Chemotaxis in the Plasmodial Slime Mold, *Physarum polycephalum*

An Experimental System for Student Exploration & Investigation

Donna M. Bozzone Denise A. Martin

Background

Physarum polycephalum, a plasmodial slime mold (myxomycete), lives in dark, moist environments such as under the bark of decaying logs and beneath decaying leaves on the forest floor. The vegetative form of this organism is the plasmodium, a glistening yellow mass of indefinite morphology. Plasmodia are negatively phototactic, display chemotaxis, and have the ability to "crawl" to seek food. Feeding on bacteria, spores and decaying organic material, a plasmodium can grow to a fairly large size (up to 30 cm in diameter). Despite this large mass, it is not composed of separate cells but is one large amoeba-like cell containing many nuclei (Sauer 1982).

When food is scarce, the starving plasmodium undergoes a remarkable transformation. The plasmodial mass develops numerous fruiting bodies, called sporangia, which serve as a resting stage. When food becomes available once more, the spores of the fruiting body germinate and release either amoeboid or flagellated swarm cells depending upon environmental conditions. It is the swarm cells which fuse together to ultimately produce another feeding plasmodium (Cummins & Rusch 1968; Sauer 1982).

For more than 35 years, *Physarum polycephalum* has been a model research organism for the study of several important biological problems including growth and differentiation, cell cycle dynamics, cytoplasmic streaming, and cytoskeletal function (Cummins &

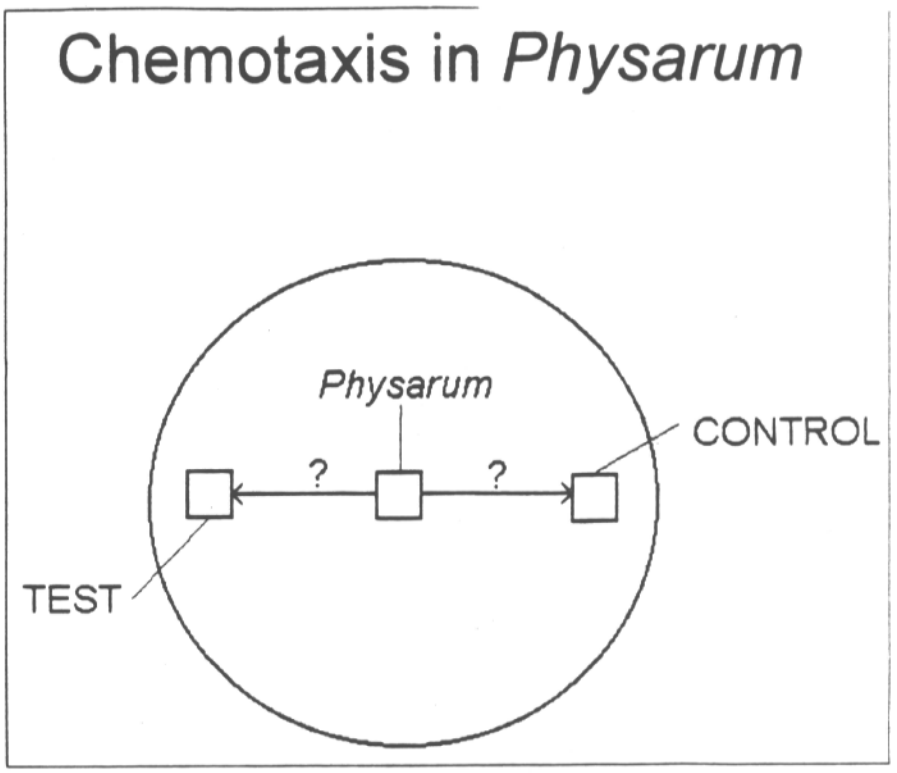

Figure 1. Experimental setup for chemotaxis assay. The control is a block of 1.5–2% agar. The test block is either agar containing a specific test substance or it can be a small pile of food or some type of solid material. The *Physarum* block consists of a piece of agar with a piece of plasmodium on its surface.

Student Instructions

Activity #1

A. Initial Observations

1. Without removing the cover of the petri dish, examine the stock plate of *Physarum*. The plasmodium is the yellow, glistening, web-like material on the dish.
2. Use a dissecting scope to examine the flow of cytoplasm in the plasmodium. Is the flow directional? Does the direction of flow change?
3. What is the probable function of the cytoplasmic streaming?

B. Chemotaxis in *Physarum*

Physarum is repelled by or attracted to some substances, and does not respond to others. In today's lab, your group will test whether *Physarum* exhibits a chemotactic response to one of several available test substances:

1. 100 mM glucose in 1.5% agar
2. 20 mM NaCl or KCl in 1.5% agar
3. Sweetened cereal (several types are available)
4. Unsweetened oatmeal
5. Fresh fruit

State the null hypothesis for your experiment. After discussion with your lab partners, predict how *Physarum* might respond to your test substance. Try to formulate an explanation for your prediction.

Experimental Procedure

There is a variety of ways to set up a chemotaxis test for *Physarum* but to make this experiment suitable for chi-square analysis, the plasmodium should be presented with two choices for directed migration. Sterile technique will be used to set up this experiment. Your instructor will review the necessary procedures with you.

a. Each group needs a plasmodium culture, five agar plates (1.5–2% agar), a test substance, and a scalpel.
b. Cut blocks of agar from one non-nutrient agar plate and from the glucose-agar plate; these blocks should be approximately 1 cm^2.
c. On each of four non-nutrient agar plates, deposit one non-nutrient agar block approximately 1 cm from the edge of the dish. If your test substance is dissolved in agar, on the opposite side of one of these plates, deposit a second agar block, also approximately 1 cm from the edge of the dish. If your test substance is not in agar, sprinkle or place a small amount of the food item on the opposite side of the plate. Be sure to mark the bottom of the petri dishes to indicate the identity of each type of agar block or food item. (See Figure 1).
d. Cut the plasmodium culture into 1-cm^2 blocks. Transfer an agar block containing a piece of plasmodium to each of the three petri dishes. When choosing areas of *Physarum* for transfer, try to select a thick "vein" located towards the edge of the plasmodium. Place the agar block, *plasmodium* side down, in the center of the dish. Be careful not to transfer pieces of oatmeal from the stock cultures.
e. Draw a line down the middle of the bottom of the petri dish to demarcate the left and right sides and label left/right.
f. Wrap the dishes in aluminum foil and incubate right side up at room temperature.
g. Observe plasmodium migration at ~20–24 hours and record its location. A plasmodium positioned anywhere besides the center can be scored as a + for that half of the dish.
h. We will pool class data. Your instructor will discuss with you how to record your results on the data sheets.

Activity #2

Followup Studies

The results from week one will be used to determine what you will do for your second set of experiments. Some possible projects include:

1. Determine the rate of response to a test substance. Transfer an agar block containing a test substance onto a non-nutrient agar plate. On the other side of the plate place a non-nutrient agar block. After 24 hours, place an agar block with *Physarum* in the center of the plate. Arrange with your lab partners to observe the plates at two-hour intervals.
2. Test for food preference. If *Physarum* is attracted to more than one test substance, set up an experiment in which it is provided a choice between two of them.
3. Test the response of *Physarum* to a combination of an attractant and a repellent.

Activity #3

*** Chi-Square Analysis of Data**

For this experiment, the null hypothesis is that plasmodia are *not* migrating directionally; migration is random. Since a minimum sample size of 15 is needed for calculating the chi-square test, you will use pooled results for analysis. We will discuss how to do the chi-square test and analyze results together in lab.

(see Appendix I, pg A-2: Helms)
Null Hypothesis:
" -that no effect occurs-
If, in testing our hypothesis, we find that our data support the null hypothesis, then we need to evaluate
(1) the design of our experiment and
(2) the usefulness of a hypothesis."

*Chi-Square - See pages A-11 → A-12 (Helms)

CAROLINA PLANT BIOLOGY SERIES

Physarum Life Cycle

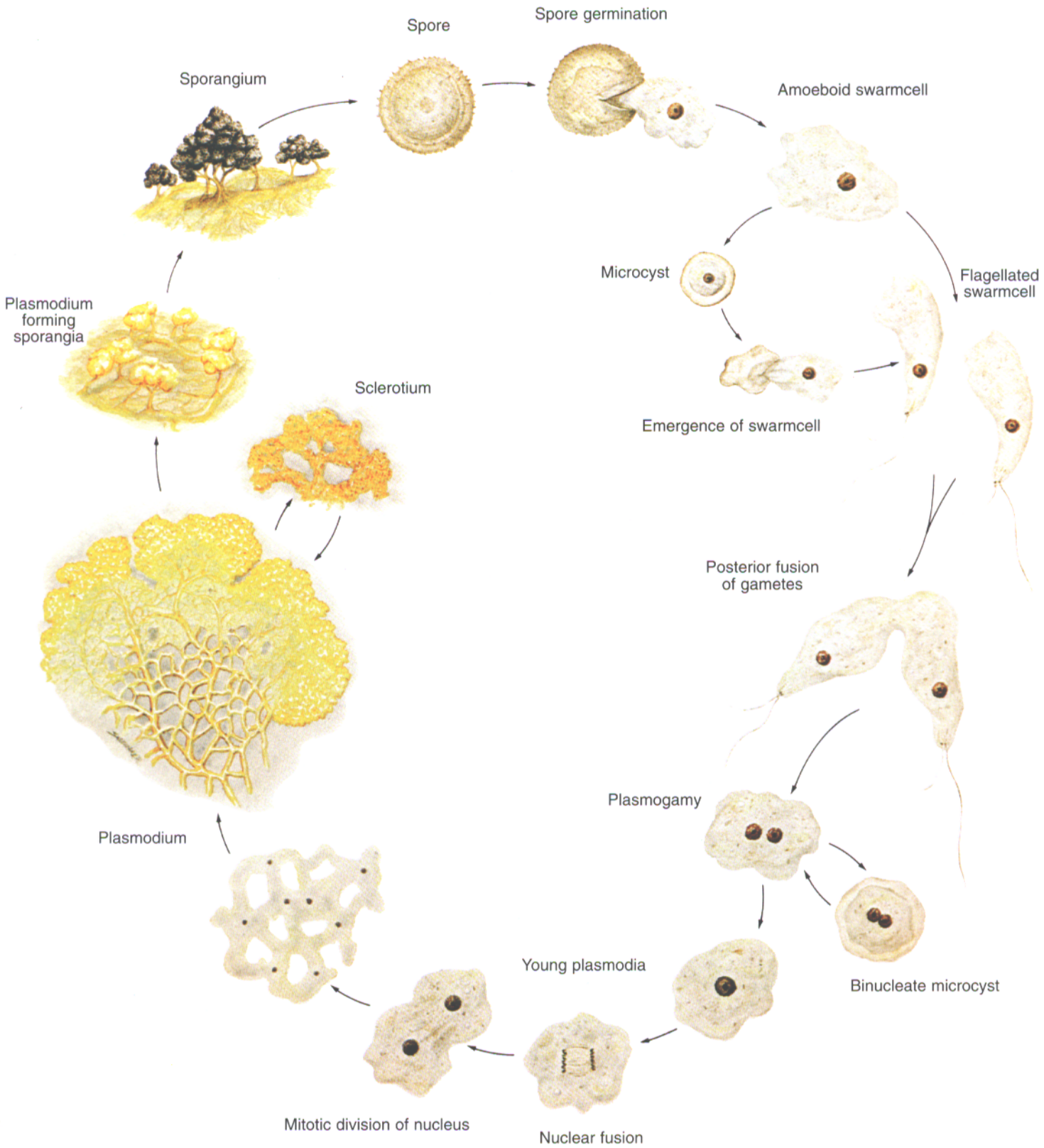

Illustration by Sandra H. Shomaker

Carolina Biological Supply Company, Burlington, North Carolina 27215

Bioreview® Sheet 8316