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Moving slime: exploring chemotaxis with slime mould

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What are slime moulds? And what do they eat for breakfast? Discover these fascinating giant microbes and explore chemotaxis and the scientific method with these slimy experiments.

Teaching eukaryotic evolution and ecology often means teaching about animals, plants, and fungi. But most of the metabolic, morphological, and ecological diversity is within the unicellular eukaryotes, called protists.^[1] To understand our world better, we need to teach students about microorganisms in all different forms, colours, sizes, and habitats.

An especially stunning protist that can help students to become fascinated with microbial eukaryotic diversity is the slime mould *Physarum polycephalum* (figure 1).^[2] Its single amoeboid cell reaches sizes up to several hundreds of square centimetres, contains multiple nuclei, and spreads out network-like while searching for food. Chemoreceptors and external memory (which consists of colourless slime that the slime mould leaves behind and can recognize if it comes across it again) allow them to be very efficient in their search. ^[3,4] Slime moulds do not just move around but seem to know exactly where they are going. But what kind of food do they like? In nature, slime moulds feed on bacteria or pieces of dead organic material that they take up via phagocytosis.^[2,3]

The following experiments allow students aged 11–16 to explore the food choices of slime moulds over a period of three lessons by first offering them different kinds of food and then determining what substances attract or repel them. The experiments allow students to learn about the sensing strategies of organisms and to learn how to test hypotheses



Figure 1: Slime mould in nature Image: Ann Evankow/ iNaturalist.org

and design experiments with control variables (i.e., how to be scientists themselves).

Activity 1: Do slime moulds prefer salami or lettuce?

In this experiment, students offer the slime mould two kinds of food to observe its preference. Students should be encouraged to hypothesize about the results and discuss and debate the reasons for their hypotheses. It is recommended that the students work in pairs or small groups. The activity takes 45 to 60 min, although time varies with the students' age. The results will be observable in the next lesson.

See the <u>teacher instructions</u> in the supporting material for details on how to prepare for the experiments (e.g., acquiring and culturing the slime mound).



Safety notes

It is important to work under conditions that are as sterile as possible. Wear gloves while performing experiments.

Materials

- 1 agar plate with a slime mould
- 1 new agar plate per group of students
- Ethanol & paper towels
- Scalpel
- Tweezers
- Salami slices (pieces of ca. 2–3 cm)
- Lettuce leaves (pieces of ca. 2-3 cm)
- Parafilm
- Aluminium foil/a box

Procedure

- 1. Clean work surfaces, tweezers, and the scalpel with ethanol.
- Cut out a piece of slime mould with the agar it grows on (size ca. 1 cm²) using the scalpel. Alternatively, take an oat flake with slime mould on it.
- 3. Use the tweezers to place the piece of slime mould in the middle of the new agar plate.
- Cut out one piece each of salami and lettuce (roughly equal in size!).
- Place the salami on one side of the slime mould and the lettuce on the other. The distance between the slime mould and the food sources should be equal (see figure 2).

- 6. Seal the plate with parafilm.
- 7. Keep the plate at room temperature in the dark for approximately 24 h, e.g., wrap it up in aluminium foil or put it in a box.

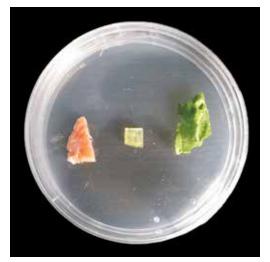


Figure 2: Slime mould (middle) with the option of roughly equal-sized pieces of salami (left) or lettuce (right) *Image courtesy of the authors*

Expected results

After several hours, the slime mould grows towards the lettuce (see figure 3). Its network-like structure will be moving towards or growing onto it.

The slime mould decides against the salami, but the students still do not know why. This leads to a second round of experiments, in which the students try to find out the reason for the slime mould's food choice.

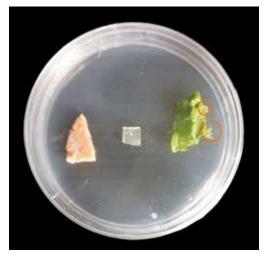


Figure 3: Slime mould hidden underneath the lettuce (red circles) *Image courtesy of the authors*

To discuss the observation, the following questions can be considered: Does the lettuce attract the slime mould? Or does the salami repel it? If it is repulsion, what exactly is repelling the slime mould? There are many different ingredients in salami. Students should be encouraged to discuss them and their possible influence on the observed repulsion. This discussion can be used to collect ideas for test substances for Activity 2.

Note: Focussing on repulsion is an advantage because it is easier to think of substances in the salami which can be used in the next experiment and that produce clear results than to isolate substances from the lettuce that could be attracting the slime mould.

Activity 2: Slime mould chemotaxis

Chemoreceptors in the slime mould's cell membrane detect chemical molecules in the environment that either attract or repel it.^[2,4,5] In this activity, the students design experiments to find out which ingredient(s) in salami is (are) repelling the slime mould. If possible, the students should design the experimental setup themselves, while the teacher functions as a guide during this process.

The slime mould is offered two stimuli: a control (oat flakes) and a test substance (oat flakes + test). Oat flakes are the slime mould's usual food in the laboratory. Therefore, oats are used for both the control and test food options. If a substance is added to the oat flakes, there are three options for the results:

- the slime mould moves towards the control (away from the test);
- 2. it moves towards the test;
- 3. it does not show any preference.

As the slime mould likes and knows oat flakes, result 1) would mean the added substance is causing the movement away from the test, which leads to the interpretations that we observe negative chemotaxis and that the substance is a repellent. Alternatively, result 2) would mean the substance is adding something to the oats, so that they appear more attractive than usual/the control; this can be interpreted as observing positive chemotaxis and the substance being an attractant. Result 3) would mean the added substance seems to be neutral for the slime mould. The students' different ideas for test substances can be collected and each group of students gets assigned one.

The activity takes 45–60 min. The discussion should be done in the following lesson when the results are available.

Safety notes

It is important to work under conditions that are as sterile as possible. Wear gloves while performing experiments.

Materials

- The slime mould <u>Infosheet</u>
- 1 agar plate with slime mould
- 1 new agar plate per group of students
- Ethanol & paper towels
- Test substances (e.g., salt, butter, ...)
- Scalpel Tweezers
 - Spatula
- Distilled water Marking pen
- Parafilm
- Aluminium foil/a box

Procedure

• Pipette

- Before starting the experiments (this can also be done in the previous lesson), introduce slime moulds using the provided <u>Infosheet</u> and/or this <u>video</u>.
- 2. Wipe the work surfaces and materials with ethanol.
- 3. Cut out a piece of slime mould with the scalpel and place it in the middle of the new agar plate using tweezers.
- 4. Use tweezers to place oat flakes on both sides of the slime mould (equal amounts and distance).
- 5. Place a drop of water on the oat flakes. This provides moisture for the slime mould and allows the oat flakes to stick in place on the agar.
- 6. Add one substance, e.g., salt, to the oat flakes on one side of the slime mould by using the spatula (see figure 4).
- 7. Mark on the plate which substance was used and on which side it was placed.
- 8. Seal the plate with parafilm and place it in the dark for about 24 h.



Figure 4: Setup for the chemotaxis experiment. Slime mould (middle) offered oat flakes with salt (left) and plain oat flakes (right) Image courtesy of the authors

Expected results

The slime mould moves towards the oat flakes without salt (see figure 5). Just like acid or caffeine, salt is a known repellent in the literature.^[2-5] The slime mould's chemoreceptors allow it to detect chemicals and direct it away from harmful substances without needing further sensing organs.



Figure 5: Slime mould on the oat flakes without salt (right) *Image courtesy of the authors*

Note: The experiments do not have to be restricted to the salami/lettuce problem. If the students are interested in trying other substances (e.g., sugar, acids) or finding out whether the slime mould prefers lettuce over its usual oat flakes, they should be encouraged to do so.

Discussion

Activity 2 teaches students about the slime mould's general chemotaxis, but also provides possible explanations for the results of Activity 1, or could be used to predict further food choices.

Students should consider the following questions:

- What kinds of food are attractants/repellents for the slime mould?
- Which results are surprising? Which are expected? Why?
- Why does the slime mould not like salami?
- How could the concentration of different substances influence the chemotactic behaviour towards them?

Some further questions to explore with older students (aged 14–16):

- Why is it important to use simple biological models to ensure easier discussion of results?
- In what sense does the second experiment clarify the importance of chemotaxis?
- What is the ecological function of slime moulds?

Slime moulds also offer opportunities for many <u>other exper-</u><u>iments</u>. They are influenced by other environmental factors, such as light (phototaxis), or can be used to map routes or solve mazes due to their habit of strengthening only the most efficient connections by creating thicker plasma streams in the pseudopods between food sources and withdrawing the pseudopods with less nutritional flow.^[4,6]



Slime mould Image courtesy of the authors

References

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- [6] Wegner C, Strehlke F, Weber P (2014) <u>Intelligent slime? A</u> <u>hands-on project to investigate slime moulds.</u> *Science in School* **30**: 16–20.

Resources

- Excellent <u>tutorial on slime moulds</u> from researcher Audrey Dussutour (French language, but the automatic English subtitles are sufficient for watching as well).
- Watch a very informative <u>video about slime moulds</u> with beautiful images and footage.
- Watch a fascinating video about how <u>slime moulds can</u> <u>solve mazes</u>.
- Try more slime mould experiments: Wegner C, Strehlke F, Weber P (2014) Intelligent slime? A hands-on project to investigate slime moulds. Science in School **30**: 16–20.
- Explore antibiotic resistance and drug development with these fun microbiology activities: Deumal Fernandez M, Lladonosa Soler M, Godinho T (2021) <u>Microbiology:</u> Discovering antibacterial agents. Science in School 55.
- Try hands-on experiments with Daphnia as a model organism: Faria HM, Fonseca PA (2022) <u>From drugs to</u> <u>climate change: hands-on experiments with Daphnia as a</u> <u>model organism.</u> *Science in School* **58**.

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Amber Buchta studies biology, mathematics, and chemistry as a Master of Education student at the University of Duisburg-Essen, Germany. Her work on this teaching exercise was funded by an Erasmus+ grant. **Micah Dunthorn** is a professor of microbial diversity and environmental DNA at the Natural History Museum, University of Oslo. His expertise spans from evaluating meiotic genes in putative asexual protists to uncovering protistan diversity in tropical forests and other ecosystems.



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