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# A Multiweek Project Examining the Chemotactic Behavior of *Tetrahymena* in an Undergraduate Biology Laboratory †

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## INTRODUCTION

Single-celled eukaryotes offer a wide range of benefits for laboratory exploration by undergraduate students. *Tetrahymena*, a free-living ciliate, has proven to be especially beneficial in laboratory exercises for both K–12 and college-level students (1). The Advancing Secondary Science Education through Tetrahymena (ASSET) program at Cornell University (https://tetrahymenaasset.vet.cornell.edu/chemotaxis/) offers modules for science exploration for all levels of education. Phagocytosis, population growth, microscopic staining, and chemokinesis have all been presented by Bozzone (2) as options for basic procedures with *Tetrahymena* as well as opportunities for student-designed experiments. Beyond its use in educational settings, *Tetrahymena* has been proposed as a test organism for the detection of pharmaceuticals and pollutants (3, 4).

Inquiry-based laboratory experiences have been employed in a variety of courses (5–7) and implemented in different ways suitable to the pedagogical needs of those courses. The consensus regarding these efforts has been that students retain as much content as they do in other, more traditional laboratory exercises, if not more, and in addition, attain a much fuller appreciation of the methods and scope of the scientific enterprise.

In an introductory biology laboratory for majors, we designed a multiweek project employing single-celled eukaryotes, with an emphasis on genetic influence on motility and chemotaxis. We aimed to combine microscopic observations of protists and their behavior, quantitative analysis of responses to changes in the environment, and the incorporation of genetic mutants to supplement course coverage of Mendelian genetics. The laboratory portion of the course consists of three distinct modules that span the semester. The first module emphasizes biological molecules and concludes with a guided inquiry lab in which students design an experiment to investigate the effects of pH, temperature, and inhibitors on the activity of lactase (8). The second module introduces students to cell biology and concludes with a guided inquiry experience on yeast fermentation in which students examine the effects of time, concentration, and the nature of sugar on rates of fermentation (9). For the third module, we have designed a three-week project in which students investigate the chemotaxis of *Tetrahymena* and examine how changes in the environment and genetics influence the behavior of these cells grown in culture. While previous studies have used *Tetrahymena* in the laboratory to study chemotaxis (10), we have modified the protocol for incorporation into an undergraduate setting and further extended the activity to include the analysis of genetic mutants. Herein, we will describe a multiweek laboratory activity for the third laboratory module of the course.

Through this multiweek laboratory experience, students will:

1. Understand the process of chemotaxis and the factors that influence this process in *Tetrahymena*
2. Understand the link between genotype and phenotype through the use of Tetrahymena mutants
3. Further develop microscopy skills through visual analyses of *Tetrahymena*
4. Practice the skill of experimental design with proper use of controls and additional variables

## PROCEDURE

### General procedure

This chemotaxis assay is based on a procedure (10) employing a two-phase density step gradient where *Tetrahymena* cells are layered on top of a Percoll solution and their chemotactic migration into the density medium monitored by spectrophotometry at 550 nm. Percoll is an iso-osmotic

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medium used in cell purification by centrifugation. The timed assay is begun with careful layering of cells on top of 1.0 mL of Percoll in a plastic disposable cuvette. The cuvette is immediately placed into the spectrophotometer, zeroed, and $A_{550}$ readings taken every two minutes. Total time for one assay is typically 26 minutes.

## Multiweek project design

In the first week, students run the chemotaxis assay with wild-type *Tetrahymena* using two concentrations of the chemoattractant proteose peptone (in Percoll) and a control (Percoll only). This initial experiment accomplishes multiple goals. First, students gain an appreciation for the effect of specific molecules on the chemotaxis of living cells. Secondly, they examine and discuss the concentration-dependent effects of the chemoattractant being used. Lastly, this assay provides the groundwork for later assays in subsequent weeks. Student-generated results are shown in Figure 1.

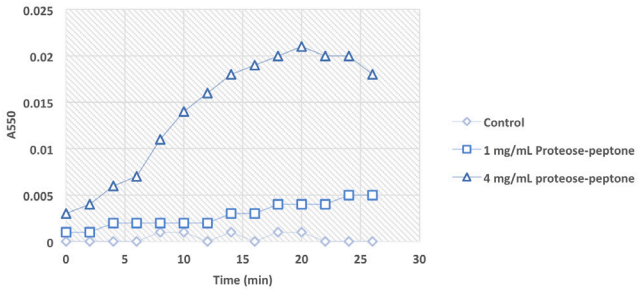


FIGURE 1. *Tetrahymena* chemotaxis toward various concentrations of proteose peptone, a known chemoattractant. Students performed the described experiment at room temperature in a Genesys 20 spectrophotometer according to the instructions provided in the Week One student protocol (described in Appendix 1).

The second week allows students to further explore the dose-response of varying concentrations of the chemoattractant and consider the meaning of their results in terms of biological effects and experimental design. In this experiment, students continued studying the effect of proteose peptone through analyses that employ significantly higher concentrations than those used in the first week. Student-generated results are shown in Figure 2.

In the third week of the project, students examined the effect of a known temperature-sensitive mutant of *Tetrahymena*, the *oad* mutant, missing outer dynein arms at the restrictive temperature. This genetic mutant has previously been reported to display decreased motility (11). This experiment allowed students a great opportunity to link the role of specific genes to a well-examined phenotype such as chemotaxis. To do this, students carried out experiments to analyze whether the *oad* mutant, when grown at the permissive temperature, would exhibit any phenotypic changes in chemotaxis (see Supplemental Materials). Student-generated data from this experiment are shown in Figure 3.

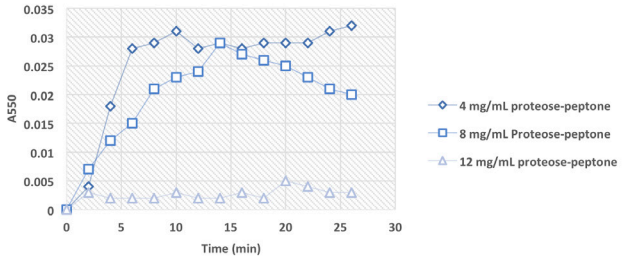


FIGURE 2. Chemotaxis of *Tetrahymena* toward increasing concentrations of proteose peptone. Students carried out a chemotaxis assay utilizing *Tetrahymena* and increased concentrations of proteose peptone (described in Appendix 2). Results varied as to whether 4 mg/mL or 8 mg/mL gave the larger response, but 12 mg/mL consistently gave the poorest chemotaxis. Students were encouraged to discuss possible reasons for that effect.

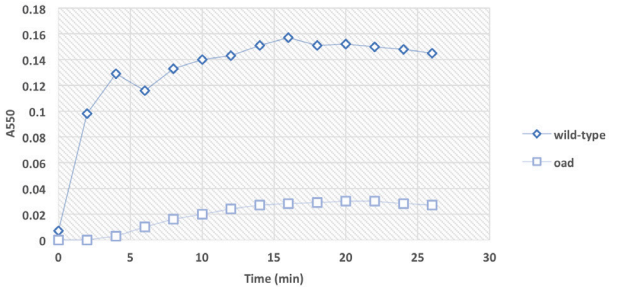


FIGURE 3. The effect of *oad* gene mutation on the chemotaxis of *Tetrahymena*. Student-generated data examining the chemotactic ability of *oad* mutants as compared with wild-type *Tetrahymena* cells (described in Appendix 3). Data show that cells lacking a functional *oad* gene display a significant decrease in their ability to migrate toward a chemoattractant in this assay.

Experiments from week three provided the opportunity for students to analyze the effect that specific genes have on observable phenotypes. It is important to note that since the *oad* mutant cells are temperature-sensitive in nature, some student groups had variable data with regard to the ability of these mutants to migrate toward the chemoattractant. However, in every case, there was a significant increase in the chemotactic movement of wild-type cells compared with the *oad* mutants.

In addition to the spectrophotometric description of cellular behavior, the experiments from week three were coupled with an activity to reinforce students' microscopy skills. Through analyses of wet-mounts, students observed both wild-type and mutant *Tetrahymena* cells microscopically, generated detailed drawings of the cells, and included written descriptions of behavior and semi-quantitative estimates of directionality of motility, the percentage of cells moving, and the relative speed of movement.

## CONCLUSION

This multiweek, inquiry-based project focused on the chemotactic response of *Tetrahymena* to a known attractant and provided multiple opportunities for students to learn a new spectrophotometric technique based on the concept of light scatter instead of absorbance. In addition, they familiarized themselves with chemotaxis, became acquainted with density step gradients, and related microscopic observations to spectrophotometric measurements.

Through faculty-led discussions following this module, students proposed a number of questions regarding the assay and how to interpret data correctly. If so desired, this lab could be extended to additional weeks. Some examples of further studies using this technique are given below:

- What will be the effect if the temperature-sensitive *oad* mutant is actually grown at its restrictive temperature?
- What if chemoattractant concentration is varied with the *oad* mutant grown at room temperature?
- How would different species of the *Tetrahymena* genus compare in chemotactic rate?
- Proteose peptone is the principal ingredient in the growth medium for *Tetrahymena*. Are there other substances which may also function as a chemoattractant in this assay?

There are several interesting modifications that can be employed to increase the level of student-directed inquiry with this system, ranging from simple exercises to multiweek projects. In our opinion, the essence of the best experiments is that they lead to further questions. We believe this assay provides a platform that encourages this intellectual pursuit by students.

## SAFETY ISSUES

*Tetrahymena* is a BSL1 organism, and all work should thus be performed in BSL1 laboratories, with appropriate personal protective equipment. Students should be trained in BSL1 procedures prior to conducting this laboratory activity. During the creation and use of these protocols, all ASM biosafety guidelines were followed (https://www.asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-Laborator).

## SUPPLEMENTAL MATERIALS

Appendix 1: Week one student protocol
Appendix 2: Week two student protocol
Appendix 3: Week three student protocol
Appendix 4: Laboratory preparation instructions

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