The Influence of Light on the Development of the Coprophilous Fungus, *Pilobolus*

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Fungi consist of about 100,000 species arranged in groups according to reproductive strategies. Fungi play an important role in the ecosystem, in nutrient cycling as decomposers, and as part of symbiotic relationships in lichens and mycorrhizae (Mueller et al., 2004). Fungi are also important plant and animal pathogens: Hyphal tips penetrate plant cell walls, insect cuticles and human skin, nails and hair (Jackson et al., 1997; Moore, 2001).

Some fungi are important drug producers and are used in the manufacturing of vitamins and antibiotics. Penicillin, the first antibiotic discovered, is produced by the fungus *Penicillium chrysogenum*. The fungal product cyclosporin is used as an immunosuppressant and ergot alkaloids first extracted from a parasitic fungus of wheat and rye have been used to treat migraine headaches (Moore, 2001).

Finally, fungi are also important in our diet. The fruiting bodies of some fungi are edible and quite nutritious. The unicellular yeast *Saccharomyces cerevisae* is used to bake bread and to brew beer. Some fungi strains are used to produce gourmet cheeses and to ferment soy sauce (Campbell & Reece, 2005)

Pilobolus Life Cycle

In this laboratory exercise, we use the coprophilous fungus, *Pilobolus*. This fungus is classified as a "Zygote Fungus", Phylum Zygomycota (Zygomycotina when treated as a Subdivision), a zygospore producing fungus. Unlike the common mushroom, zygote fungi do not produce a fruiting body but they do undergo both sexual and asexual reproduction (Figure 1).

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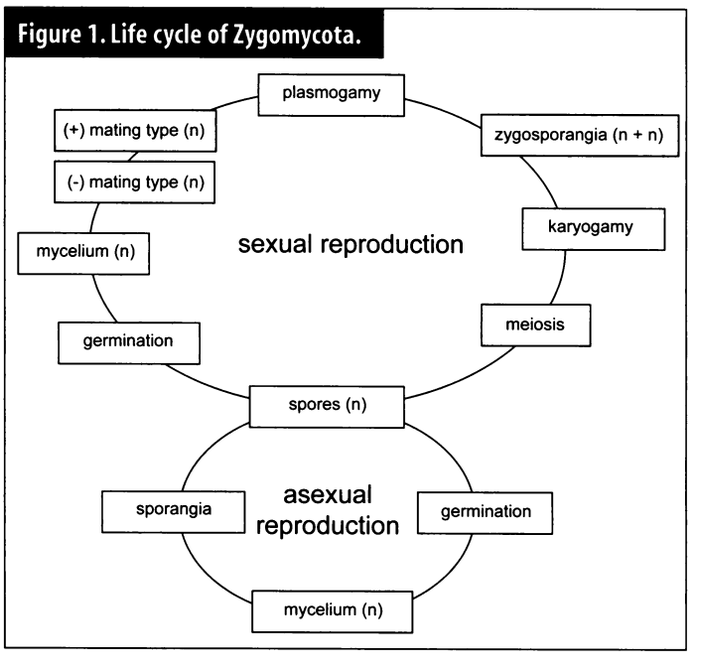
During sexual reproduction, hyphae of different mating types make contact and their cell walls break down. Following plasmogamy several nuclei pair and fuse to form a large heterokaryotic zygosporangium, the resistant stage that becomes dormant and characterizes phylum Zygomycota. As the zygosporangium germinates into a short sporangiophore its nuclei undergo meiosis to produce haploid spores that are released to form new mycelia (Mauseth, 2003). During asexual reproduction, the sporangium releases haploid spores which germinate to form horizontally spreading mycelia that eventually produce vertical sporangiophores. This is the part of *Pilobolus* life cycle covered in this exercise.

Figure 1. Life cycle of Zygomycota.



In nature, *Pilobolus* spores are present in herbivore's feces after passing through the animal's gut. The spores germinate and decompose the dung by an extensive hyphal growth (mycelium). This growth is followed by the development of slender stalks (described in this

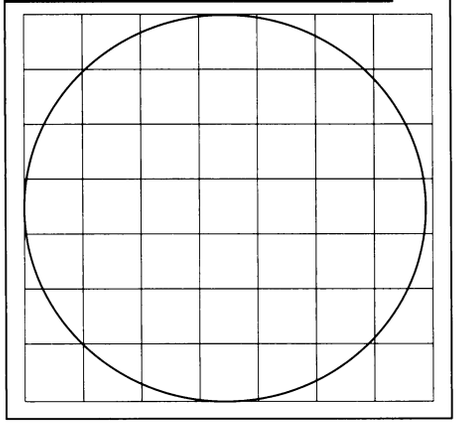
exercise as developing sporangia) that elongate to form the sporangiophores. At the tip of each sporangiophore a clear, swollen vesicle forms, and on top of it a black sporangium develops. The swollen vesicle (also called subsporangial vesicle) works like a lens, focusing light on the ring of flavonoid molecules directly below (Deacon, 2003). Upon light detection, the sporangiophore bends and shoots the sporangium toward the light source. In order to continue its life cycle, *Pilobolus* spores must be eaten again by an herbivore. This means that spores must reach a fresh, new grass leaf far away from the pile of dung where they are growing. The combination of a phototropic response and an explosive sporangia discharge mechanism allows this to happen. When the sticky sporangium touches a grass leaf, it attaches and is eventually eaten by a grazing animal.

Light Effects

In *Pilobolus* and many other fungi, aspects of the life cycle are controlled by light in the blue or near UV region. Light stimulates the formation of trophocysts (orange-brown swellings in the hyphae) and is required for the production of sporangia on the sporangiophores. In addition, *Pilobolus* shows a phototropic response upon exposure to light. It has been suggested that the same photoreceptor may be involved in both the light growth response of sporangiophores and the phototrophic response (Bergman, 1972).

In general, the phototrophic response has its peak at 450 nm (blue region of visible light). Cultures grown in the dark will develop trophocysts and some tall sporangiophores, but no sporangia (Ellis, 1996).

Figure 2. Sample grid for measuring mycelium growth.



In order to study the effects of light on the life cycle of *Pilobolus,* we expose it to three different light conditions: incandescent light, blue light, and no light. We emphasize the similarities between the light response of this heterotroph and plant responses to light.

Why Use *Pilobolus*?

Pilobolus, also known as the "Shotgun Fungus," is easy to obtain, culture, and maintain. It completes its asexual cycle in about 10-14 days and is not a human pathogen. The phototropic growth response to light and the shooting of mature sporangia are unusual phenomena attractive to students.

Experiments on *Pilobolus* and other coprophilous fungi have been done with fresh cow dung (Coble & Bland, 1974) or with deer or rabbit pellets collected by students and cultured in corn meal agar, malt extract agar, or potato-dextrose agar (Chamuris & Counterman, 1999). To save time and to get consistent results, *Pilobolus* culture plates and agar plates with sterile rabbit dung pellets can be obtained from biological suppliers. Several variations of this lab can be done, for example, testing diffuse light, light intensity, or different wavelengths, (Coble & Bland, 1974), assessing optimum growth temperature (Foos & Royer, 1987) or optimum growth moisture (McGranaghan et al., 1999).

Materials & Methods

We use *Pilobolus* culture kits from Carolina Biological Supply Co. that include a culture plate, sterile plates with rabbit dung agar, scalpels, and aluminum foil. We use a *Roscolux* filter (Rosco Laboratories, Inc., Standford, CT) in primary blue #80. Other materials needed are:

- plastic wrap
- 95% ethanol for surface sterilization
- transparency grid with 1 cm^2 squares that fits the culture plate lid (Figure 2)
- dissecting microscopes
- permanent markers
- lab bench with growth lights (preferably in a separate room without windows or exposure to other sources of incidental light) where all Petri dishes can be incubated under incandescent light at room temperature.

Because cultures take about 10–14 days to grow, this lab is set up in one week and completed two weeks later. For a class of 20 students, allow approximately one hour for setup in the first lab session and one to one and a half hours in the second lab session for data collection. As part of the first laboratory period, we have students view the life cycle of *Pilobolus* using a segment of the video recording "*The Biology of Fungi*" (BioMedia). We review concepts of light, wavelength, light intensity, as well as terms such as spore, culture plate, and tropism and ask students to think about the hypotheses of their investigation. We have students discuss the different stages of the life cycle and analyze which ones may or may not be influenced by light. Students often do not realize that mycelium growth can occur in the absence of light.

General aspects of the experiment such as sterile technique, labeling, handling of cultures, and data gathering are also addressed. As students observe the mycelium, sporangiophores, and sporangia on the commercial plate (with a dissecting microscope) we stress the fact that the mycelium must grow in the agar before sporangiophores and sporangia can develop. The mycelium appears as thread-like lines, while the sporangiophores are upright clear stalks, visible on the agar surface, which may or may not have black tops (sporangia). To see the mycelium, the students must hold the dish against the light or focus the dissecting scope on a plane within the agar layer. Students disinfect the work surface with 95% ethanol and wash their hands, before and after making their observations. The student lifts the lid of the agar dish inoculated with *Pilobolus* just enough to cut out a 1 cm^3 agar cube with a sterile scalpel and transfers the cube

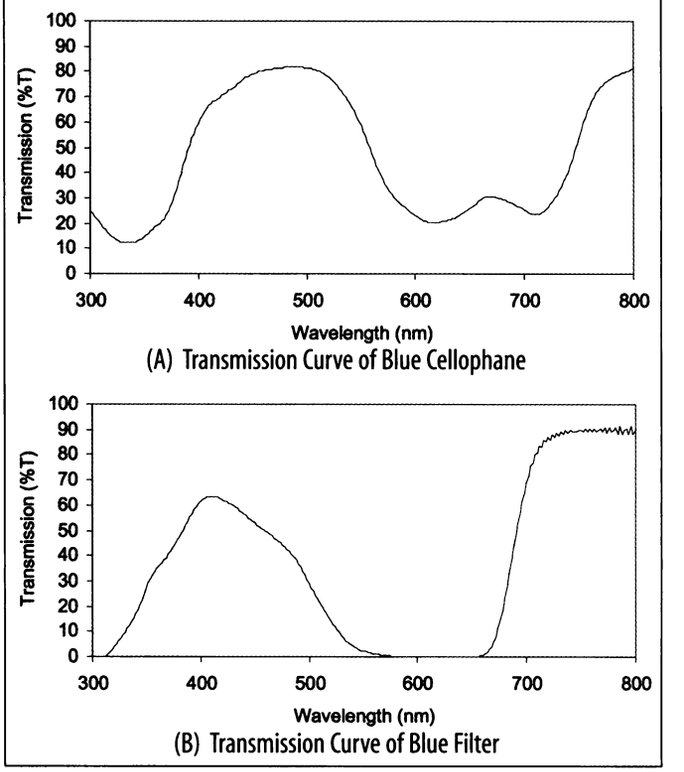
to a new sterile agar/rabbit dung dish. It is best to make sure that at least one (black) sporangium is included and to place it in contact with the agar surface of the sterile agar/rabbit dung dish as close as possible to a dung pellet and close the lid. Every group inoculates three dishes, one per treatment (incandescent light, no light, and blue light). The groups then wrap their incandescent light treatment in saran wrap and the no light treatment in foil. The inoculated plates for the blue light treatment are placed on a tray for the instructor to cover with the blue filter. All culture plates are placed next to each other on the lab bench about 24 in below an incandescent light source that is on a 12h : 12h light/dark cycle for the duration of the experiment (approximately two weeks).

It is important to emphasize the need for true filters when doing this experiment since cellophane paper, often used in biology experiments that deal with light treatments, does not transmit the correct wavelength and results can be ambiguous (Coble & Bland, 1974). Parallel to the design of this experiment we compared light transmission curves between blue cellophane paper and blue color filter. Samples of cellophane paper were placed in a spectrophotometer and transmission was measured at intervals between 300 and 800 nm (Figure 3A). We compared these measurements with transmission curves of professional color filters (Figure 3B).

Differences between true filters and cellophane paper are of consideration. Blue cellophane paper transmits up to 80% while the true filter transmits only to 60%. In addition, blue cellophane has an extra transmission peak ~670 nm while the true filter has ≤5% transmission at the same wavelength. One important difference is that the blue # 80 filter is better able to filter out > 500 nm wavelength light. Using two layers of cellophane paper did not alleviate the problem (graph not shown). To achieve the best results, we strongly suggest that true filters be used. Two blue filter sheets (20" x 24") are enough for four lab sections of 20 students, cost about $7.00 each, and can be reused almost indefinitely unless they are damaged.

During the second lab period (approximately 14 days later) students remove and /or unwrap their dishes and inspect them with a dissecting microscope. Using a transparency grid with 1 cm^2 squares (Figure 2), students count and record (Table 1) the number of squares that have mycelium growing through the agar, the number of developing sporangia (orange-colored short stalks or sporangiophores), the number of developed sporangia (mature, black-topped stalks), and the number of black sporangia "shot" toward light. Note that sporangia may have adhered to the dish lid, so students need to inspect the lid as well. Class data are pooled and recorded (Table 2). Students use class data in their lab report to qualitatively describe trends in the average number of grid squares that contain mycelium, the average number of developing and developed sporangia, and the average number of sporangia shot off.

Figure 3. Comparison of transmission curves of common blue cellophane (A) and *Roscolux* #80 Primary Blue filter (B). Transmission curves were done on a Cary UV 500 Spectrometer.



Discussion

We have done this lab with classes over several semesters and have had good results. Students should find that mycelium growth is not different between treatments since mycelia grow within substrates and do not require light for growth (Graham et al., 2003). Students should also find that developing and developed sporangia and the number of sporangia shot are higher in the incandescent light treatment as compared to the no light treatment. The absence of light inhibits *Pilobolus* from completing its life-cycle (Sanchez-Murillo et al., 2004). Finally, students should find that the blue light treatment has the most sporangia

Table 1.

Light conditions	Extent of mycelium growth (number of squares per grid)	Number of developing sporangia (without black sporangia)	Number of developed sporangia (with black sporangia on top)	Number of sporangia "shot"	Other observations
Constant light					
No light (foil treatment)					
Blue wavelength					
Red wavelength					

development and numbers of sporangia shot off. The effects of blue light have been well studied in fungi with results indicating that the increased intensity of the blue light has an effect on tropisms, growth, sexual and asexual reproduction (Galland & Lipson, 1987; Corrochano & Cerda-Olmedo, 1991; Sanchez-Murillo et al., 2004).

Table 2.

Light conditions	Extent of mycelium growth (number of squares per grid)	Number of developing sporangia (without black sporangia)	Number of developed sporangia (with black sporangia on top)	Number of sporangia "shot"	Other observations
Constant light					
AVERAGE					
No light (foil treatment)					
AVERAGE					
Blue wavelength					
AVERAGE					
Red wavelength					
AVERAGE					

Lab Report, Assessment & Grading Rubric

This exercise is taught to college introductory biology students of which > 75% are majoring in biology. Students set up the experiment and gather, analyze, and interpret data. They work in teams of four and prepare individual lab reports using class data. Upon successful completion of this exercise, students achieve competency in the areas of scientific thinking (understanding and applying the scientific method), analytical skills (understanding how to produce and interpret quantitative and qualitative information), and social skills (working effectively with others for a common goal). The instructor also explains the components of the lab report, the use of scientific writing style, and the grading rubric.

Figure 4. Grading rubric for lab report.

	MAX POINTS	YOUR SCORE
Hypotheses		
1. A written hypothesis is done for every light treatment	1	
2. Hypotheses include predictions for the outcome of the experiment	1	
3. Hypotheses address all aspects of *Pilobolus* life cycle	1	
Results		
4. Paper includes completed data sheet and tables	1	
5. Paper describes results and addresses experimental problems	4	
Discussion		
6. Paper explains if results support or reject each hypothesis	1	
7. Paper discusses results from a biological standpoint	2	
8. Paper summarizes the overall effect of light on *Pilobolus* lifecycle	2	
References		
9. A minimum of two references are cited	0.5	
10. Electronic references have authors	0.5	
11. References are cited in the body of the paper	0.5	
12. References follow suggested format	0.5	
Further points may be deducted for poor grammar/spelling and/or poor presentation		/ 15

As part of the biology curriculum we like to teach this lab exercise at the beginning of the semester so students become familiar with a simple asexual life cycle. This knowledge will be beneficial when learning more complex plant life cycles.

The student report includes hypotheses for the three different conditions, results (including group's data and class data), discussion, and references. We have developed a grading rubric that is given to students as a guide on how their reports will be graded (Figure 4).

Conclusions

Overall, we found a wide range of outcomes from this experiment. For the writing component, students gather, analyze, and report data. As members of a team, students collaborate in the collection of data and supervise each other in the culture transfer and handling of cultures. Students also practice the scientific method as they develop and state their working hypotheses.

As with any other biology experiment, however, there are things that can go wrong. In our experience, contamination can be a problem. Students do not have a good grasp of sterile techniques and contamination often occurs when transferring the agar blocks, so we discard plates that become too heavily contaminated to quantify. We insist that students thoroughly wash their hands before inoculation to reduce possible contamination. Furthermore, students should take care to label the bottoms and lids of the agar plates with group information. We had an instance of a group switching lids and confusing treatments. Finally, unexpected results

can occur in the no light treatment in that sporangia can develop and be shot off if plates are not completely wrapped in foil. Even if the exposed places are small, there may still be enough light for a few *Pilobolus* to go through all the stages of development since *Pilobolus* needs very little exposure time to light to complete its life cycle (Sanchez-Murillo et al., 2004).

This laboratory accomplishes several objectives of the Organismal Biology curriculum. It studies the lesser known Fungi Kingdom and introduces students to a simple asexual life cycle. In particular, the ability to observe the growth of the mycelium through the agar media is essential for understanding how fungi work!

Supply Sources

Carolina Biological Supply, 2700 York Rd., Burlington, NC 27215-3398. 1-800-334-5551. Catalog 73 (2003-04); *Pilobolus* kit.

Rosco Laboratories Inc., 52 Harbor View, Stamford, CT 06902. 1-800-767-2669. Online catalog http://www.rosco-ca.com/intl/index.html; Roscolux color filters.

BioMEDIA Associates: Learning Programs for Biology; http://ebiomedia.com/.

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