dr. Wood spends a lot of time studying cyanobacteria and their close relatives. Most of the time she finds these organisms free-living in the water column as independent individuals. But a few years ago, while she was studying cyanobacteria in the Indian Ocean, she found some dinoflagellates that she had never seen before. Upon closer inspection of these organisms, she found that they had cyanobacteria living inside of them.

### Dr. Wood's Experiment

**Observation:**

Some dinoflagellates living in the Indian Ocean have cyanobacteria living inside of them.

**Broad Question:**

What do scientists know about this symbiotic relationship?

**Thinking:**

She began to do some research to find out what other scientists might know. She found that only a few people had written papers about this relationship, and no one had figured out how the relationship was helpful to its members. She became particularly interested in Dr. Carpenter's team and their research. Building on what Dr. Carpenter's team had learned she continued to develop their model that the **symbiosis** was some form of mutualism in which both partners got a benefit from living together. She formulated another question.

**Specific Question:**

Is the enzyme for fixing carbon (Rubisco) that is found in the cyanobacteria actively fixing carbon; and, if it is, is the carbon fixed by the cyanobacteria transferred to the dinoflagellate?

**Thinking:** Dr. Wood knew that during carbon fixation (part of the photosynthetic process), carbon enters the cell as carbon dioxide. Carbon dioxide is a gas that is **soluble** in water. The processed carbon dioxide becomes part of the sugar made during photosynthesis. If the carbon is now part of the cell material it cannot be easily washed away. She also knew that she could trace the carbon in carbon dioxide and find out if it was turned into sugars by labeling it with a **radioactive** form of carbon.

**Hypothesis:**

1. If the cyanobacteria were actively fixing carbon then the labeled carbon from carbon dioxide would be incorporated into cell material during photosynthesis.

2. If the model of mutualism that involves the cyanobacteria providing sugar to the dinoflagellate is true, then labeled carbon would first appear in the cell material of the cyanobacteria and only later in the dinoflagellate.

**Thinking:** An autoradiographic technique could be used to label the carbon. This would allow us to trace it through the cell. **Autoradiography** is similar to photography. In photography, a special chemical (photographic emulsion) on the film is exposed (or turns dark) when light hits it. This creates a negative. In autoradiography, the radioactive material acts as the light. Wherever there is radioactivity, the film is exposed and has dark spots on it.

**Plan:**

Using autoradiographic techniques, trace carbon flow in samples that have been incubated in sunlight and compare to a known photosynthetic cyanobacteria for verification (a control).

**Procedure:**

1. Collect and prepare specimens from the ocean.
2. Add radioactive carbon dioxide (a tracer) to the sample.
3. Incubate in the light for about one hour (for photosynthesis to take place).
4. Remove the soluble carbon dioxide and expose the sample to photographic emulsion.
5. Run a control on a photosynthetic cyanobacterium using the same experimental technique.

**Results:**

1. The control shows that dark spots would indicate photosynthetic activity.
2. The autoradiographic techniques show evidence of fixed carbon (in the form of dark spots) in the area of the cyanobacteria.

**Conclusions:**

The results of the two experiments seem to indicate that the cyanobacteria are contributing products of photosynthesis to the dinoflagellate. It appears that the dinoflagellate cannot do this on its own. It appears that at least one side of a mutualistic relationship is being described by the experiments that Dr. Wood and Dr. Carpenter and their colleagues have done.

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## Activity: Symbiosis Search

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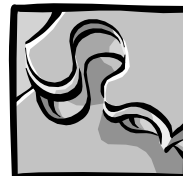
Scientists generally follow the scientific method to set up an experiment. This method has several steps but there are also variations within the method depending on the type of problem to be solved or the information that the scientist is seeking. One important part of any scientific endeavor includes research. Scientists gather materials to find out what has all ready been done in their field. They look for information that will help them with their next scientific experiment. Today, with so much information available, it is critical for scientists to consider the source of their information and its reliability.

**Objectives:** Students will be able to do the following:

1. Search the internet to find answers to questions.
2. Compare information from two sources.
3. Propose criteria for determining sources of reliable information.

**Materials:**

- Internet access
- Question page
- Writing instrument



**Procedure:**

1. Have students use the following websites to answer the questions in the Symbiosis Search.
  - Source 1-<http://oceanworld.tamu.edu/students/coral/coral3.htm>
  - Source 2-  
<http://mgd.nacse.org/hyperSQL/lichenland.html/biology/meeting.html>
  - Source 3-<http://www.imm-km.unibe.ch/projekte/symbiosis/Sym.html>
  - Source 4-<http://www.seacave.com/sym.html>
2. Have students look for the answer to either question 3 or 4 in another resource. (Use other websites, written materials, human sources, etc.) What conflicting information (if any) did they find?
3. Brainstorm with students how to determine the best source when conflicting information is given.
4. Devise a plan to test the proposal. Did it take into account all variables? Does it need to be revised? Where in the real world do we find similar problems?

**Answer Key:** The number in parentheses indicates the internet source for the answer.

- |   |                                |
|---|--------------------------------|
| 1. alga and fungus (2)                              | 6. seawater (3)                |
| 2. bacteria ( <i>Vibrio fischeri</i> ) (3)          | 7. lichen (coral, etc.) (2)    |
| 3. “sym” means together and “biosis” means life (1) | 8. mucous coating (4)          |
| 4. less than 1% (1)                                 | 9. Cnidaria (1)                |
| 5. thallus (2)                                      | 10. antibiotics, food, dye (2) |

### Symbiosis Search

1. What two organisms join together to make a lichen?
2. What kind of organism produces light for the Hawaiian squid?
3. What does the word symbiosis mean?
4. What percent of the world's oceans are covered by coral reefs?
5. What is the name of the lichen "body"?
6. Where does the squid find its bioluminescent symbionts?
7. Name one organism (from the website) that is an indicator organism?
8. How is the anemone fish protected from the anemone's sting?
9. To what phylum does the coral animal belong?
10. Name one use for a lichen.

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**Activity: Can You Fix This?**

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Organisms need to have all of the components of their living tissues in order to live and grow. One important nutrient that all living things need is nitrogen. Nitrogen is commonly found in the atmosphere, but this nitrogen cannot be used directly by organisms. It must be changed into another form. Organisms that can change atmospheric nitrogen into usable forms are called nitrogen-fixers.

**Objectives:** Students will be able to do the following:

1. Construct organic molecules using the correct ratios of nutrients.
2. Describe a limiting factor.
3. Analyze the importance of nitrogen-fixers.

**Materials:**

- Items that can be connected and come in a variety of colors such as colored paper clips, legos, gum drops and toothpicks, etc. If nothing else is available, paper squares of various colors can be stacked or glued together.
- Four colors of the chosen item
- Plastic or paper bags to hold items-one per group
- One extra bag for the nitrogen-fixer

**Prior to the Activity:**

(Paper clips will be used for this demonstration. Any four colors may be used.) Prepare the nitrogen-fixer by placing at least 30 green paper clips into a bag. Prepare one bag for every four students using the following ratios of paper clips. You may make doubles if necessary.

- 36 white, 8 yellow, and 3 blue
- 36 white, 8 yellow, and 2 blue
- 36 white, 8 yellow, and 1 blue
- 36 white, 8 yellow, and 0 blue



**Procedure:**

1. Discuss the importance of nutrients to living organisms. Explain that these nutrients are incorporated into organic molecules that make up living tissue. In order to construct the correct molecule, the components must be combined in a particular ratio. Explain that the job of the nitrogen-fixer is to change one of these components, nitrogen, into a form that can be used in these organic molecules. In this activity, the molecule being made consists of 9 white paper clips, 2 yellow paper clips, and 1 blue or green paper clip. (This information can be written on the board.) The blue or green paper clip represents the nitrogen that is necessary to complete the molecule.
2. Give the nitrogen-fixer bag to one student. (This student will not be part of a group.)



3. Give one bag to every four students, and tell them that they will be given a limited amount of time to construct their molecules using the correct number and colors of paper clips.
4. Give students a signal to begin.
5. Have students build their molecules with only the paper clips they have in their bags. Discuss what problems they encountered and how many organic molecules they were able to complete. Record their results.
6. Explain that nitrogen is the limiting nutrient in this case. If organisms could get more nitrogen, they could use all of their other nutrients (paper clips). Ask for ideas on how they could get more nitrogen.
7. Tell them that in this round they will again only have a limited amount of time to build their substances. This time they may signal the nitrogen-fixer to supply them with nitrogen. The trick is that nitrogen can only be asked for when the rest of the molecule is complete. Each group may only receive one nitrogen at a time. (This limits the number of compounds that can be completed, because the nitrogen-fixer can only move so fast. This represents processing time.)
8. Have students take their materials apart and redistribute bags to other groups.
9. Give students a limited amount of time to complete their molecules. (This will vary with group size.) Remind students that they may ask for nitrogen from the "fixer".
10. When time is up, have students compare the results from the first round to the results in the second round. Could they build more substances in round two? What would happen if there were more than one nitrogen-fixer? Can they relate this to what happens in real life?

**Possible Extensions:**

1. Have students build a complex compound for instance start with having them build a sugar ( $C_6H_{14}$ ) and then put in phosphate or nitrogen to make other compounds. Try this with and without a nitrogen-fixer.
2. Supply students with varying amounts of paper clips and try using a variety of time periods to complete the task. Are there other limiting factors in addition to the amount of nitrogen available?

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**Student Information: The Case of the Curious Observer**

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Are you curious and creative? Do you like to explore nature and try to

figure out how things work? This is what scientists do. They observe things in nature and become interested in learning more about what they observed. They begin to ask questions. They want to know why things happen or maybe how they happen. They begin to look up information. Scientists may find books at the library or information on the internet. They look for papers written by other scientists, and they talk with their colleagues. They gather lots of information and begin to form an idea about what is happening.

Scientists try to fit all the pieces together in a **conceptual model**. Then they try to predict what will happen if the model is true. They develop a testable prediction based on the model; this is called a **hypothesis**. This hypothesis helps the scientists focus their research. Next they need to design an

experiment to test their hypothesis. This takes planning and careful study of what scientific knowledge already exists. Once the planning is finished, the real fun begins. Researchers get to conduct the experiment! Each experiment is designed to test a particular hypothesis. Researchers may use the latest technical equipment or simple tools developed in the past while conducting their experiments.

After the experiment, the scientists study their results.

Researchers ask more questions. What do the results tell us?

Did the results support or falsify the hypothesis? What new prediction can we make to test the model in a different way? Do we need to change the model based on the results? At this point, scientists create new experiments or redesign old ones. They continue to search for answers.

Does this sound like something you would like to do?

