Guiding Student Inquiry into Eukaryotic Organismal Biology Using the Plasmodial Slime Mold *Physarum polycephalum*

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**ABSTRACT**

In order to challenge our undergraduate students’ enduring misconception that plants, animals, and fungi must be “advanced” and that other eukaryotes traditionally called protists must be “primitive,” we have developed a 24-hour take-home guided inquiry and investigation of live *Physarum* cultures. The experiment replicates recent peer-reviewed research regarding speed–accuracy tradeoffs and reliably produces data with which students explore key biological concepts and practice essential scientific competencies. It requires minimal resources and can be adapted for high school students or more independent student investigations. We present statistical analyses of data from four semesters and provide examples of our strategies for student engagement and assessment.

*Key Words:* Behavior; data analysis; model organisms; protists; speed–accuracy tradeoffs.

Our prior approach to teaching eukaryotic biology had done little to challenge our undergraduate biology majors’ enduring misconception that plants, animals, and fungi must be most closely related as well as “advanced” and that all other eukaryotes, traditionally called protists, such as the unicellular protozoan *Paramecium*, must be “primitive.” For example, discussions with our students about the evolutionary innovations of the eukaryotic lineage were previously limited to cell structure (e.g., organelles) but bypassed exploring the significance of the other traits that make all eukaryotic organisms fundamentally unique, such as their ability to become multicellular and to undergo sexual reproduction.

In response to this pedagogical challenge, we have developed a laboratory using only protist lineages to demonstrate the shared, derived traits of all eukaryotic organisms (Weeks, 2013). The laboratory comprises (1) a brief investigation of the paralytic effect of colchicine on the eukaryotic cytoskeleton using live *Paramecium*; (2) an extended microscopic survey of other protist lineages that demonstrate common themes in eukaryotic biology, including photoautotrophy via secondary chloroplast endosymbiosis (Euglena and Fucus), multicellularity and sexual reproduction (Fucus and *Saprolegnia*), biomineralization (foraminifera and diatoms), and organismal mutualism (*Trichonympha*); and (3) a 24-hour guided inquiry and investigation of live *Physarum polycephalum* cultures (based on Latty & Beekman, 2011). Here, we describe the pedagogical value of the *Physarum* guided inquiry and investigation as well as its implementation.

The plasmodial slime mold, *P. polycephalum* is a useful model for student investigations of universal eukaryotic traits because its appearance contradicts most students’ preconceptions of “advanced” organisms. As a member of the Amoebozoan clade (Katz et al., 2012), *Physarum* is not closely related to animals, plants, or fungi yet is large enough to be held and observed with the naked eye, can readily move, and has well-characterized behavior. Its ease of culture and experimental manipulation have made it a favored subject for eukaryotic biology research and education (Dussutour et al., 2010; Bohland et al., 2011). Our *Physarum* inquiry and investigation highlights four essential biological concepts for students (National Research Council, 2003): (1) the idea that all eukaryotes share common ancestry (e.g., what does *Physarum* have in common with humans?); (2) the value of model organisms to reductionist and holistic thinking in eukaryotic biology (e.g., what is the purpose of a model organism?); (3) the idea that all organisms have behavior (e.g., is behavior or intelligence limited to animals?); and (4) that communication networks must integrate multicellular bodies to effect organismal behavior (e.g., how does *Physarum* make decisions without a brain?). The process of conducting the experiment and analyzing the data also guides students through practicing three key scientific competencies (AAAS, 2011): (1) applying the process of science, (2) reasoning quantitatively, and (3) communicating results. Lastly, by replicating a peer-reviewed
experiment, students confront the realities of a highly variable data set that has true signal while learning data-analysis skills that they can use in the future. This last component answers a recent call by students for more practical learning experiences in the laboratory (AAAS, 2011, p. 30).

Materials

• Fine-tipped permanent markers
• Sterile scalpels
• Sterile forceps
• Disinfectant or ethanol lamp for sterilizing scalpels and forceps
• 90 x 25 mm plastic Petri dishes
• Printed acetates showing 1-cm² grid spacing or precise plate setup (see Figure 1)
• Parafilm strips for sealing Petri dishes
• Aluminum foil
• Bacto agar (Beckton, Dickinson & Company, no. 214010)
• Oat flour (Arrowhead Mills or other brand, typically available in health food stores)
• Physarum polycephalum plasmodium on agar (Carolina Biological Supply, no. LJ-156193)

Methods

Prior to lab, 2% agar Petri dishes are poured to a depth of 5 mm. We make one dish per student plus 10% to account for student errors and one dish per four students for the starved Physarum culture setup. We also pour 2% agar stock plates containing four different oatmeal flour concentrations (2%, 6%, 8%, and 10% w:v) to 5 mm depth. The oatmeal flour is added to the medium prior to autoclaving, and a sterile spatula is used to spread the thick medium into the Petri dishes while it is still fluid. Twenty-four hours prior to lab, cultures of starved Physarum are initiated by inoculating 2% agar Petri dishes with plasmodia from purchased stock plates. If necessary, additional cultures of well-fed Physarum, beyond those purchased, can be initiated on 2% oatmeal agar plates several days before lab.

On the day of lab, each student receives one 2% agar Petri dish and is assigned either the Easy Choice treatment or the Difficult Choice treatment to set up and take home for observation (Figure 1). Students census each migrating Physarum plasmodium at 8, 12, 16, 20, and 24 hours to determine whether or not it has made a decision and which quality of food it has chosen. At the conclusion of the experiment, students email their results to their instructors, and the instructors collate all of student data into a single, five-column MS Excel spreadsheet (Table 1) for analysis the following week.

Student Analysis & Assessment

Students analyze the Physarum data collaboratively in small groups that meet in the week following the take-home experiment. In the group meetings, the instructors briefly share multimedia presentations regarding Physarum biology to engage the students (see Supplemental Resources for examples), review the original experimental setup, and then distribute a worksheet that includes an empty table for summary statistics and analysis and interpretation questions (Tables 1 and 2). Students then download the MS Excel spreadsheet containing the collated experimental data to their laptops (Table 1). Many of our undergraduate students have little experience using MS Excel, so this exercise also serves as an introduction to the software. During the meeting, the instructors demonstrate Excel analysis functions necessary to calculate the summary statistics (e.g., “=COUNTIF”, “=SUM”, “=AVERAGE”), and student groups apply them independently. After student groups calculate summary statistics, they develop answers to questions on the worksheet (Table 2). They must use quantitative reasoning to evaluate trends in the accuracy and speed of the experimental treatments and the well-fed and starved Physarum. Students are also challenged to explain the experimental setup and to design a future experiment. Students are often surprised to learn, especially after they have come to appreciate the variability of the data set, that all the Physarum individuals used are genetically identical. Students are assessed on the accuracy of their summary statistics and the quality of their responses to the questions.

Results & Discussion

Over the past four semesters of using this teaching experiment in our course, trends in the data have been relatively consistent (Table 3). Compiled data from all semesters (N = 158–161) indicate a tradeoff between speed and accuracy in both Difficult and Easy Choice treatments, with well-fed Physarum taking longer and choosing more accurately than starved Physarum. Compared with starved Physarum, well-fed Physarum took longer to decide (+1.16 hours, Easy Choice; +2.66 hours, Difficult Choice) and made proportionally more accurate food decisions (+11%, Easy Choice; +15%, Difficult Choice). Speed–accuracy tradeoffs in Physarum are correlated with physiological state, and these results are consistent with the hypothesis that...
starving, stressed organisms would choose more quickly and with greater error as a means for survival. This finding replicates that of Latty and Beekman (2011). However, differences in the time-to-decision between physiological states reveal a counterintuitive behavioral pattern related to risk management that replicates the most biologically significant findings of Latty and Beekman (2011). Well-fed Physarum decrease their time-to-decision between the Difficult and Easy Choice treatments as expected (–0.16 hours), whereas starved Physarum increase their time-to-decision between the treatments (+1.38 hours). This contrasting behavior is not consistent with the hypothesis that more easily discerned food qualities would result in shorter decision times universally. One might expect that all Physarum would detect and respond faster to food selections having 4% differences in quality as in the Easy Choice treatment (2%, 6%, and 10%), rather than 2% differences as in the Difficult Choice treatment (6%, 8%, and 10%).

Table 1. Example of experimental results and summary statistics calculated by students. The metric “proportion most accurate” is calculated by dividing the number of individual Physarum that choose 10% by the total number of observations. Students can report null values if Physarum does not move or does not choose a single piece of food.

<table>
<thead>
<tr>
<th>Raw Data</th>
<th>Assigned Treatment</th>
<th>Starved Time to Decision (Hours)</th>
<th>Starved Choice (%)</th>
<th>Well-fed Time to Decision (Hours)</th>
<th>Well-fed Choice (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student 1</td>
<td>Difficult</td>
<td>20</td>
<td>8</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Student 2</td>
<td>Difficult</td>
<td>12</td>
<td>6</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Student 3</td>
<td>Easy</td>
<td>Null</td>
<td>Null</td>
<td>12</td>
<td>10</td>
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<tr>
<td>Student n</td>
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<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
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</table>

<table>
<thead>
<tr>
<th>Summary Statistics</th>
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<tbody>
<tr>
<td>Average</td>
<td></td>
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<td></td>
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<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of observations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Number choosing 10%</td>
<td></td>
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<tr>
<td>Proportion most accurate</td>
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</table>

Table 2. Examples of student analysis and interpretation questions.

1. What is a speed–accuracy tradeoff?
2. Do you predict that starving Physarum will be more accurate or less accurate in its choice than well-fed Physarum? How about time-to-decision?
3. What aspects of the 0% agar cube make it a good experimental control?
4. Explain some of the possible causes of null data points that were reported in the raw data set. Keep in mind that all Physarum inocula were genetically identical.
5. Within the Difficult Choice treatment, what evidence do you see for a tradeoff of speed and accuracy?
6. Within the Easy Choice treatment, what evidence do you see for a tradeoff of speed and accuracy?
7. Between Difficult and Easy Choice treatments, which physiological state (starved vs. well-fed) shows the greatest difference in accuracy?
8. Between Difficult and Easy Choice treatments, which physiological state (starved vs. well-fed) shows the greatest difference in time-to-decision?
9. What biological factors might explain the answer to question 7?
10. What biological factors might explain the answer to question 8?
11. Our experimental setup was not perfect. The starved and well-fed Physarum inocula were initially added to your Petri dish on pieces of different substrates. How might this have affected their behavior over the duration of the experiment?
12. Describe a similar experimental setup using just one Petri dish that would test whether starved Physarum behaves differently in response to food type rather than just the quality alone. For instance, compare oatmeal flour (predominantly carbohydrate) versus whey powder (predominantly protein).
13. How has this activity changed your perceptions, if any, of the emergence of intelligence in the tree of life?
The proposed explanation for starved Physarum’s extended decision time during the Easy Choice treatment lies in this organism’s ability to detect risk associated with available food sources and to modify its behavior in response to the perceived risk. The Easy Choice treatment exposes all Physarum to a greater risk of choosing poorer-quality food (2%) than the Difficult Choice treatment (6%). However, starved Physarum will be more negatively affected by a less-accurate choice than well-fed Physarum because it does not have the physiological energy stores to hedge against the consequences of a poor decision. In response to the greater risk of the Easy Choice treatment, starved Physarum increases its deliberation time slightly (+1.38 hours) and receives a greater gain of accuracy (+14%) than the well-fed Physarum (+11%). Latty and Beekman (2011) noted that this nuanced behavioral response, while well characterized in animals, is just beginning to be recognized in other eukaryotic lineages such as plasmodial slime molds.

We analyzed our data with the statistical procedures followed by the original study in order to assess how closely our student-collected data replicated the work of Latty and Beekman (2011). A Cox proportional hazards (CPH) model was used to check for the effects of difficulty and stress on decision speed. Decision accuracy for both task difficulty and stress (well-fed vs. starved) was assessed using logistic regression. Physarum were then divided into fast and slow decision categories. Those that decided by the 8-hour mark were placed in the fast category, and those that decided by 12–24 hours were placed in the slow category. This criterion does not precisely mirror that of Latty and Beekman (2011), who used a 4-hour category that our experiment does not use. A chi-square test was used to determine the relationship between these decision speeds (fast vs. slow) and accuracy of decision (i.e., 10% vs. all other possible choices). Likelihood of choosing the lowest-quality food (2% or 6%) versus decision speed was also tested using a chi-square test. All analyses were carried out using the R statistical package, version 2.15.

The data was compiled over four semesters and is shown in Table 3.

### Table 3. Student data compiled over four semesters.

<table>
<thead>
<tr>
<th>Treatment by Semester</th>
<th>Starved Physarum</th>
<th>Well-fed Physarum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg-time to decision (hours)</td>
<td>Avg-food choice (oatmeal-agar %)</td>
</tr>
<tr>
<td>Difficult (6%, 8%, 10%)</td>
<td>Fall 2011</td>
<td>14.08</td>
</tr>
<tr>
<td></td>
<td>Spring 2012</td>
<td>15.42</td>
</tr>
<tr>
<td></td>
<td>Fall 2012</td>
<td>12.26</td>
</tr>
<tr>
<td></td>
<td>Spring 2013</td>
<td>12.21</td>
</tr>
<tr>
<td></td>
<td>All semesters</td>
<td>13.25, SD=5.50, N=158</td>
</tr>
<tr>
<td>Easy (2%, 6%, 10%)</td>
<td>Fall 2011</td>
<td>14.93</td>
</tr>
<tr>
<td></td>
<td>Spring 2012</td>
<td>13.45</td>
</tr>
<tr>
<td></td>
<td>Fall 2012</td>
<td>15.06</td>
</tr>
<tr>
<td></td>
<td>Spring 2013</td>
<td>14.82</td>
</tr>
<tr>
<td></td>
<td>All semesters</td>
<td>14.63, SD=5.68, N=162</td>
</tr>
</tbody>
</table>

We found that decision time was significantly affected by starvation (CPH model, P = 0.000601) but not by task difficulty (CPH model, P = 0.439127). Interactions between starvation and task difficulty were not significant. This differs somewhat from the results of Latty and Beekman (2011), who found that both starvation and task difficulty significantly influenced decision time. Both starvation and task difficulty had a significant effect on decision accuracy (logistic regression, P = 0.000502 for task difficulty, P = 0.001791 for starvation). Slow decision-makers were not significantly more likely to choose the 10% food than fast decision-makers when faced with the Difficult Choice treatment (chi-square test, P = 0.1953) but were somewhat more likely to pick the 10% food during the Easy Choice treatment (chi-square test, P = 0.028295). Finally, our results for choosing the worst-quality food were opposite those of Latty and Beekman (2011). We found that in the Easy Choice treatment, Physarum was somewhat less likely to pick the low-quality food (2%) (chi-square test, P = 0.017625) but noted no such tendency in the Difficult Choice treatment (chi-square test, P = 0.17555).
Although some outcomes of our teaching experiment do not have the statistical significance of those of Latty and Beekman (2011), who had greater ability to control variability among replicates, more stringent census criteria, and slightly different culturing techniques for introducing the inocula to the experimental plates, our experiment is surprisingly reliable and amenable to adoption by other educators. The needed preparation equipment is minimal, the consumable items are relatively low-cost, and Physarum nearly always performs for the students. Consequently, there is always a data set worth discussing at the end of this experiment. By giving students a model of an accurate experimental design, this activity can also be used to transition students to more independent explorations of Physarum, which many of our students have requested. High school teachers could also use the experiment to introduce the concept of a scientific control and to distinguish arithmetical concepts used in biological research (e.g., means, proportions, and percentages). In this way, our experiment can be adapted to a range of student audiences yet still deliver key biological concepts in a way that allows students to develop their scientific competencies.

Supplemental Resources


Tokyo Rail Network Designed by Physarum Plasmodium. http://www.youtube.com/watch?v=BZUQsMcR5-g

References


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