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The Power of Seeing the Whole Picture: A Preregistration Style Approach in a Remote Introductory Lab

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A growing number of scientific publications are allowing authors to preregister their experiments to improve the transparency and quality of results. We asked introductory biology lab students to complete a preregistration-style activity by submitting a series of narrated PowerPoint slides. Students were given an experimental question about cell signaling to investigate, as well as a summary of a previous experiment that served as a model for the experimental setup. Students were asked to describe what experiment they would design (including data collection and statistical analysis) and the possible outcomes. Then, for each outcome, they were asked to discuss the results from their analysis that would match that outcome, and to interpret what the results mean in the context of the experimental question. This lab activity was done remotely so students were not able to conduct the proposed experiment. We believe a preregistration approach to scientific work eventually conducted in an in-person lab setting would be just as valuable.

Keywords: Preregistration, remote, C-Fern, chemotaxis

Introduction

A preregistration approach to experimentation and scientific communication requires researchers to define their research question and analysis plan before the data is collected and analyzed. While there are varying thoughts on this, some suggest this will increase transparency and reproducibility in the scientific literature (Nosek 2018). When planning for a remote lab, brought about due to the COVID-19 pandemic, instructors saw a preregistration-style lab approach as a way to expose students to almost all steps of an in-lab experiment minus the actual in-person data collection. To be successful, students needed to think through not only the experimental design, but also the possible results and implications of the results on the experimental question.

Our introductory biology students had been using C-Fern plants in lab before leaving to go remote and these ferns were chosen to be the model system for our remote lab. C-Fern gametophytes are multicellular, haploid, gamete producing stages of the fern, that exist as male or hermaphrodites (Banks 1997). They are useful teaching tools for many areas of investigation including chemotaxis. Sperm is released from structures on the gametophytes called antherida and under the microscope students can see the sperm gather near the egg-containing archegonia of the hermaphrodite (Hickock 1998).

To prepare students, a Moodle forum was used to ask students to reflect on the movement of sperm to an egg and to comment on how sperm can find the egg in an aqueous environment. Students commented on the presence of some "signal" that could be produced and this led us to a discussion about chemotaxis. Instructors described an experimental setup to test the idea that sperm moved to the egg due to a chemoattractant. This led us to the rest of the lab including the preregistration assignment; a proposed investigation into the "players" of C-Fern sperm chemotaxis. Instructors discussed with students that in order to understand a cell signaling pathway, researchers need to describe the members of a pathway such as receptors, ligands, and second messengers. One way to do

this is to observe what happens when a chemical agonist or inhibitor of a receptor, ligand, or second messenger is introduced into a system (Svoboda 2002).

Student Outline

A. Experimental setup: is chemotaxis occurring?

Based upon postings to the Moodle Forum, we will hypothesize that C-Fern sperm are guided to archegonia (egg producing area) by a chemical signal sent out by archegonia. This chemical signal is called a "chemoattractant," and the movement of sperm toward that chemoattractant is called chemotaxis. Below is a description of a protocol by which our hypothesis can be tested. Protocol adapted from *C-Fern* Investigations: Chemical Attraction *C-Fern* Sperm Chemotaxis Kit, Student Version.

1. Place 200 µl of buffer in the well of a depression slide. (A depression slide is a microscope slide with a concave depression in the middle of it. The buffer is a medium in which sperm can survive and perform normal movements. It also helps the antheridia (sperm producing area) release their sperm.)
2. Transfer 5 male gametophytes from a culture dish of mature wild-type C-Fern gametophytes to the buffer in the depression slide. Males are the smaller, light bulb shaped gametophytes. Observe under a compound microscope at low power (10x objective), and within several minutes, sperm should begin to be released from antheridia.
3. After you have added the male gametophytes to the slide as in the step above, obtain a culture dish of "cold-treated" mature, wild-type C-Fern gametophytes. This treatment (refrigerated for 15 min then returned to room temperature for 30 min) causes archegonia to exude a small drop of viscous liquid. This can be seen quite clearly with a dissecting microscope. Take a sharpened toothpick and touch it to the exudate drop in the region of the archegonia on 5 different hermaphroditic gametophytes. This should collect enough "archegonia exudate" for the next step.
4. While looking through the compound microscope and focused on the top surface of the buffer with male gametophytes in the well of the depression slide, gently and briefly touch the sharpened end of the toothpick (that now has "archegonia exudate") to the surface of that drop of buffer (that now has released sperm).
5. After observing for a minute, you should see a high-intensity swarm of sperm in the area that was touched by the toothpick.

Some questions to consider:

a) If we want to better test whether sperm were truly attracted to the liquid exuded from the archegonia, what type of control test could we do?
b) We can count the number of sperm (from digital image capturing) in a swarm around the area where the toothpick touched the surface. Using a control from the answer above, how could we statistically test whether sperm were truly attracted to the liquid exuded from the archegonia

Think about your answers to these questions; you will be asked about them on a Moodle quiz as your next activity.

Students answered that they could have a control toothpick with no exudate and they could analyze sperm counts with Chi-square analysis.

B. Question selection: what players are involved in C-Fern sperm chemotaxis?

As discussed in the narrated screencast you watched, the final assignment is to propose an experiment that could investigate one of the following 3 questions (Choose one):

a) Some research seems to suggest that an increase in calcium ions (Ca^{2+}) in the cytosol is involved in the control of swimming direction in C-Fern sperm. The question though, is whether this increase in Ca^{2+} is from extracellular or intracellular sources.
b) Research is unclear as to the activity of the second messengers that may be involved in the signaling pathway that controls swimming direction in C-Fern sperm. Is the signaling pathway one in which the second messenger activates calmodulin, or one in which the second messenger activates protein kinase A?

c) Some research seems to suggest that an increase in calcium ions (Ca^{2+}) in the cytosol is involved in the control of swimming direction in C-Fern sperm. The question though, is whether this increase in Ca^{2+} is from a signal pathway started by ligand-gated Na^{+} channels or directly from ligand-gated Ca^{2+} channels.

Experimental design:
You will use the general protocol you already read about where exudate and control stimuli were introduced to the sperm on toothpicks (*Part A, above*). In order to investigate your question, you will introduce 5 µl of your selected chemical (described below) to the 200 µl of buffer containing the sperm from male gametophytes.

You need to figure out which two chemicals (from Table 1, below) to use in order to answer the research question you've chosen. For example, consider the following:

- For question (a), which chemical would be used to see if extracellular calcium is involved in the signaling pathway that regulates the swimming of C-Fern sperm? Which chemical would be used to see if intracellular calcium is involved in the signaling pathway that regulates the swimming of C-Fern sperm?
- For question (b), which chemical would be used to see if protein kinase A (PKA) is involved in the signaling pathway that regulates the swimming of C-Fern sperm? Which chemical would be used to see if calmodulin is involved in the signaling pathway that regulates the swimming of C-Fern sperm?
- For question (c), which chemical would be used to see if sodium channels are involved in the signaling pathway that regulates the swimming of C-Fern sperm? Which chemical would be used to see if external calcium channels are involved in the signaling pathway that regulates the swimming of C-Fern sperm?

Table 1. A list of available chemicals and their targets of inhibition.

Chemical	Possible action
Curcumin	inhibits PKA
EDTA + Strontium chloride	binds extracellular Ca^{2+} and inhibits surface Ca^{2+} channels
Lanthanum trichloride	inhibits IP_3-activated intracellular Ca^{2+} channels
Lidocaine	inhibits Na^{+} channels
Tetraethylammonium chloride	inhibits K^{+} channels
Trifluoroperazine	inhibits calmodulin

Table modified from "Signal Transduction", Johnson 2009.

C. Create a narrated PowerPoint: how will you investigate?

For this final assignment, you need to submit a narrated PowerPoint file of slides that very briefly describe your proposed experiment and its possible outcomes. Imagine you are working in a lab, and this is a proposal you need to submit to a review board in order to be approved to conduct the research. Follow the guidelines below:

Slides 1-2: Study Information

- Title that is specific and informative
- Background:
    - Explanation of existing data (sample data) that shows chemotaxis toward archegonium exudate
    - Explain experimental question/purpose of study
- Hypothesis you're testing (in your own words, derived from the experimental question you've chosen)

Slides 3-4: Methods

- Brief overview of experimental setup, including:
    - Independent variable, controls, dependent variable
    - Statistical analysis you would use – be specific about what the analysis is comparing and testing

Slides 5-6: Outcomes

- (a) Present each possible outcome from your study, (b) refer to results of statistical analysis (name the test and the P-value) that would indicate each outcome, and (c) interpret each outcome in the context of your experimental question. For example:
    - If chemical 1 disrupts chemotaxis (give the general statistical result that would provide evidence of this outcome), and chemical 2 does not (give statistical result), then that suggests that…
    - If chemical 2 disrupts chemotaxis (give the general statistical result that would provide evidence of this outcome), and chemical 1 does not (give statistical result), then that suggests that…
    - If both chemicals disrupt chemotaxis (give the general statistical results that would provide evidence of this outcome), then that suggests that…
    - If neither chemical disrupts chemotaxis (give the general statistical result that would provide evidence of this outcome), then that suggests that…

Cited References

C-Fern Investigations: Chemical Attraction *C-Fern* Sperm Chemotaxis Kit: Student Version. Available from: http://www1.biologie.uni-hamburg.de/b-online/library/cfern/cfern.bio.utk.edu/teaching/Chemotaxis/ChemotaxisStudent.html

Materials

This lab was done remotely and therefore students only needed use of a computer. The instructors made use of asynchronous Moodle forums, quizzes, and narrated Microsoft PowerPoint slides to interact with students and to introduce the lab activity in stages.

Notes for the Instructor

This lab was designed for introductory biology students. The students had previous experience with C-Ferns but not with C-Fern fertilization. Images and videos were used in place of in-lab observations. Because this lab was made for a time when remote labs needed to come together quickly we thought it best to explain the initial experimental setup of chemotaxis where we may have done this collaboratively with students had we been in person. The instructors found the hardest part for students was to determine what the possible results meant and how these results would look in terms of P-values. This assignment encouraged students to think about these ideas in a meaningful way, especially important since this lab was done at the end of the semester and served as the students' final project. Students had experience with experimental design at this point and experience with Chi-square analysis.

Students were asked to narrate the slides they made as part of the assignment. The instructors feel that this was helpful as it encouraged students to have a thorough understanding of their slides. Because slides can be narrated one at a time and rerecorded if the speaker is dissatisfied, students could approach this in a time frame that worked well for them. This asynchronous approach helped the instructors to create an inclusive and rigorous assignment with plenty of opportunities for questions and discussions. The instructors look forward to trying this approach in-lab when students can preregister their experiment and then collect data. We hope that thinking ahead to the outcomes earlier will help students to understand their findings more thoroughly.

Cited References

Banks JA. 1997. Sex determination in the fern *Ceratopteris***.** Trends in Plant Science. 2(5):175-180.

Hickok LG, Warne TR, Baxter SL, Melear CT. 1998. Sex and the *C-fern*: not just another lifecycle. Bioscience. 48(12):1031-1037.

Johnson AD. 2009. Forty Inquiry Exercises For the College Biology Lab. NSTA Press. 514.

Nosek BA, Ebersole CR, DeHaven CA, Mellor, DT. 2018. The preregistration revolution. PNAS. 115(11):2600-2606.

Svoboda KKH, Reenstra WR. 2002. Approaches to studying cellular signaling: a primer for morphologists. Anat. Rec. 269(2):123-139

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