Chemotaxis in *Physarum*, a Plasmodial Slime Mold (a Simple Experiment to Teach Chi^2 Analysis)

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Physarum polycephalum, a true or plasmodial slime mold (myxomycete) can exist in several distinct forms including fruiting bodies, plasmodia, and amoebae. The experiment described here studies the response of the plasmodium, a yellow slimy mass of indefinite morphology. Plasmodia are negatively phototactic and "crawl" to seek food. In nature, they are found under the bark of decaying trees feeding on microorganisms and organic material. A plasmodium can grow to a fairly large size (up to 30 cm in diameter; Sauer, 1982) and despite this large mass, it is not composed of separate cells but is one huge amoeba-like cell containing many nuclei (Sauer, 1982).

While many aspects of the behavior and physiology of *Physarum* plasmodia are quite intriguing, the protocol delineated below focusses on the chemotactic response of this organism. The exercise has several objectives:

1. Initial exploration of the phenomenon of chemotaxis in *Physarum* plasmodia.
2. Generation of testable questions about chemotaxis in *Physarum* and implementation of experiments.
3. Analysis of data using the chi^2 test.

Protocol

A. Chemotaxis Experiment

1. *Growing Physarum*. Preparing cultures of *Physarum* plasmodia is quite straightforward. You can obtain cultures from Carolina Biological (2700 York Rd., Burlington, NC 27215-3398) or other supply companies; order either the plasmodium or preferably, sclerotium stage (a resting structure; Catalogue #15-6190). Cultures are grown on sterile 1.5–2% non-nutrient agar (15-20 g agar per liter of distilled or deionized H_2O) with oatmeal sprinkled on top after the agar has solidified. Using a sterile scalpel, cut a block of agar on which a piece of plasmodium is present, and transfer it to the agar - oatmeal plate. If culturing a sclerotium, use sterile forceps to transfer a piece of filter paper containing the resting stage (this is how they arrive from Carolina Biological) to the agar-oatmeal plate. Wet the filter paper with a drop of sterile H_2O.

   Once cultures are set up, seal the edges of each plate with parafilm, and wrap dishes in aluminum foil to keep out light. Cultures need to be transferred every 3-4 days if kept at

room temperature. However plasmodia will go into "suspended animation" for weeks (probably months) if refrigerated.

2. *Chemotaxis Assay*. There are a variety of ways to set up a chemotaxis assay for *Physarum* but to make this experiment suitable for chi^2 analysis, the plasmodium should be presented with two choices for directed migration.

   Agar Block Method

   a. Each group needs a plasmodium culture, four non-nutrient agar plates (1.5–2% agar), one plate containing 1.5–2% agar in 100 mM glucose, 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 three non-nutrient agar plates, deposit one agar block approximately 1 cm from the edge of the dish. On the opposite side of one of these plates, deposit a second agar block, also approximately 1 cm from the edge of the dish, and on two dishes, place a glucose-agar block. Be sure to mark the bottom of the petri dishes to indicate the identity of each type of agar block.
   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. Place the agar block, plasmodium side down, in the center of the dish.
   e. Wrap the dishes in aluminum foil and incubate at room temperature.
   f. 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.
   g. We will pool class data.

   Testing Food

   Using the above method, you can test a variety of chemicals. To test foods, set up the assay as described above but instead of depositing a block of agar containing a test chemical, sprinkle a small amount of the test food opposite the agar block. Deposit the plasmodium in the middle, incubate in the dark, and record observations.

B. Chi^2 Analysis of Sample Data

For this experiment, the null hypothesis is that plasmodia are <u>not</u> migrating directionally; migration is random.

Information about chi^2 analysis can be found in most General Biology or Genetics lab manuals (for example, see Eberhard, 1990). Tables 1 and 2 show the results and chi^2 values for several representative experiments.

Table 1. Chi^2 Analysis of Results of a Test of *Physarum* Plasmodium Migration in Response to 100 mM Glucose.

Migration	Observed (0)	Expected (E)	O-E	$(O-E)^2$	$(O-E)^2/E$
Moved Towards Glucose	14	7.5	6.5	42.25	5.63
Did Not Move Towards Glucose	1	7.5	-6.5	42.25	5.63
Null hypothesis is rejected			$Chi^2 = 11.27$ degrees of freedom = 1 $p < 0.01$		

Table 2. Sample Results of Various *Physarum* Chemotaxis Tests.

Test Substance	Movement Towards Test Substance	Movement Not Towards Test Substance	Chi^2	*p* value
100 mM glucose	14	1	11.3	< 0.01
50 mM glucose	11	4	3.3	< 0.10
10 mM glucose	12	2	7.1	< 0.01
2 mM glucose	12	5	2.9	0.10
oatmeal	20	0	20.0	<< 0.01
agar	8.5	7.5	0.06	0.80

Suggestions for Further Experiments

The references listed below provide lots of ideas for tests including various sugars, amino acids, vitamins, etc., at different concentrations and combinations. You also can test a variety of cereals, or other foods (I have tried Rice Krispies, Cheerios, Rice Chex, oatmeal, and Special K!). You can provide choices between attractants or combine attractants and repellents to see which response dominates.

References

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