Mosses and the Plant Life Cycle

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Physcomitrium pyriforme can be used to demonstrate fundamental characteristics of both bryophytes and the plant life cycle in general. Using mosses in the classroom, students can see phenomena such as the formation of antheridia, the male sex organs, and archegonia, the female sex organs. Moss reproduction can also be taught, as students will have the opportunity to see fertilization and sporophyte development firsthand. This real-world visual also aids in the understanding of haploid and diploid stages experienced during bryophyte development and sexual reproduction. Furthermore, concepts such as totipotency can be explored, which is the ability of all moss cells to give rise to new plants. More generally, this sample may be used to communicate concepts encompassing all plants including alteration of generations, meiosis, and mitosis.

How to produce *Physcomitrium pyriforme* sporophytes:

- This sample of *Physcomitrium pyriforme* includes gametophytes— the dominant, haploid generation. At this stage, the sex organs of the moss, antheridia and archegonia, have not yet begun to develop. In the laboratory a growth chamber is used to simulate fall conditions to stimulate the development of antheridia and archegonia. The conditions are 8 hours of light at 10°C (50°F). If a growth chamber is not available, we have several ideas for sex organ production. If it is fall or spring, cultures can be placed outside to experience cooler temperatures and shorter day lengths. Alternatively, a refrigerator can be modified to simulate a growth chamber by adding a small battery-powered light inside (a touch light that sticks to the inside of the fridge may work best). The light should be turned on for 8 hours per day. Refrigerator temperatures are typically 4°C (39°F). Increasing the refrigerator temperature closer to 10°C may help with sex organ formation. You may need to experiment with light and temperature conditions using the equipment you have to initiate sex organ formation. Simulating fall conditions may enable the production of sex organs so that sporophytes can be formed via sexual reproduction:
    1. Samples in cool temperature and short day length conditions should be checked once a week through some type of magnifying lens to see if antheridia or archegonia have been formed. A dissecting microscope or handlens is good for this type of evaluation. Antheridia should form after approximately one month and archegonia after approximately two months.
    2. When mature antheridia and mature archegonia are observed, flood (Figure 1) the sample by pouring enough water (about 75 mL) into the pot to completely saturate the

soil and come into contact with both the antheridia and archegonia. The water should rise up the edges of the pot and may even overflow slightly. This water creates a pathway that allows the sperm from the antheridia to swim to and fertilize the archegonia. If snapping on the lid tightly causes leaking, the lid may be lightly placed on top of the pot. The sample should remain flooded for 24 hours.

3. After 24 hours, the excess water should be drained out of the sample by holding the lid to the side and allowing the water to drain out of one of the corners.

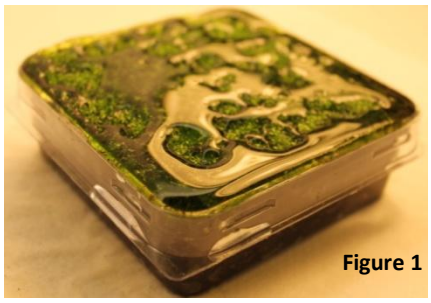
Figure 1

4. Once the excess water is drained from the pot, the sample should be placed back into the refrigerator for one week.
5. After one week, the sample should be moved to a room temperature (20.5°C/ 68.9°F) spot on a windowsill that receives light 12-16 hours per day. Supplement with a fluorescent lamp if needed. Tiny sporophytes (Figure 2) should be visible within a week. After about a month, mature sporophytes (Figure 3) that are dark brown in color should be fully formed. While the sporophytes are growing, the lid may sit loosely on top (Figure 4) of the container.


Figure 2

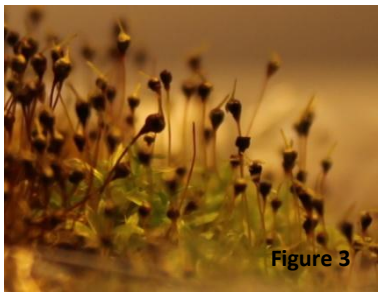
Figure 3

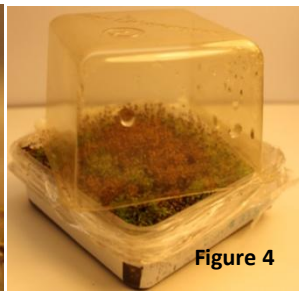
Figure 4

When is the sample ready for flooding?

- The sample is ready for flooding when the antheridia and archegonia are mature.
    1. Mature antheridia (Figure 6) are typically plentiful and a tan/orange color at maturity. They are found in clusters at the tips of the leafy gametophyte stems. Brown antheridia indicate that they are old, and green/bright yellow that they are immature (Figure 5). Ideally, the sample should be flooded while the antheridia are a tan/orange color.

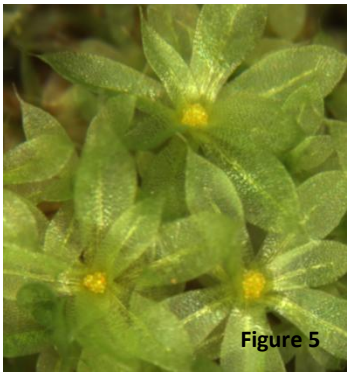
Figure 5

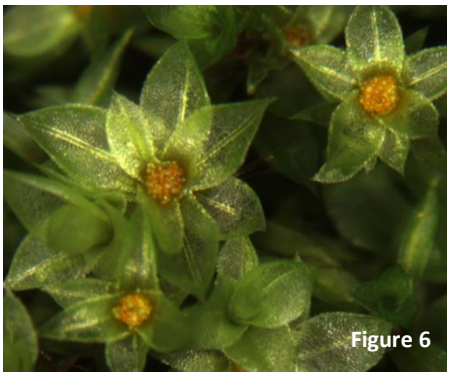
Figure 6

    2. Mature archegonia (Figures 9 & 10) are typically not as plentiful as antheridia. Archegonia are found on leafy gametophytes with apical leaves that are wrapped tightly around the apex, in a swirled arrangement (Figures 7 & 8). Often you can look down the center of the inside leaves to see if the archegonia are mature. If the leaves are too tightly wrapped together, tweezers can be used to gently pull off the leaves surrounding a couple of archegonia to see if they are brown at the base where the egg is located. If all of the archegonia are green, they are still immature. Archegonia develop sequentially with only a couple of archegonia ready for fertilization in each cluster at any one time. If there are some brown archegonia in each cluster and many clusters, they are likely mature and should be flooded.


Figure 7


Figure 8


Figure 9

The brown archegonium is visible here at the base of the swirl of leaves.

Figure 10

Several of these archegonia have opened up and are brown in color, indicating that there are archegonia in the cluster that are mature.

How to make additional samples of *Physcomitrium pyriforme*:

If sporophytes were successfully produced, their spores can be used to create additional leafy gametophyte filled pots using the following procedure:

Materials:

- Small container such as a hexagonal weighing dish (Figure 11)

Figure 11

- 2 tweezers
- Small plant pot— PlantCon Plant Tissue Culture Container (see below for more info)
- Potting soil (rich sandy loam)
- Dissecting microscope/ magnifying eyewear (Optional)

1. Add a small amount (about 3 mL) of water into a small container.
2. Use the tweezers to remove a mature sporophyte from your sample. The sporophyte's capsule should be brown and closed.

3. Add the sporophyte to the water (Figure 12) and use your tweezers to tear apart the capsule by breaking it apart against the wall of the container. You should observe the spores from inside the capsule being released into the water. Make sure that the capsule is sufficiently torn apart to ensure that the maximum number of spores is suspended in the water.

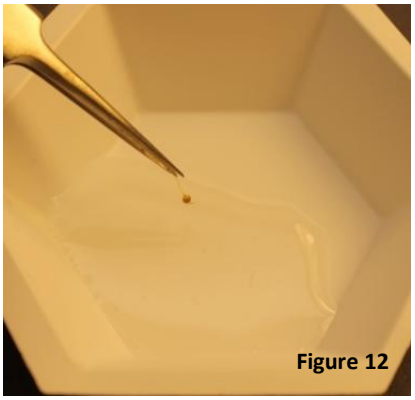
Figure 12

4. Repeat steps two and three, 4-5 additional times so that the spores from 5 or 6 sporophytes are suspended in the water. (Figure 15)

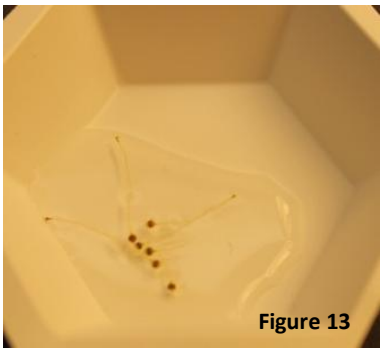
Figure 13

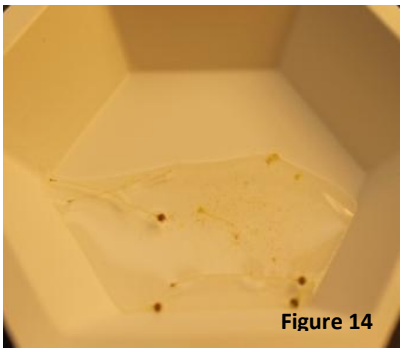
Figure 14

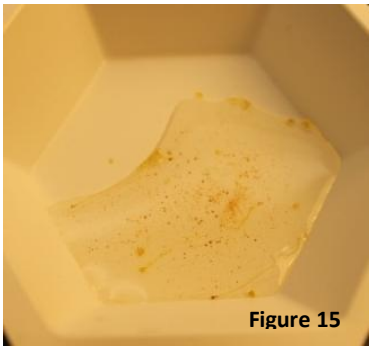
Figure 15

5. Add more water (about 15 additional mL) so that the small container is about ¾ full. (Figure 16)

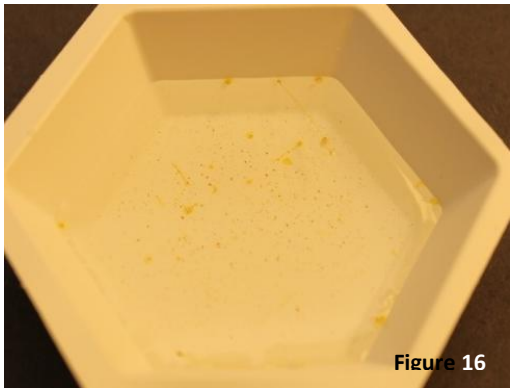
Figure 16

6. Use the tweezers to mix the spores around in the water, scraping any off of the walls of the container if necessary. (Figure 17)


Figure 17

7. Pour the solution onto a new pot of soil. The new pot of soil should be slightly watered so that with the addition of the sporophyte solution the sample is saturated but not so wet that there is a puddle of standing water. Avoid pouring in additional water after, as this may push spores deeper into the soil.

If sporophytes are not successfully produced, it is still possible to create additional leafy gametophyte filled pots. The leaves of *Physcomitrium pyriforme* are totipotent, meaning that by grinding them and adding them to a new soil pot they can create new protonema and eventually new leafy gametophytes. The following process describes this technique:

Materials:

- Mortar and pestle (Figure 18)


Figure 18

- 2 tweezers
- Small plant pot— PlantCon Plant Tissue Culture Container (see below for more info)
- Potting soil (rich sandy loam)
- Dissecting microscope/ magnifying eyewear (Optional)

1. Add a small amount (around 5 or 6 mL) of water to the bottom of the mortar (Figure 19). Be cautious about adding too much because this can make grinding difficult.


Figure 19

2. Use tweezers to remove leaves from the moss sample and add them to the mortar (Figure 20). The leafy gametophytes should be added, but soil should be avoided because it makes grinding more difficult. Furthermore, be cautious about adding plant matter that does not have the same appearance as the rest of the leaves, as contamination with another species can occur. If there appears to be another species contaminating your sample, simply avoid adding any of it to the mortar.


Figure 20

3. When enough leaves have been added to the mortar to cover the base, this is a sufficient amount for grinding. Begin grinding using the pestle. Make slow circles for about 30 seconds using enough force to tear up the leaves. The final mixture should have tiny pieces of leafy gametophytes, but no entire leaves. (Figure 21)

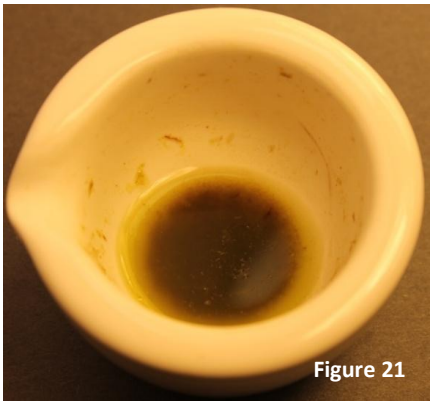
Figure 21

4. Pour additional water (about 25 mL) into the mortar until it is about ¾ full, rinsing off the plant material on the pestle into the mortar as the water is poured.
5. Quickly pour contents of the mortar into a new soil pot so that the solution does not have time to settle. As the last bit of liquid is poured onto the soil, pour faster to assure that the maximum amount of plant material is added to the soil.

Steps to Avoid Contamination:

To avoid any interference or competition from other species, it is important that all materials be kept uncontaminated and free of spores from other species. This way, when growth is seen in the soil pot there is confidence that it is the species that is intended to grow rather than a contaminating species. The following guidelines help to establish a degree of confidence in the samples:

- Whenever tweezers are used they should be cleaned in a 70-95% ethanol solution. Additionally, they can be quickly run through a flame to dry the ethanol and kill any remaining contamination. (Exercise caution when using a flame and alcohol.)
- When adding water to the samples, it should be kept in a covered container and re-covered while not in use.
- The lid should be kept on top of the sample as much as possible to avoid contamination.
- When soil is retrieved, it should be kept covered unless being actively added into pots.
- Hands should be washed or gloves should be worn when potting up new soil pots.
- The work surface or bench top should be sprayed down with a 70% ethanol solution before potting soil or any of the other culturing tasks above.

Potting soil using PlantCon Plant Tissue Culture Containers:

PlantCon Plant Tissue Culture Containers can be purchased on the Fisher Scientific website (http://www.fishersci.com). Search for "MP Biomedicals PlantCon Plant Tissue Culture Container". A set of 10 covers and 10 bottoms costs approximately $65 total (covers and bases sold separately; June 2012).

1. Pour water into the soil and mix with washed hands to saturate the soil so that it is moist but not dripping water.
2. Take handfuls of the moist soil and add it to the pot bottom, pushing it into corners and flattening it with the palm of your hand so that the surface of the soil is flat. There should be a space of about 5 mm from the top of the pot to the upper edge of the soil.
3. Snap the cover tightly on top until you are ready to pour leafy gametophytes or spores onto the soil.