Process of Science: Paramecium Investigations

The best way to truly understand the scientific process is to do it yourself! In these two labs, you will investigate Paramecium behavior to explore many aspects of the process of science – doing background research, collecting observations, constructing a hypothesis, developing an experimental procedure to test your hypothesis, and analyzing your data to draw conclusions. The main goal of the first week is to gather relevant background information and observations in order to develop a hypothesis regarding the ability of chemicals to act as Paramecium attractants (or repellants). In a follow-up assignment, you’ll address questions that get you thinking about how to test your hypothesis. The second week of lab, you’ll design and perform an experiment to test your hypothesis, and learn how to analyze your data. For homework, you’ll generate graphs and/or tables to clearly present your results, and draw conclusions from your analysis. Through your experiments, you will also develop your ability to use two common types of microscopes and learn more about Paramecium, a single-cell eukaryote. The specific species you’ll use is *Paramecium tetraurelia*.

**IMPORTANT NOTES:**

- In addition to this handout, review your notes from our discussions in lecture of the scientific process.
- You need to bring this handout to the first TWO weeks of lab. Keep track of it!
- If you have a flash (jump) drive, you might want to bring it with you to lab. You’ll have the opportunity to take pictures and videos. The files are too big to send via e-mail, but if you don’t have a flash drive, you can send them to yourself by file transfer.

**LEARNING OBJECTIVES**

- Gather background information and observations to aid in hypothesis development.
- Generate a hypothesis and an experimental procedure to test your hypothesis.
- Gain experience analyzing results, performing statistical analyses, and generating appropriate tables and graphs to present your data.
- Be able to draw appropriate conclusions based on your data.

**INTRODUCTION**

*Process of Science*

The word *science* comes from the Latin word meaning “to know.” Ultimately, science uses objective information to build a body of knowledge that grows and changes as we obtain more information. As you know, there is no rigid formula or series of steps for carrying out scientific endeavors. However, scientific analyses do share a set of common components, including a few that are usually left out of textbook descriptions like imagination, serendipity, and luck. Biologists and scientists in all fields make observations, pose questions, gather relevant information, develop hypotheses, conduct experiments to test hypotheses, collect and analyze data, interpret results, and draw conclusions. Science is a never-ending process. Conclusions most often lead to more questions, hypotheses, and analyses. In many ways, that’s the best part of science!
**Experimental Design**

Hands-on experience is particularly important when it comes to designing experiments. It is one of the most challenging parts of the scientific process as there are numerous factors to consider including what’s actually feasible in terms of time and resources (e.g., equipment). The procedures for experimental analyses vary greatly. Observational studies carried out in the field differ from analyses undertaken in a laboratory setting. The paragraphs below focus on specific aspects of experimental design relevant to the investigations you’ll perform in this course.

When designing an experiment to test a hypothesis, it is essential to identify and carefully consider the variables – factors that can change during an experiment or vary between groups/conditions. There are three basic categories of variables – dependent, independent, and controlled.

- **Dependent variables** are factors that are actually measured, counted, or observed. They are the variables you expect to change in response to the experimental treatment.

- **Independent variables** are factors that are intentionally manipulated in an experiment by the researcher. They are your experimental treatment or treatments. Independent variables are selected because the researcher predicts that they will effect the chosen dependent variable(s).

- **Controlled variables** are conditions held constant between each group. **IMPORTANT:** Controlled variables are not your control group/condition! Controlled variables are all the things that you want to be sure to keep the same between your experimental and control group/condition to reduce the chance these variable could influence your results.

Your dependent variable(s) and independent variable(s) must be clearly defined before you develop your procedure. Although it is impossible to identify all possible factors that could be different between groups, you should identify other variables that are likely to impact your results, and determine the best ways to ensure they remain constant between your groups/treatments.

The procedure or method refers to the series of steps and materials used to conduct an experiment. When designing the procedure, you need to consider the level of treatment (magnitude of the independent variable), the sample size (number of participants/samples you will assess for each group/condition), and the number of replicates (how many times the experiment will be repeated). Importantly, most experiments need to include a control group – a group or condition where the independent variable is NOT manipulated. It is important to note, however, that in comparative analyses there is often no well-defined control group. For example, a scientist comparing the effectiveness of a particular drug for treating people of different ages might include two or more groups with different age ranges (e.g., 20-40 years old and 40-80 years old) that are both given the drug. In this case, there’s no inherent reason to consider the “young” or the “old” group the control group.

Although scientists may make some general predictions based on their hypothesis, more specific predictions are typically made after deciding on a particular set of experimental methods. Experiment design and prediction development is a somewhat circular process – how you design your experiment informs your predictions, and vice versa. Predictions can be expressed in the form of if/then statements: If the independent variable is X and an experiment is conducted in which X is varied, then the dependent variable will be altered in this way. Predictions are useful for evaluating the results of an experiment. If the results match your prediction, then the hypothesis is supported. If the results do not match, then the hypothesis is not supported. Although it seems straightforward, in many cases your data may be quite different from anything you would have predicted and/or fall somewhere in between matching and not matching your prediction. This is why hypotheses are tested multiple times in multiple ways, and researchers frequently revise their hypotheses as they gather more data.
**Microscopes**

Technological advances have allowed us to delve deeper and deeper into the mysteries of organisms. One of the most widely used tools is the microscope (aka scope). There are numerous different types of microscopes that are used for different purposes. The two that are available in lab are described below. In addition, there will also be cameras that you can use to capture images and videos of your Paramecia.

**IMPORTANT:** Read the descriptions carefully so that you have a basic understanding of how to use the scopes BEFORE you come to lab. Your lab TA will demonstrate use of both types of scopes and the cameras.

**Stereo (dissecting) microscopes** are used to examine surface features of solid objects. They illuminate samples with reflected light – light that strikes the outside of objects. Dissecting microscopes magnify objects between 10 and 75 times (10X to 75X), and provide a 3D image to the viewer. Typically, the magnification is a zoom type that allows smooth adjustment of any magnification within the range of the microscope.

**Compound microscopes** are used to view very small objects, and are capable of magnifications up to 1000 fold (1000X). Light is transmitted through a sample to illuminate the interior. Compound microscopes must be used with glass slides that have cover slips over the specimens to be viewed. The slide is moved around under the lens by a finely adjustable mechanical stage, and the magnification is changed by selecting a particular lens. Unless connected to a computer with image processing software, compound microscopes provide a 2D image to the viewer.

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**Paramecium Techniques**

Like all organisms, Paramecium must obtain energy (food) from their environment. Additionally, there are certain conditions that are harmful, and these conditions must be avoided for a Paramecium to survive. In your analysis, you'll be focusing on the ability of certain chemicals/conditions to attract or repel Paramecium. The steps below describe how to perform a basic Paramecium “attraction assay.” You'll refine this procedure as you develop a specific method to test your hypothesis. Also included are steps for getting an up-close and personal view of the Paramecia cells using a compound microscope.

**IMPORTANT** technical details:
- Use clean plastic pipets and pipettor tips to avoid contaminating the stock solutions.
- Keep all the solution bottles and Paramecium tubes CLOSED when not in use.
- Dispose of used plastic pipets and pipettor tips in the lab waste containers provided!
- Wipe up any liquids that you spill on the microscope stages using kimwipes.
- Clean your slides with 70% ethanol between uses. When you are done with your slides, wash them well with soap and water.
- Put all used kimwipes in the lab waste.

**Paramecium Attraction Assay**

1) Obtain a sample of Paramecium and test solution(s).
   - Mix the stock tube of Paramecia gently. With the pipettor provided, use a clean tip to transfer 0.5 mLs (500 μL) of the Paramecia solution to a clean microfuge tube.
   - Label the top of the microfuge tube so that you can keep track of it.
   - Using a CLEAN tip, transfer 0.3 mLs (300 μL) of a test solution into a clean microfuge tube. Label the top.
   - Repeat the step above to obtain additional test solutions.

2) Clean a glass slide using 70% ethanol and a kimwipe. Thoroughly dry your slide carefully with a clean kimwipe. A dirty slide will not allow you to conduct the assay well!
3) Place your cleaned slide on the dissecting microscope.

4) Gently invert your microfuge tube of Paramecia cells to mix. Using a clean plastic transfer pipet, put one drop of the Paramecium solution just to the left of the center of the slide.

5) With a CLEAN plastic pipet, put a drop of a test solution next to your Paramecium drop (on the right). IMPORTANT: Put the drop of your test solution far enough away so that it doesn’t merge with your Paramecium sample, but close enough that you’ll be able to connect the two drops (see next step).

6) Using the TOP of the plastic pipet you used for your test solution, carefully connect the two drops, dragging the test solution into the drop of Paramecium. (Think about why you do NOT want to drag the Paramecium drop into the test solution!) Note – Your lab TA will demonstrate how to do this in lab.

7) Observe whether the Paramecia are attracted or repelled (or neither) by the test solution.

8) To carry out additional assays, repeat steps #3 through 8.

**Viewing Paramecia with a Compound Microscope**

1) Follow step #1 from the method above to obtain a sample of Paramecium if you do not have one.

2) Clean a glass slide using 70% ethanol and a kimwipe. Dry your slide with a clean kimwipe.

3) Gently invert your microfuge tube of Paramecia to mix. Using a clean plastic transfer pipet, put one drop of the Paramecium solution on the slide.

4) Starting at one edge of the drop, carefully lower a coverslip over your Paramecia. Blot any excess liquid that spills out the sides using a kimwipe.

5) Adjust your compound microscope to the lowest power (4X), and place the slide on the stage.

6) Using the coarse and then fine focus adjustments focus the scope on the Paramecia sample. IMPORTANT: Make sure that the coverslip does NOT hit the lens!

7) Adjust the mechanical stage to scan the slide and look for Paramecia.

8) When you have focused on the Paramecia, switch to a higher magnification for a closer look. IMPORTANT: Note that the higher power lenses are longer than the 4X lens. Make sure that the lens does NOT hit the coverslip!

9) Using ONLY the fine focus adjustment, focus on the Paramecia at the higher power.
PART I: Background, Observations, and Hypotheses

For your Paramecium analyses, you’ll work in groups of two. BOTH partners need to be active participants and complete their own Paramecium Hypothesis & Experimental Design Questions. You’ll complete the first two sections in lab, and finish it prior to your next lab.

I. Quiz
As described in your lab syllabus and noted in the lab schedule, you will have a pre-lab quiz at the beginning of most lab periods. To prepare for the quiz, read this handout carefully AND review your notes from our discussions in lecture on the process of science. You should focus particularly on what you’re doing in lab this week, and hypotheses.

II. Lab Discussion
- logistics and expectations
- the scientific process

III. Gather background information
Before developing hypotheses or experiments to address a question, it is important to do some background research in order to determine what’s already been reported that relates to your question. Your goal is to search the internet for information that could be helpful for exploring the question of what chemicals attract (or repel) Paramecium. The questions below are designed to help guide your search for information, but are certainly NOT the only questions worth answering! As you gather information, think carefully about the validity of the sources you’re using (i.e. how much do you trust the information?). BOTH partners need to WRITE DOWN the background information you collect in the first section of your Paramecium Hypothesis & Experimental Design Questions. You should use at least THREE sources.
- What type of organism is Paramecium?
- Where are Paramecia found? In what conditions do they typically live?
- What do Paramecia eat?
- How do Paramecia move?
- Have any similar analyses been performed? If so, what did they find? NOTE – Google scholar is probably your best bet for searching for information related to this question.

IV. Lab Discussion:
- Paramecium background and source validity
- microscope and camera demos
- attraction assay set-up

V. Make observations and develop a hypothesis
Using the basic procedures described in the Introduction section, collect observations that will help you construct a hypothesis regarding the ability of chemicals/conditions to act as Paramecium attractants (or repellants). Record your observations in the second section of your Paramecium Hypothesis & Experimental Design Questions. As you perform these initial analyses, THINK about how you are going to measure attraction (or repulsion). Work with your partner to develop a hypothesis regarding the ability of chemicals/conditions to act as Paramecium attractants (or repellants).

Your completed Paramecium Hypothesis & Experimental Design Questions are due at the BEGINNING of lab next week.
PART II: Experimental Design, Data Collection, and Data Analysis

This week, you’ll design and carry out an experiment to test the “Paramecium attraction” hypothesis you developed in lab last week, and learn how to analyze your data. **IMPORTANT:** In addition to reviewing this handout, please read the Excel Guide posted on BlackBoard appropriate for the Excel version available in your lab room.

I. Quiz
   To prepare for this week’s quiz, review this handout carefully AND review your notes from our discussions in lecture on the process of science. Focus on what you’re doing in lab this week and experimental design.

II. Lab Discussion: hypotheses & experimental design (part 1)

III. Experiment Design
   Work with your partner to define the specific experimental procedure you’ll use to test your hypothesis. BOTH partners should write down the procedure. You’ll need it for your Paramecium Results and Conclusions assignment!

IV. Lab Discussion: data analysis

IV. Data Collection & Analysis
   On a sheet of paper, create a table in which to record your data. Work with your lab partner to carry out your experiment. BOTH lab partners must record ALL data! In addition, note any relevant observations and deviations from your methods. Once you have all your data, use the remaining time to start your data analysis. Copies of the Excel Guide will be available in lab, and you are strongly encouraged to talk to your lab TA if you are having trouble with Excel and/or the calculations needed to analyze your data. Remember that the more you complete in lab, the less you’ll need to do later!

Make sure that you have your written procedure and ALL the data from your experiment before you leave lab! E-mail any files you’ve generated to yourself so that you have them to complete your Paramecium Results & Conclusions assignment (posted on BlackBoard). Your assignment is due at the beginning of your next lab period. You are encouraged to discuss the assignment with your partner, but each person must turn in their OWN work!