Inducing Mutations in *Paramecium*: An Inquiry-Based Approach

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Abstract- A major challenge in teaching any college level general genetics course including a laboratory component is having the students actively understand the research part of an experiment as well as develop the necessary laboratory skills. This laboratory experience furthers the students' knowledge of genetics while improving their laboratory skills. It provides the students with experience in the design and implementation of their own experiments. This inquiry-based approach will provide them an opportunity for a deeper appreciation of how scientists perform their investigations. The students were given four weeks to induce mutations into *Paramecium* that will alter their physical and/or behavioral traits.

Keywords: Undergraduate, *Paramecium*, mutation, genetics, inquiry-based

INTRODUCTION

A major challenge in teaching any college level general genetics course including a laboratory component is having the students actively understand the research part of an experiment as well as developing the necessary laboratory skills. Most laboratory exercises published for undergraduate genetics courses fall into the traditional cookbook approach and do not provide the students the opportunity to design their own experiments. The goals of this laboratory experience were to: further the students' knowledge in the field of genetics while improving their laboratory skills; provide the students experience in the design and implementation of their experiments; provide the students experience in literature searches; and provide the students the experience of presenting their results from their experiments in a symposium format. The National Science Education Standards state that students "must actively participate in scientific investigations, and they must actually see the cognitive and manipulative skills associated with the formulation of scientific explanations" (National Academy of Sciences, 1995). This inquiry-based approach is designed to give them a deeper appreciation of how scientists conduct their investigations. The students were provided with four weeks to induce mutation into *Paramecium* that would alter physical and/or behavioral traits.

Since the extensive and classic work by Jennings (1906), *Paramecium* (Figure 1) has been one of the favorites for the study of behavior in unicellular organisms and has been used in many studies that are important for understanding the physiological mechanisms of behavior (Van Houten, 1992). This organism has been shown to have relevance to investigations of cellular processes and signal transduction pathways in higher organisms. *Paramecium* is an excellent model for studying the diverse aspects of behavior including chemical sensation and response, detection of chemical gradients, changes in cellular membrane potential, and responses to stimuli (Kung et al., 1975; Kung & Saimi, 1982). *Paramecium* incorporates the complexity of many larger life forms in a compact package. It possesses motility, electrical activity akin to nerve cells, and cellular signaling pathways similar to those found in humans. *Paramecium* has been likened to "swimming neurons" (Hinrichsen & Schultz, 1988). They have been used as a primitive system to study taste and olfaction, galvinotaxis, and vertigo, to name a few. One particular advantage of studying this organism is its swimming behavior- swim speed and turning frequency, which are easily recorded and are well documented in the literature for both wildtype and known mutants. Moreover, *Paramecium* is responsive to a wide range of environmental stimuli, including chemicals (taste/smell), light, touch, osmolarity, gravity, and electrical fields (Hinrichsen & Schultz, 1988; Machemer, 1988; VanHouten & Preston, 1987).

Figure 1. *Cell of wildtype* Paramecium. *Photo by John Wayne Johnston*

Paramecia, like other ciliates, have both a germline and a somatic nucleus. The germline micronucleus is diploid, and intervenes in sexual processes (Fujishima, 1988). The somatic macronucleus is polyploid and is the seat of all transcription. Because of the elimination of repeated sequences, the macronuclear genome of *Paramecium* is very "compact"; it is estimated that its coding region is over 70% compared to 1% found in the human genome (Genscope). The introns are small (from 18 to 35 bases) and the intergenic regions generally less than 50~100 bases (Figure 2). Because of this, mutations are very likely to affect genes and therefore produce a measurable phenotype.

Paramecium is an ideal classroom organism because it is easily cultured, can reproduce readily, is accessible to microscopic study, inexpensive and readily available, (as are all the materials necessary for the following exercise). Additionally, studies of *Paramecium* raise no ethical issues inherent in many animal studies. This organism is an excellent model to incorporate scientific inquiry based activities into the classroom. Today it is important for educators to provide their students with the opportunity to experience good basic science, which includes questioning, experimentation, observations, data collection, analysis and finally drawing conclusions based upon their findings. This *Paramecium* based activity represents how scientific inquiry can be built into the classroom curriculum and be in compliance with the National Science Standards as seen in Table 1.

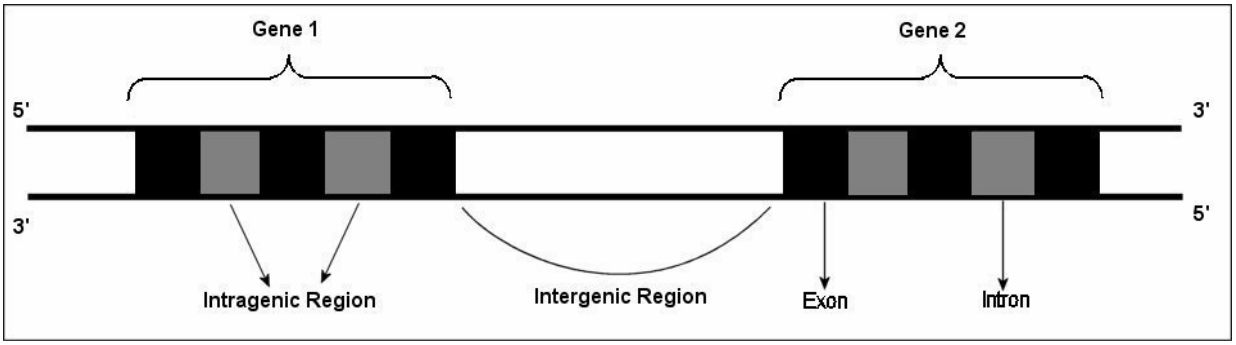


Figure 2. *The schematic of DNA sequence shows two genes. Note the intragenic regions known as introns. Introns are non-coding DNA segments, which are removed after transcription to produce a functional messenger RNA. Also included in the figure are exons and intergenic regions between the genes.* Paramecium tetraurelia *has very few intragenic and intergenic regions. Image created by Olivia Cauthorn.*

Table 1. *National Science Standards, (National Academy of Sciences, 1995)*

TEACHING STANDARD	FOCUS OF STANDARD	DOMAINS OF INCLUSION
Standard A	Teachers of science plan an inquiry-based science program for their students	In doing this, teachers select teaching and assessment strategies that support the development of student understanding and nurture a community of learners
Standard B	Teachers of science guide and facilitate learning.	In doing this, teachers ✓ focus and support inquiries while interacting with students ✓ orchestrate discourse among students about scientific ideas ✓ challenge students to accept and share responsibility for their learning ✓ recognize and respond to student diversity and encourage all students to participate fully in scientific learning ✓ encourage and model the skills of scientific inquiry, as well as the curiosity, openness to new ideas and data, and skepticism that characterizes science
Standard D	Teachers of science design and manage learning environments that provide students with the time, space, and resources needed for learning science.	In doing this, teachers ✓ structure the time available so that students are able to engage in extended investigations ✓ identify and use resources outside the school
Standard E	Teachers of science develop communities of science learners that reflect the intellectual rigor of scientific inquiry and the attitudes and social values conducive to science learning	In doing this, teachers ✓ display and demand respect for the diverse ideas, skills, and experiences of students ✓ nurture collaboration among students ✓ structure and facilitate ongoing and informal discussions based on the shared understanding of rules of scientific discourse

METHODS AND MATERIALS

The students were supplied with a bacterial culture of *Klebsiella pneumonea*, regular wheat media, and a stock of *Paramecium tetraurelia.* The student teams (2-3 students) were responsible for maintaining a stock solution of *Paramecium tetraurelia* wildtype cells. *Paramecia* were grown in wheat media that had been inoculated with bacteria. The bacteria, *Klebsiella pneumonea*, were grown overnight at 37^{o} C in 50 mL wheat medium; the following day, the inoculated wheat medium was allowed to cool to room temperature, and 500 ul of Paramecium was added to the inoculated medium. Following the overnight incubation, the wheat medium was cloudy in appearance due to the bacterial growth. As the cell population increased over time, the cloudiness of the wheat medium culture decreased due to the cells feeding on the bacterial within the culture. A new stock cell culture would have to be started every 8-10 days. This involved inoculating 50 mL of wheat medium with *Klebsiella pneumonea*, growing the culture overnight at 37^{o}C, allowing the flask to cool to room temperature and then transferring 500 µl of cells from the previous stock culture into the freshly inoculated wheat medium. In addition the students were instructed on how to video tape and calculate their cells swim speeds and turning frequencies. They were also provided with stage micrometers as a means to measure the size of the cells. Stage micrometers are available through any biological supply catalog.

OUTLINE OF THE LABORATORY SCHEDULE

This activity was implemented over a four-week part of the semester that used a weekly 3-hour laboratory period. All the students were familiar with working with *Paramecium* in the lab because their two previous laboratory activities involved these cells. In addition, the students received the necessary information about these cells from both their laboratory notebook (Elwess & Latourelle, 2003) and lecture notes. These sources included two well-documented facts about *Paramecium tetraurelia*, its average swim speed is 1.0 mm per second, and it is typically 120-140 µM in length.

We presented the students with the challenge of inducing measurable phenotypically sustained changes in *Paramecium tetraurelia* one week in advance of the start of their projects (Figure 3). Students were advised, that once they had evidence that they had achieved phenotypic changes, their cells were to be placed back into the regular wheat medium for at least two to three days. This would ensure the change would be carried over many generations since Paramecium populations double every 70 minutes under ideal conditions (Kippert, 1996). However with the transfer of 500 ul of experimental cells back into 50 mL of regular wheat medium, the authors recognize that a small amount of mutagen might be transferred. A future inclusion for this laboratory experience will include a washing step prior to transfer. This would involve concentrating the cells by centrifugation (350 x g) for 1 minute in 15 mL conical tubes. The cell pellet would be collected and suspended in 50 mL of regular wheat medium that had not been inoculated. Then 500 ul of culture would be placed into a 50 mL of inoculated wheat medium. This would reduce or eliminate the transfer of the mutagen. It was important for the students to recognize the difference between a mutagen versus the action of an agent simply causing a physiological effect on behavior.

The students were informed of materials available to them in the lab, and told they could bring in products (with instructor's approval). They were asked to consider what possible agents in the cells' natural environment could cause mutations, and suggest some ideas including such as farm runoff, fertilizers, and ultraviolet light exposure. Each team of students was required to submit an experimental design matrix to the instructors before starting their preliminary investigation (Figure 4). The students were provided time in the departmental computer laboratory to research primary literature using databases such as PubMed. The students also started the necessary cell cultures that they would need to perform their initial investigations. The cultures usually took 4-6 days to be at their optimal growth in terms of number of cells.

Week 1

Upon approval of their experimental design, teams set up their initial experiments. A primary objective was to determine the concentration range and time of exposure for their chosen mutagens. They determined a workable concentration range as well as exposure time through simple trial and error as well as obtaining information from the primary literature. Monitoring of the cells demanded frequent laboratory visits for microscopic observations. This served to reinforce the students' understanding and preparation of solutions, dilutions, and concentrations. Experiments included exposing the cells to Miracle-Gro®, UV light, Vitamin A, cupric sulfate, microwave radiation, sodium azide, sterobol, and caffeine. At the end of this week the teams were to submit a revised matrix based on their preliminary results, which had a more defined approach with a better-stated hypothesis.

Weeks 2-3

During this time the students focused on refining their concentrations and/or length of exposure for their chosen approach. There was a computer laboratory across the hall from the genetics laboratory where the students could research their approach and/or journal articles related to their experiments.

Week 4

The students used this time to document and analyze their results. This included videotaping the experimental cells to quantify or qualify their swimming behaviors compared to wild-type cells and/or taking pictures of their cells to determine differences in cell structure and/or physiology. The students used the computer lab for data analyses.

End of the Semester

At the end of the semester each team gave a 12-15 minute oral presentation in a symposium format. This involved each team bringing the results of their research to their peers in a more formal manner than simple classroom discussion. Their presentations included their experiment design, background information from literature reviews on their approach, results, conclusions, and finally suggestions for follow-up experiments.

RESULTS

The students obtained a wide variety of results with this laboratory experience. Fifteen (55.55%) of the 27 student teams had quantitative success in inducing mutations in their cells. Six teams *appeared* to have some type of mutation but could not produce the data to support this finding. This in itself was an importance lesson for them, that in scientific experimentation data are needed to form conclusions. Six teams generated data indicating that they did not induce any physical or behavioral mutations. This helped enforce the concept that negative results are an important part of scientific investigation.

Inducing Mutation
(Design Matrix Required)

Introduction:
Paramecium is an excellent model for studying the diverse aspects of behavior including chemical sensation and response, detection of chemical gradients, changes in cellular membrane potential and responses to stimuli. As a unicellular organism, *Paramecium* incorporates the complexity of many larger life forms in a compact package. It possesses motility, electrical activity akin to nerve cells, and cellular signaling pathways similar to those found in humans. Paramecia have been likened to "swimming neurons". The genome of *Paramecium* is very "compact": it is estimated that its coding region is over 70% compared to 1% found in the human genome. *Paramecium* is an ideal classroom organism because it is easily cultured, can be grown in large numbers, is accessible to microscopic study, inexpensive and readily available, as are all the materials necessary for the following exercise. Additionally, *Paramecium* raise no ethical issues inherent in animal studies. This is the organism that will be used for this laboratory activity-Inducing Mutations. Mutations are useful in Science because they are an indication of where problems are occurring and what might be causing them.

Purpose and Objectives:
Your group will design an experiment(s) to induce a measurable mutation(s) within wildtype cells. You might want to consider what agents might be affecting the quality of these cells natural environment OR what type of mutagen in general can induce a mutation. The goal here is to induce mutations but not kill the cells. Be sure to include controls in your experimental design! Remember that you are going for mutation not just abnormal behavior in response to test conditions in which you have placed your cells. As an example, if you placed your cell sample in wheat media that is much colder than that to which they have been accustomed, the cells will probably respond with "abnormal" behavior. Is this a mutation? Would their response be the same if the temperature of their environment were brought up to normal? If your cells have indeed been mutated, they should display their mutation over time. When the cells are removed from the test conditions, returned to normal wheat media and environmental surroundings, the mutation(s) should still be evident. **Be sure to have your experimental design approved by the instructor prior to initiating the experiment.**

Initial Materials:

Wheat grass media
Test tubes
Depression slides
Dissecting microscope
Pasteur pipettes
Wildtype cells (Amount dependent on design)

Expectations:
1. Induced mutations within your cell population
2. A completed design method for determining and measuring the mutation and an approximate % of mutated cells within your *Paramecia* population.
3. **In addition to recording your results in your laboratory notebook, you will be giving an oral presentation on your investigation during the week of _____**

Figure 3. *Student directions presented prior to investigation of inducing mutation(s).*

Title of the Experiment					
Hypothesis					
Independent Variable					
Levels of Independent Variable					
Number of Repeated Trials					
Dependent Variable					
Controlled Factors or Constants (at least 3)					
Control or Explanation *or* Why It Is A Controlled Experiment					

Figure 4. *Design matrix used in the general genetics laboratory sections.*

Paramecium behavioral mutants are named according to their behavior, for example the *pawn* mutant was named for the chess piece. The *pawn* mutant in *Paramecium* has a defective calcium channel and therefore cannot move backwards just as the chess piece cannot move backwards. Each team that had produced a mutation was allowed to assign a name to its mutant.

We have included some figures demonstrating the effects of some mutagens. Figures 5 and 6 represent differences in swim speeds between the control and test cells grown in Miracle Gro® and Caffeine respectively. In both cases the cells were video taped and their swim speeds calculated. In both cases the test cells statistically swam faster than the control cells. One team saw the number of changes in direction/second as the difference between the control and the test cells (Figure 7). The cells were video taped, and the tape was played back frame-by-frame (30 frames per second) so the students could count the number of changes in direction per second. Figure 8 shows the overall structural differences between the test (UV light exposure) and control cells and Figure 9 illustrates the analysis of the generated length data supporting those structural differences.

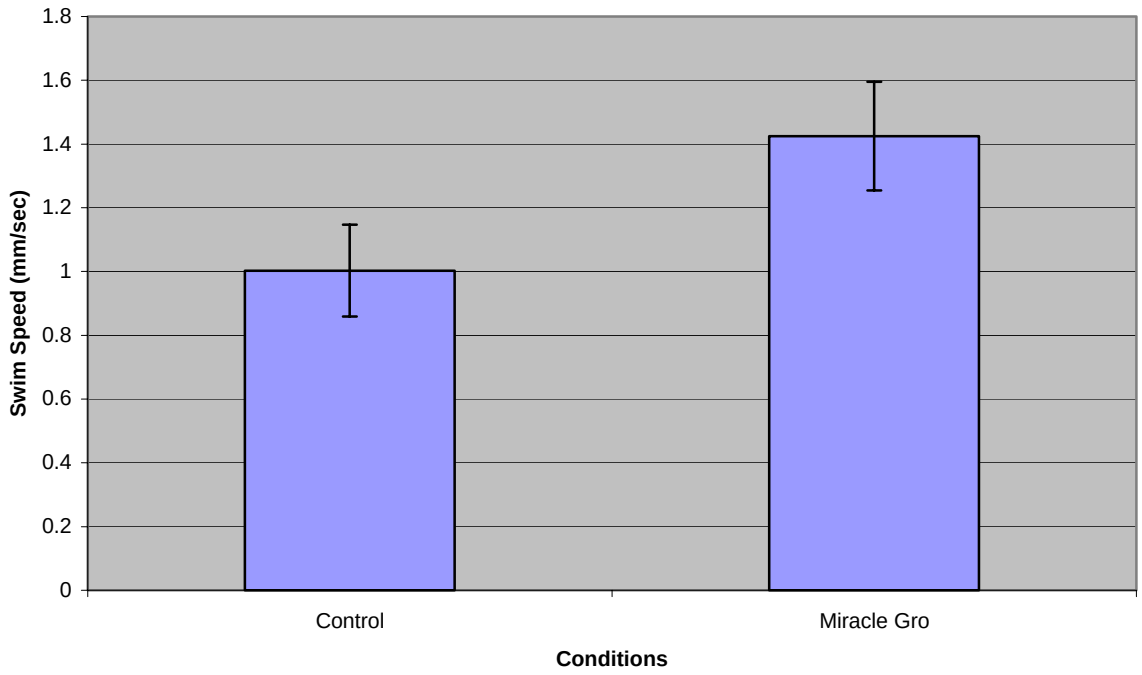


Figure 5. *Comparisons of swim speeds in the control and test cells. Cells were placed in medium containing 0.25% of Miracle-Gro® for 48 hours, then placed back into the regular wheat medium. The control cells (N = 10) swam at a rate of 1.00 mm/sec ± 0.144; whereas, the test cells (N = 10) swam at a rate of 1.42 mm/sec ± 0.17. The students named their cells JJK for Jackie Joyner Kersey, since their cells swam faster than the control.*

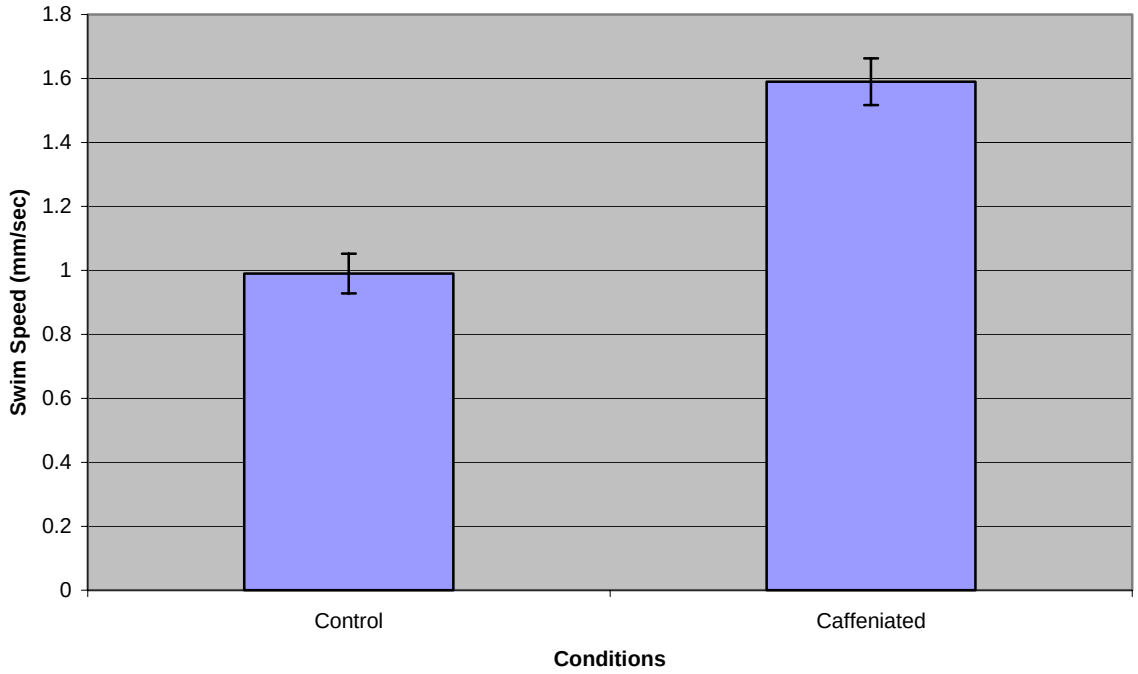


Figure 6. *Comparisons of swim speeds in the control and test cells. Cells were placed in 50 mL of inoculated wheat medium with 50 mg of caffeine for 48 hours, and then placed back into the regular wheat media. The student teamed named their cells Tanked. The control cells (N = 8) swam at a rate of 0.99 mm/sec ± 0.06; whereas, the test cells (N = 8) swam at a rate of 1.59 mm/sec ± 0.07.*

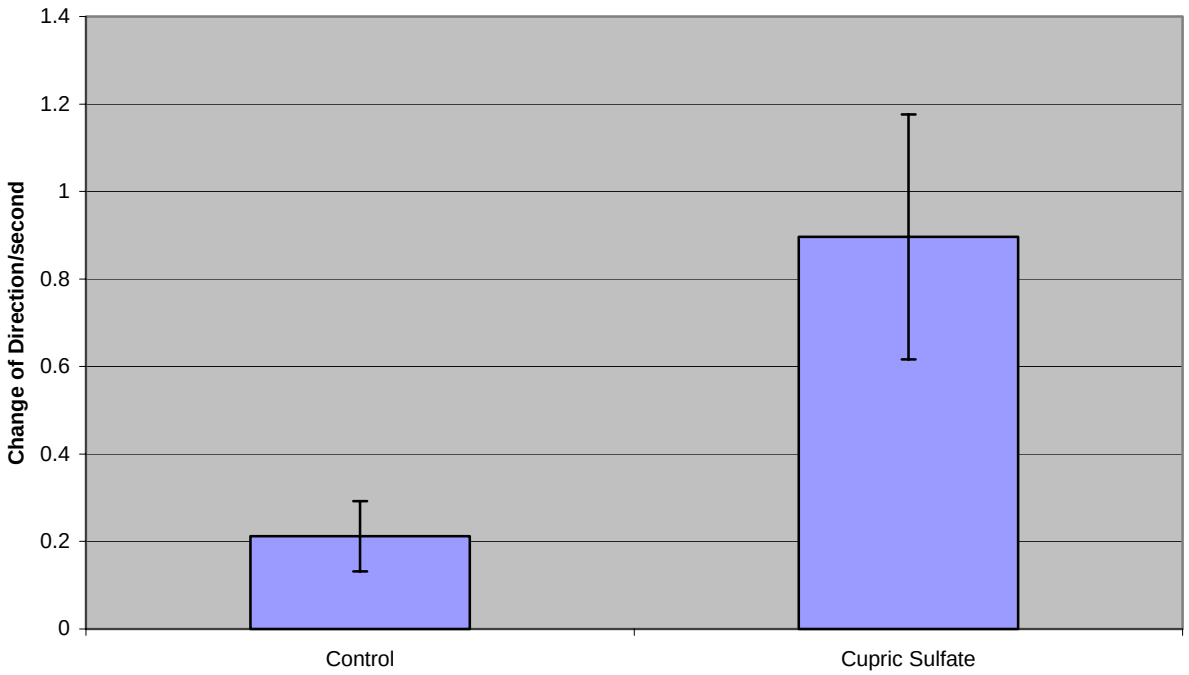
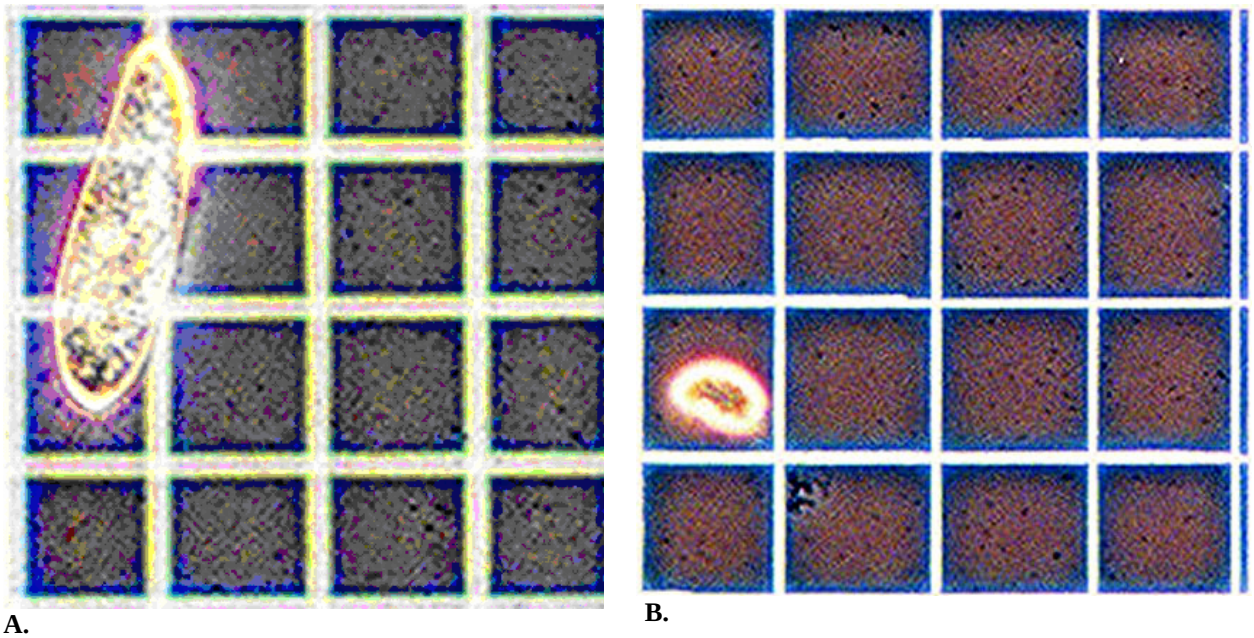


Figure 7. *Comparisons were made for changes in direction between the control and test cells. Test cells were exposed to a medium containing 25 µM cupric sulfate overnight then transferred to regular wheat media. The cells were video taped and the tape played back frame-by-frame (30 frames = one second) to count the number of changes in direction. Five control cells and five exposed cells were measured. The control cells had an average change of direction of 0.212 ± 0.08 per second; whereas ,the test cells had an average change of 0.896 ± 0.28. The students named their cells Tune-Up because the swimming behavior reminded them of a car that needed a tune-up.*



A. **B.**

Figure 8. *Side by side comparison of a wildtype cell (A) compared to a cell that has been mutated by a 30-minute exposure to UV light (UV range 280-315 nm) for 4 consecutive days (B). The mutated cells were named Minimecium by the student team.*

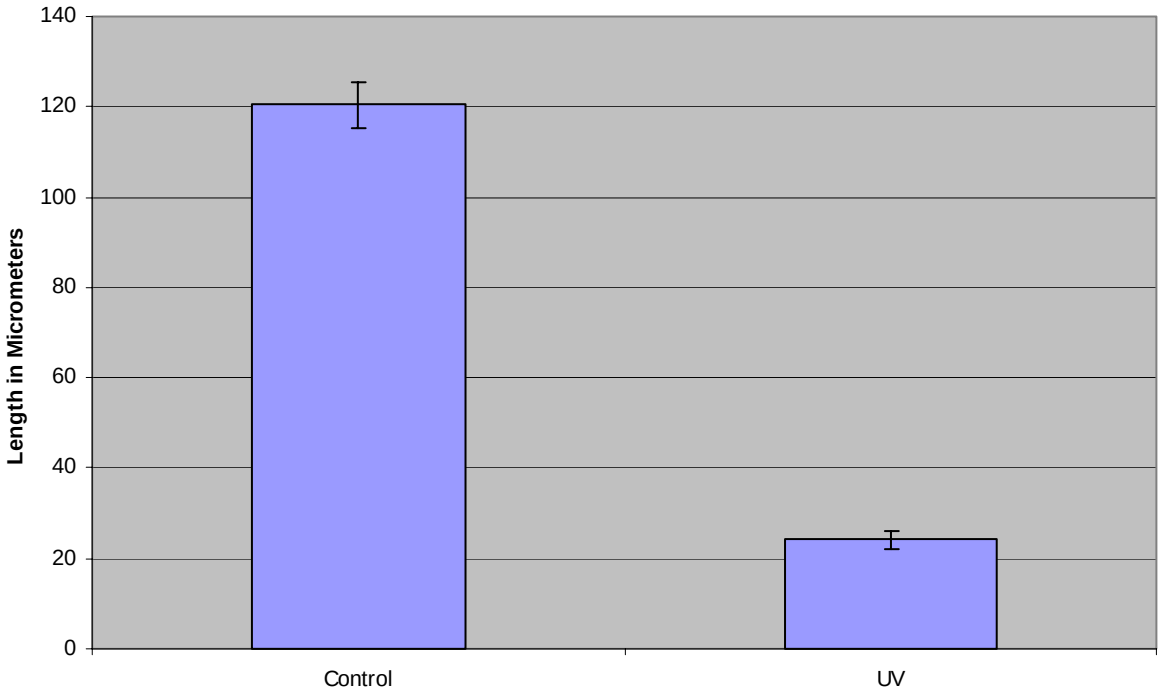


Figure 9. *Comparisons of the length of the cell between the control and test cells. Test cells were exposed to UV light (UV range of 280-315) for 4 consecutive days for 30 minute each day. The cells were photographed on a stage micrometer. Five control cells and five UV exposed cells were measured. The control cells had an average length of 120.4 µm ± 5.18; whereas, the test cells had an average length of 24.2 µm ± 1.92. This team of students named their cells Minimecium.*

ASSESSMENT OF STUDENTS

The students were assessed on the basis of five different categories: The Design Matrix, they submitted two, the initial matrix, and the revised matrix (10% of activity grade); Literature review on the agent they were using to induce the mutations (10% of activity grade); Effective use of the laboratory equipment and instruments (20% of activity grade); Recording of results in their laboratory notebooks (30% of activity grade); and Formal Presentation of their results (30% of activity grade).

DISCUSSION

This laboratory experiment provided our junior/senior level general genetics students with a valuable learning experience that gave them the opportunity to enhance their laboratory skills, conduct literature research on their topics, design their own experiments, collect and interpret their data, and prepare an oral presentation. What surprised us the most was the student response to our questionnaire about their research experience.

Student Outcomes/Responses

The students were asked at the end of this experience to respond to the following questions:

1. Approximately how much time outside of your scheduled laboratory time did you invest in this experiment?
2. How do you feel about this particular inquiry based approach to learning as opposed to other more prescribed approaches in other science labs?
3. What were the positive aspects of this 4-week project?
4. What were the negative aspects of this 4-week project?
5. If you had to talk to next year's general genetics class about this experience, what would you tell them?

Figure 10 is a summary of the student group response to question number 1. There was a wide range of time (1-5 hours), the overall average was 2.56 hours ± 1.32.

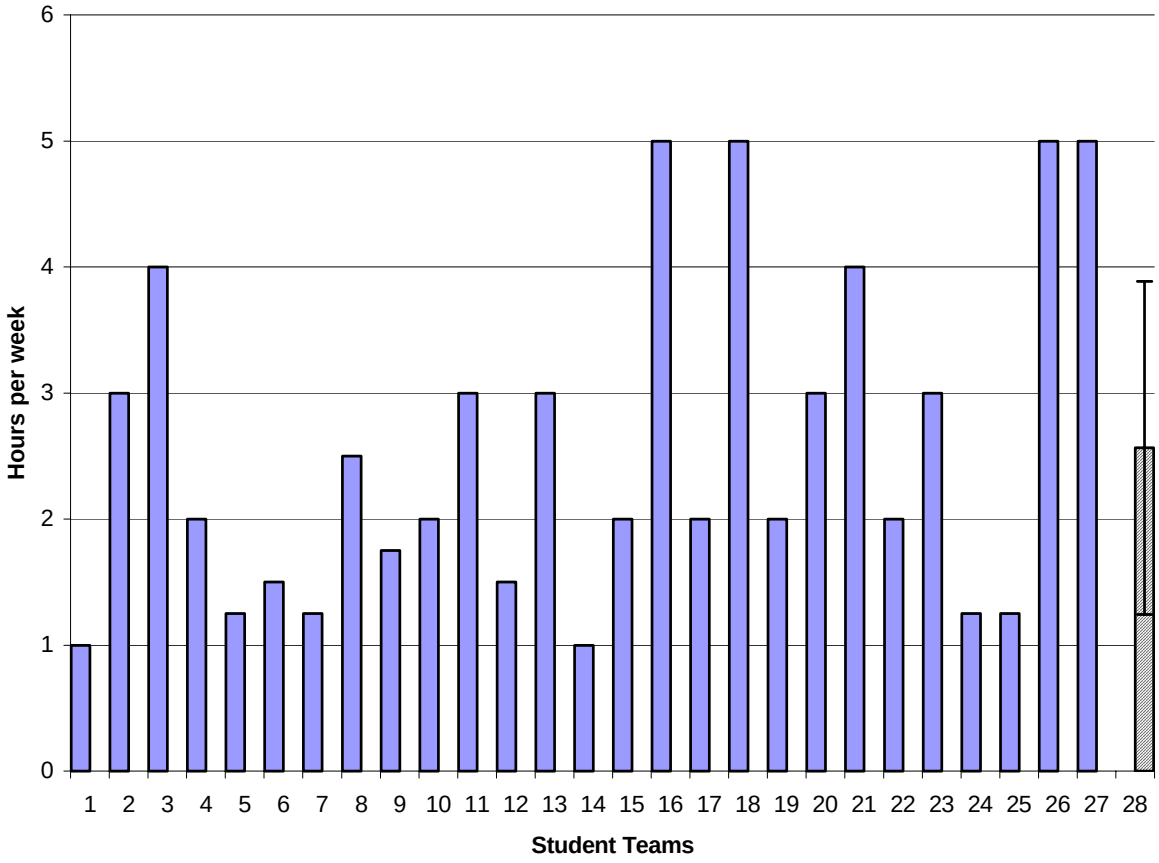


Figure 10. *The individual team responses to the question of how much time outside of the scheduled laboratory time did they spend per week in the lab with this laboratory experience. The overall average (far right column) for all the teams was 2.53 hours/team ± 1.31 hours.*

The students had very positive responses to question 2 above. We have included several of their comments concerning this inquiry-based approach to learning:

> "This format allowed for free-thinking and creativity in a laboratory setting. This was very beneficial in the understanding of the scientific method."

> "This helps you think independently; you are given a chance to do hands-on work using your own ideas."

> "It was very challenging but exciting."

The survey also asked the students to share what they felt were both the positive and negative aspects of this project. The students in general felt that the most positive aspects of this activity were the independence and creative freedom they were given.

> "We had a lot of freedom to choose how to design our experiment."

> "Results were more exciting and rewarding since we were given the freedom to design our own experiments."

The students felt that the extra time they had to invest was the major negative aspect of this activity. One student summed it up well. "The only negative aspect was the amount of time invested. However, with the freedom to design your own experiment comes the responsibility, so I can't complain."

The students expressed their feelings when asked what they would tell next year's genetics class about this inquiry-based laboratory experience. Most of the students gave positive and encouraging responses. Some of these are:

> "It's an excellent experience; just be prepared to dedicate some time."

> "It's one of the best labs you will ever get to do."

> "This type of activity not only helps develop necessary skills to conduct lab procedures, it prepares you for the outside world. The ability

to design, analyze, and learn new techniques is an asset to any institution."

Our students did provide the comment that a good laboratory partner is crucial when working on any type of team effort and/or activity. Creating opportunity for our students to be engaged in active learning based on their own questions and experimental design has been a realized goal. On the way to this goal, students developed skills to enhance the understanding of natural phenomena. They cultivated the art of questioning both themselves and the statements of others. Above all, they formed positive attitudes toward science.

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Message from Our President-Elect:

Dear fellow members of ACUBE,

The next annual meeting is still months away, but I am already looking forward to it and hope the other members of ACUBE are as well! The excellent facilities at Wabash College are most appropriate for the theme "Technology in Biology Education." Please share your teaching ideas by submitting a proposal for a paper, workshop, or poster to the Program Chair, Joyce Cadwallader at jcadwall@smwc.edu.

So much of how I teach has been a direct result of my interaction with the membership of ACUBE. I would like other potential and current members to benefit as much as I have. Bring a colleague to experience the collegial interactions, present a paper, and learn more about our peer-reviewed journal Bioscene. The best way to get our colleagues interested in ACUBE is to have them directly experience what our organization has to offer. I always return home from the annual meeting rejuvenated, with at least one useful idea that I try to use in the classroom or laboratory right away.

Submit that proposal, and I will see you in October in Crawfordsville, Indiana!

Sincerely,

Lynn Gillie
President-Elect