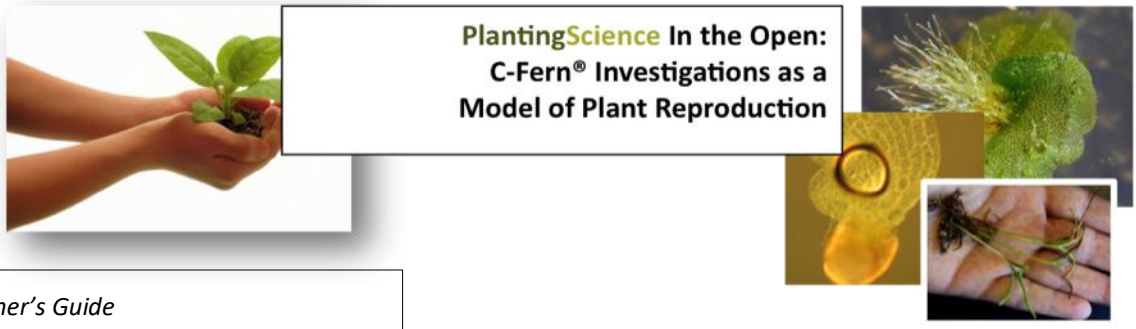


PlantingScience In the Open: C-Fern® Investigations as a Model of Plant Reproduction

Teacher's Guide

Table of Contents

Module Big Ideas and Design: This module is designed to expose students to the plant life cycle. They will gain first-hand experience with the alternation of generations, a phenomenon that is typically difficult for students to understand. Students work in teams to grow, observe and document developmental stages of the model organism, *Ceratopteris richardii* (C-Fern®). They sow spores and maintain their cultures in a way that is typical of biological research laboratories. Each stage of the *C-Fern®* life cycle will be examined as students remove one or two plants from their culture each week. Students will learn how to make a wet mount for observations under a compound light microscope as well as whole culture observations made under a dissecting microscope. As this is an observational study, data will take the form of hand sketches and qualitative descriptions made by each student. Students communicate with scientist mentors online to discuss their findings. **Guided and Open Inquiry:** Growing C-Fern® requires special conditions, so these are strongly guided in the document for optimal growth and development from one stage to the next in classrooms. Opportunities for open inquiry within the framework of the C-Fern® life cycle are indicated.

Targeted Grade Level: 9-12, Biology courses, especially suitable for Botany (portions can be adapted for middle school and college classes)

Prior Student Background: None required. Extensions depend on student background.

Time Requirement: 4-6 weeks (possibilities for longer extensions).

Collaboration and Support: This module was developed for the PlantingScience program in collaboration with Renee Lopez-Smith and Karen Renzaglia at the University of Southern Illinois (in Carbondale) and Allison Landry at the Louisiana School for Math, Science, and the Arts (in Natchitoches). All material reproduced here from the C-Fern® Manual and website (www.c-fern.org) is used with generous permission from Thomas Warne. Additional funding has been provided by the National Science Foundation and the Monsanto Foundation.

Concepts

- All living organisms have a life cycle.
- A diversity of plant life exists — not all plants are flowering plants.
- The phenomenon of Alternation of Generations exists in all plant life cycles:
  - The haploid (1n) gametophyte generation
  - The diploid (2n) sporophyte generation
- Environment affects plant growth and germination.
- Science investigation often involves good lab skills, including measurement, microscopy, and culturing of organisms under sterile conditions.
- Careful observation and data recording are part of scientific investigation and understanding phenomena.
- Science inquiry relies on good communication and sharing ideas.

What You'll Need

Plant Materials, Lab equipment, and tools

A growing system for C-Ferns®

- Light system
  - fluorescent light banks, or
  - screw-type fluorescent bulb in a light box, or
  - C-Fern® Growth Pod available from Carolina Biological Supply
- 60 x 15 mm plastic Petri dishes (enough for individuals or teams to sow spores, plus extras for the whole class)
- Basic C-Fern® agar medium available from Carolina Biological Supply
- Pre-sterilized C-Fern® spores (1 vial of wild type strain, RNW*T1*, will sow approx. 30 cultures)
- Clear plastic 2-liter soda bottles (for transplanting ferns)
- Potting soil

Wet lab or table space that can get dirty

- Microscopes
  - Compound microscope, and
  - Dissecting microscope
- Hot plate, gloves, goggles, beakers, etc.
- Sterilized plastic pipettes for each team
- Sterilized distilled water
- Sharpies for labeling

Time for dialog with mentor

- Scheduled time for students to communicate with mentors online
- Time for teachers to monitor dialogues
- Time for teachers to assess and award credit for dialogue with mentors

Regular access to computers

- Necessary for online communication
- Helpful for analyzing data, creating charts, presentations

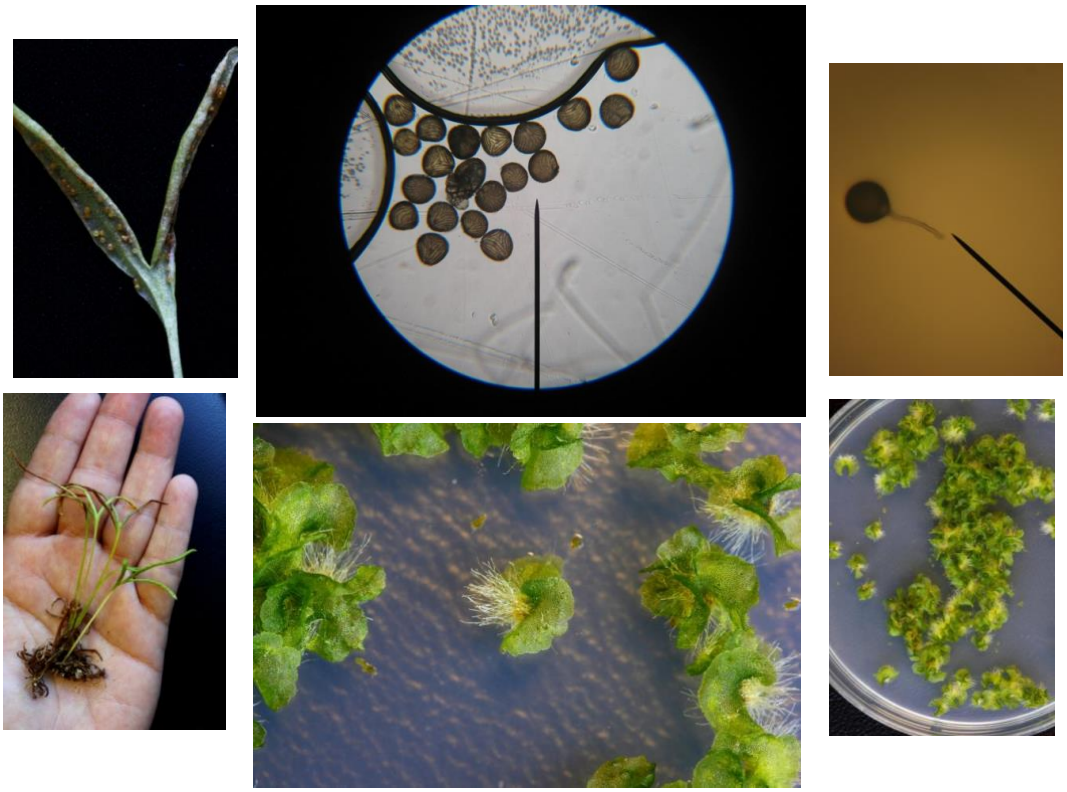
Lab notebooks

- Important for ongoing record of observations, data collection, sketches, notes, concept maps, and ideas
- Optional: Selection of pens, pencils &/or paints; Digital camera for taking pictures of student drawings and illustrations.

Learning Goals

Students will:

1. Understand that a diversity of plant life exists
2. Understand plants need specific environmental conditions in which to grow and reproduce
3. Understand that plants, like all organisms, have a life cycle
4. Understand that plant life cycles consist of alternating generations of gametophytes and sporophytes
5. Develop careful and accurate lab techniques
6. Be able to use compound and dissecting microscopes to view and manipulate organisms
7. Carefully observe and collect data
8. Analyze and make sense of findings



Life cycle moving clockwise. *Middle top*: spores with distinctive markings under compound microscope. *Upper right*: germinating spore. *Lower right*: Petri dish with many gametophytes. *Middle bottom*: close-up of gametophytes. *Lower left*: sporophyte in hand. *Upper left*: sporophyte with sporangia on the underside of leaves.

Sample Sequence

This suggested outline follows the life cycle of the C-Fern®. Once the growing system is set up and spores are sown, you can decide how far you want to go, and what activities you wish to engage in at each stage. Pick and choose activities based on your learning goals and what your students are curious about. All concepts and learning activities are not mandatory – they are designed for you to adapt to your classroom. The time needed to grow plants to full sporophyte maturity (so they produce spores) exceeds the length of the PlantingScience online sessions. If you grow them to full maturity, we ask that

students post a report and/or concluding remarks prior to the online session ending. See specific lessons for guiding questions associated with learning activities.

Please let your scientist mentors know which lessons you will be implementing.

Days after sowing	Life Cycle Stage	Learning Goals	Learning Activities
Prepare		2,5	Prepare growing system; monitor temp for 3 days Prepare tyndallized water (optional) Register online and introductions to mentors
Prior to sowing		1,2,3	**Engage Options:** *Seeds and Spores* PowerPoint Spore Brainstorm What is C-Fern®? Communicate with mentors
0-1	Sowing Spores	1,2,4,5, 6,7	**Exploring with Wet Labs:** Melt and pour agar into petri plates. Sow spores Make wet mounts of spores; observe under microscope Record activities and observations in journal Communicate with mentors
~7	Germination of Spores	1,2,3,5,6,7 ,8	**Explore and Explain:** Make wet mount to observe germinating spore Record activities and microscope observations Communicate with mentors
~14	Gametophyte Development	1,2,3,4,5,6 ,7,8	**Explore and Explain:** Make wet mount of gametophytes; microscope work Record activities and observations in lab journal Communicate with mentors
~14-21	Swimming Sperm	1,2,3,4,5,6 ,7,8	**Explore and Explain:** Make wet mount of male gametophyte; release sperm Record activities and microscope observations Communicate with mentors
~14-21	Fertilization	1,2,3,4,5,6 ,7,8	**Explore and Explain:** Make wet mount of female and male gametophytes Mass fertilize gametophytes in petri dishes Record activities and observations in lab journal Communicate with mentors
~28-60	Sporophyte Development	1,2,3,4,5,6 ,7,8	**Explore and Explain:** Observe sporophytes under dissecting microscope Transplant sporophytes into terrarium Communicate with mentors (post reports / conclusions)
~80-90	Spore Development	1,2,3,4,5,6 ,7,8	**Explore and Explain:** Observe sori where spores develop
Wrap-Up	Conclusions	1,2,3,4,5,6 ,7,8	**Sense Making:** Discussion, Presentations, Papers, Exam, Interpretations, Additional Questions

Suggested Calendar

	Monday	Tuesday	Wednesday	Thursday	Friday
Prep Week	Prepare growing system (e.g., light box, pod, or light bank) Test temperature		If using tyndallized water, begin preparing (3-days)	Students register online	Students introduce themselves to mentors
Week 1	Prepare and pour media in petri dishes Sow spores Observe (compound microscope)	Post to mentors Monitor temp of growing system			
Week 2	Observe spore germination (compound scope)	Post to mentors Monitor temp of growing system			
Week 3	Observe gametophytes (dissecting scope -- ♂and ♀?) Observe swimming sperm (♂ gametohpytes mature first)	Post to mentors	Observe fertilization (compound scope) Mass fertilization in all plates (add water)	Post to mentors Monitor temp of growing system	
Week 4	Fertilization (if didn't occur last week)	Post to mentors			
Week 5	Observe new sporophytes growing on gametophytes Post to mentors	Monitor temp of growing system			
Week 6	Monitor temp of growing system Observe growing sporophytes Prepare reports and presentations				
Week 7					
Week 8					
Week 9					
Week 10	Transplant sporophytes to terrarium				

Evaluation: The module is designed so that students can be assessed *continuously* for changes in understandings. It also allows alternate conceptions to be revealed. Online dialogs, regular postings of notebooks and data, and classroom discussions all serve as embedded assessment tools. Final class presentations and roundtable discussions, an individual reflection and conversation with mentor, and the post-experience survey serve as summative assessment tools.

Is a plant's life cycle like a mammal's?
What is a spore? What grows from a spore?

Lesson Plans and Activities:

Engaging Student Interest

We offer a variety of initial engagement activities in this section – please choose and/or adapt them or substitute other options as appropriate for your class. Students are more motivated to learn when they see connections between new material and previous knowledge they may have, and these activities are designed to help facilitate that process. They can also be used as formative assessments: you can discover misconceptions your students labor under, as well as understandings that prime them to move forward. **A primary goal of these lessons is for students to build foundation knowledge and generate questions** about the C-Fern® life cycle, and plant life cycles in general. These questions should then be posted on the student team web pages.

Within your teaching toolbox, you probably have favorite techniques for leading classroom discussions and guiding students to reveal their understandings through diagrams, charts, and illustrations. All of these techniques can be integrated with the three engagement options included here. The options were selected to provide background on plant reproduction and C-Fern to enhance what is available in high school texts. As students are more familiar with animal reproduction and life cycles of animals than plants, it can be helpful to begin with student prior knowledge of animal reproduction and then move to comparisons and contrasts between animal and plant reproduction and life cycles.

Evaluation Opportunity

As a gauge of student understanding, you may want to reserve a space to display drawings or charts created by students or teams during initial discussions and refer back to them throughout the full inquiry.

Guiding Questions

How is a plant life cycle similar to a mammal life cycle? How is it different?

In mammals, how can you tell the offspring belong to certain parents? Is it the same in plants?

What is a generation?

Where do plants come from? Where does a seed come from?

Do all plants grow from seeds? What plants might not?

What is a spore? What grows from a spore?

What are the differences between a spore and a seed?

What are the differences between plants that grow from a spore and those that grow from a seed?

Do all plants produce spores?

Mentor Moment

The scientist mentors have a lot of experience conducting field and a laboratory research. It would be a good idea at the start of the unit to have the students introduce themselves. Have the students ask questions about working in a research laboratory and encourage them to ask questions about fern or plant reproduction.

Seeds and Spores PowerPoint Presentation

Lesson Overview and Goals: The PowerPoint slide presentation entitled *Seeds and Spores* is a tool for engaging students in a discussion of these two important botanical structures. It starts by asking leading questions on the topic of seeds which students are familiar with. Then the discussion turns to the topic of spores, a less familiar botanical structure. 1. to pique the interest of students in studying plant reproduction 2. to function as an assessment of what prior knowledge your class has 3. to introduce some of the properties of spores and the structures that produce them	**Materials:** ○ A copy of the PowerPoint slideshow, "Seeds and Spores" ○ A means of projecting the slides ○ Guiding Questions at the beginning of this section
Timeline for this lesson: 15-45 minutes, depending on length of discussion. This slide show is not designed to be presented as a lecture, but instead to be used as a tool to encourage student discussion and development of ideas.	**Advance Preparation:** Familiarize yourself with the slides and notes.
Teacher Background: There are notes associated with each slide to guide you as the teacher.	***Resources:*** The PowerPoint slides are available at: https://plantingscience.org/resources/201

Mentor Moment

Prompt students to contact their mentor and brainstorm about the function, purpose and anatomy of spores.

Spores Brainstorm

Lesson Overview: This open discussion facilitated by the teacher explores what spores are. Students often don't know what a spore is. They may think it is the same thing as an egg or sperm, or confuse it with pollen. This lesson lets you see how where students' conceptual understandings are, and what misconceptions they have about spores.	**Materials:** ○ Poster board, white board or other means of capturing brainstorming ideas ○ Guiding Questions at the beginning of this section
Timeline for this lesson: 15-30 minutes.	**Advance Preparation:** Be familiar with the differences between seed-bearing and non-seed-bearing plants, and the differences between seeds and spores.
Teacher Background: see elaboration below	**Resources:**

Spores are part of the reproductive cycle of all plants and have some characteristics similar to seeds, but are not seeds. All plants produce spores. This includes mosses, lichens, ferns, flowering plants. Fungi, organisms from another biological kingdom, also produce spores. Some plants, like ferns, produce obvious spores that are visible, especially with a little magnification. However, flowering plants produce spores which are internal to the plant, and are not easily seen.

Spores give rise to gametophytes. Gametophytes (literally, *gamete-producing plants*) produce gametes, that is, egg and sperm. Fertilization takes place when egg and sperm unite. The resulting embryo grows into a sporophyte (literally, *spore-producing plant*). The sporophyte produces spores. In flowering plants the male spores give rise to pollen grains (the male gametophyte), which are very obvious in spring time. The female spores of flowering plants are not obvious at all since they are contained within the flower.

Flowering plants produce seeds, but not all plants produce seeds. Ferns, lichens and mosses do not produce seeds. In seed-producing plants, fertilization (union of egg and sperm) occurs within the flower. In seedless plants, fertilization takes place after the spores have left the parent plant. Each spore matures into a gametophyte and produces either eggs and/or sperm. Since the spore leaves the parent plant before fertilization, the spore must be able to survive in what may be less-than-ideal conditions. This survival must last until conditions improve. In this way seeds and spores are similar.

Like seeds, spores contain food reserves. Since spores are destined to produce a gametophyte plant that is fully capable of photosynthesizing, spores generally contain less nutrition than a seed. Seeds need to carry a food reserve to allow the embryonic plant to grow until it is able to photosynthesize on its own, which takes longer than the development of the gametophyte.

What is C-Fern®?

Lesson Overview: This lesson is a student reading written to introduce your students to the fern genus, *Ceratopteris*, including its morphology, taxonomy and life cycle. Plants are often overlooked by students and seen as irrelevant. Ferns and other non-flowering plants are even more often dismissed. This lesson attempts to familiarize the students with the organism they will soon be working with. After students have read the assignment, lead a class discussion to summarize the information. We present a prompt at the end of the reading, asking students to write down or discuss in groups some questions they have about the life cycle of ferns, that is, how these plants reproduce and survive.	**Materials:** ○ Copies of "What is C-Fern®" in Appendix A ○ Guiding Questions at the beginning of this section ○ Optional – A fern in the classroom – not necessarily a C-Fern® – a houseplant fern will do. It will be especially useful if the fern you bring in has the small brown dots (sori) on the underside of the leaves. These sori contain the spore producing organs called sporangia.
Timeline for this lesson: A student with average reading abilities will be to complete this assignment in 20 minutes. You could also assign the reading as homework, leaving the time for class discussion.	**Advance Preparation:** Read student assignment and make copies.
Teacher Background: Familiarity with Linnean system of biological nomenclature; use of model organisms for research and teaching.	**Resources:** C-Fern official site: http://www.c-fern.org/ C-Fern on Facebook: http://www.facebook.com/pages/C-Fern/112463705418 Boimages site: http://www.cas.vanderbilt.edu/bioimages/species/ceri2.htm

Mentor Moment

Prompt students to contact their mentor and discuss the anatomy of ferns. Have them explore the differences between flowerless, vascular plants like ferns and flowering vascular plants like lilies or roses.

What causes spores to germinate?
What are the conditions necessary for fertilization?
What happens to the fertilized cells?

Exploring the C-Fern® Life Cycle in Wet Labs

The primary goal of this series of explorations is to use this model organism to understand plant reproduction. An observational study of the alternation of generations acts as the backbone of this module. There are also excellent points of departure for other inquiries. These are identified as inquiry elaborations. The wet labs are structured to give you the best chance of success at growing C-Fern®.

Section Overview

Each exploration details a particular phase of the C-Fern® life cycle. Each will have students performing wet labs in which they work directly with the spores, the gametophytes, the gametes, and the sporophytes. Observation labs are spaced about a week apart, however development of the plants can vary considerably in time. It is good to monitor the ferns and allow flexibility to observe earlier or later, if necessary.

Students should sketch and note their weekly observations in their lab journals. These drawings and careful observations form their qualitative data, which will be analyzed over time and shared with their team mates and online mentor. It is important that students record the dates of their observations and drawings. This information will be meaningful when analyzing their qualitative data.

Below are suggestions for sequencing investigations. Refer also to the General Sequence (p. 4) to plan.

1. Have students perform all of investigations, making observations and drawings. This allows for the complete observation of alternation of generations.

2. Have students do only selected parts of the life cycle if you are only trying to illustrate a single concept using a model organism. For example, perhaps you only want to show germination of the spores into gametophytes. This will mean that you need to use only one of the parts.

3. Have students who understand the alternation of generations use only one life stage of C-Fern® to perform individualized inquiries using just a single part of the life cycle. For example, you may have students interested in how the sperm finds the egg or others may be interested in what pH is necessary for sperm motility.

4. Have students run through the whole life cycle, as in #1 above so that they can see the chronological order of events in real time. Then concurrently, either you or the students can also be growing C-Fern® at various different stages so that students will also be able to do individualized inquiries as in #3 without having to wait for another full cycle. You or the students would start one set of spores for the whole life cycle and then start fewer spores each week so that at the end of the whole cycle you would also have various stages for the individualized inquiries.

Wet Lab 1: Preparing the Plates

Lesson Overview

First, students prepare Petri plates for germinating and growing the C-Fern® spores. This lesson is specifically concerned with preparing the growing medium for sowing spores. As it involves creating the growing environment for the C-Ferns®, it is an opportunity to discuss general growing conditions of all plants, natural habitats, and controlled lab conditions. If you have a long lab period, you can combine this lab with Wet Labs 2 and 3 (sowing and observing spores).

Timeline for this lesson: 40-50 minutes.

Teacher Background

An agar medium is melted and poured into Petri plates in preparation for sowing C-Fern® spores. This should occur at least 30 minutes prior to sowing the spores. The Petri plates can be stored in a refrigerator for over a month if needed. The number of Petri plates you prepare depends on the number of students you have. One Petri plate can be allocated per team, or per person. **Prepare several extra plates for use in elaboration inquiries and possible demonstration use**.

Guiding questions for discussion:

- What do you think the natural environment of C-Ferns is?
- What affect(s) would manipulations to the environment have?
- Why are lab conditions carefully controlled?
- What do you think might be extreme conditions for C-Ferns in their natural environment?
- How might human-made changes to tropical forests affect C-Fern populations?
- What wavelengths of light do you think C-Ferns utilize?

Materials for preparing the Petri plates

The amounts below are for preparing 20 Petri dishes.

- 1 pk of 20 - 60 x 15 mm plastic Petri dishes (depends on class size and you will want to sow a few extra cultures) (available from Carolina Biological Supply)
- 1 - 400 mL bottle of Basic *C-Fern*® ® medium (Carolina Biological Supply)
- Latex (or nitrile) gloves
- Hot plate
- Sauce pan for hot water bath
- Oven mits
- Safety goggles
- Black Sharpie® pen
- 80% ethanol or 80% isopropanol
- Parafilm (if storing for later sowing of spores)

Elaboration Inquiry
(possible extension lab)

Use another set of Petri plates, but this time prepare a different type of medium. Have the students brainstorm new substances to sow the spores onto. Some suggestions would be:

•gelatin (plain or flavored)
•agar or gelatin with additives such as soup broth cubes, sugar, salt, spices
•no medium, just a liquid like distilled or tap water, sugar or salt water or pond water

Any suggestion is usable, but students should have sound reasoning behind why they choose a particular substance. We are trying to investigate biologically relevant ideas.

Mentor Moment

Prompt students to contact their mentor and discuss the upcoming wet lab experiments. Throughout the wet labs the students will want to contact their mentor to get tips on how to proceed and to discuss their observations.

Advance Preparation
You may want to start melting agar before the students arrive. This is especially true if you're using a hot water bath (the preferred, safe way) to melt the agar since it takes a while.

Procedure

CAUTION
Be very careful if using a microwave to melt your agar. We do not recommend it. Uneven heating can make the hot agar boil over when the flask is removed. Use very short, 10 second microwave heatings and carefully swirl the contents after each heating. The safer way to melt agar is with a hot water bath.

Notice
You will want to keep your work area and equipment as antiseptic as possible to avoid mold growth in the Petri plates. Use the plastic bag the plates came in for storage of the prepared plates.

Preparing the culture medium

If using a hot plate:

1. Place sauce pan with 2 cups tap water onto hot plate set at 250-300°C. Bring water to a slow boil.

2. Next, shake the bottle of Basic C-Fern® ® medium in order to break up the solidified medium (this will speed up the melting process).

3. Loosen the cap and place the bottle in the hot water bath for 50 min or until medium is completely liquefied.

4. Remove from hot water bath carefully while wearing an oven mit and allow medium to cool but not solidify.

If using a microwave:

1. Shake the bottle to break up the medium (as above), remove lid and place jar in microwave. Set to high for 3.5 min. Remove carefully using an oven mitt and allow to cool but not solidify.

Preparing Petri dishes

1. Meanwhile, prepare a clean area to pour medium into Petri dishes. Begin by wiping the entire area down with 80% ethanol or isopropanol. If alcohol is not available, wash down area with soapy water followed by wiping down with a clean damp cloth or sponge.

2. Put on gloves (latex or nitrile) and then spray your gloved hands with the 80% ethanol or isopropanol.

3. Open your package of 20 plastic Petri dishes by cutting through the end of the sleeve with scissors. Save the sleeve for storage. Line the dishes up in rows of 5.

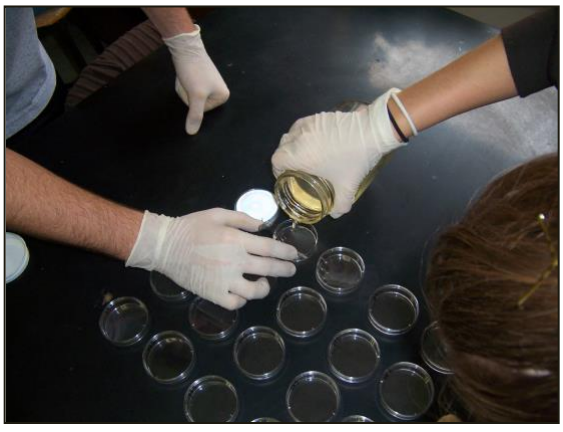
Pouring culture medium

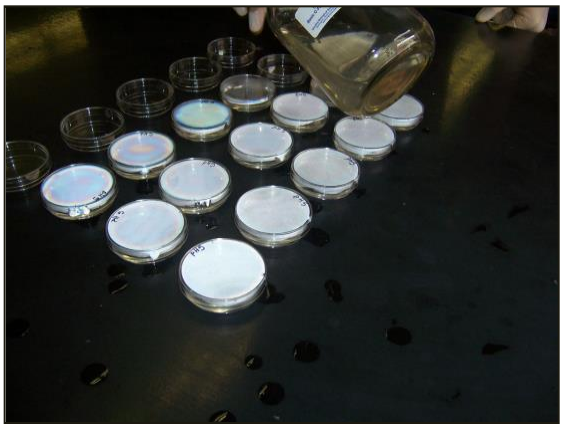
1. Spray gloves again with the 80% ethanol or isopropanol.

2. Remove the cap from the liquid medium, then lift the lid of Petri dish just enough to permit pouring of the medium into the Petri dish. Pour medium until each dish is ¾ filled and place the lid back down on the Petri dish.

3. After all Petri dishes have been filled with medium, allow the dishes to solidify undisturbed for at least 30 minutes.

4. If you will sow spores the next day, the dishes can be left undisturbed over night. If not, wrap parafilm around the dish where the lid and bottom meet and store in the refrigerator. If you store the prepared Petri dishes, you can stack them and put them back into the plastic sleeve they came in before putting them in the fridge.





Left: Pouring melted agar medium carefully into Petri dishes. *Right*: Lids are labeled and placed on top. You will notice condensation on the inside of the lids.

Wet Lab 2: Sowing the Spores

Lesson Overview
The students sow the spores onto the prepared plates. They learn to keep conditions sterile as they work with C-Ferns®. Spores are placed in suspension and allowed to soak, then dispensed and spread over the prepared agar medium in the Petri dishes. Petri dishes with sown spores will then be placed in the growing system (see Preparation). Over subsequent days and weeks, students will watch the spores germinate on the Petri plates and grow into gametophytes. (We recommend conducting Wet Lab 2: Observing Spores on the same day, if possible.)

Timeline for this lesson: 30-45 minutes

Teacher Background
Keeping contaminants (bacteria, fungi) at a minimum is important as they can readily grow on the agar medium. Therefore:

- Use sterilized spores, which are available from Carolina Biological Supply.
- Use sterile pipettes – if they are not pre-sterilized, you can do so using the isopropanol or ethanol.
- Water should be distilled and ideally sterile. An autoclave is ideal for sterilizing water. Alternatively, a procedure is in the appendix for preparing tyndallized water. This consists of boiling the water three times, allowing enough time between boiling events for bacteria or fungi propagules that may have survived to germinate. (Distilled water may be sterile enough if kept carefully covered.)
- Removing the lid from the Petri dishes introduces contamination into the culture. As this is inevitable, it is wise to pour and sow extra cultures.

Save some spores in suspension for observing under the microscope – see Wet Lab 3.

Materials for sowing the spores
The amounts below are for preparing 20 Petri dishes.

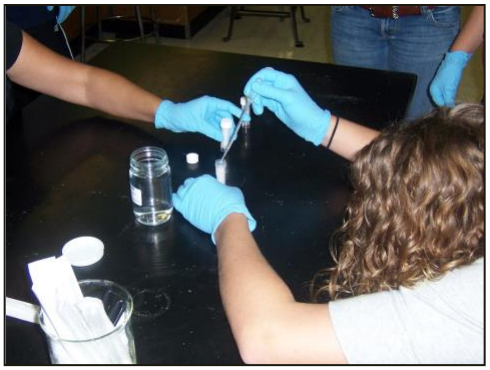
- 1 - 10 mg vial of wild type strain, RNW*T1,* pre-sterilized C-Fern® spores (1 vial will sow 25-30 Petri dishes) (available from Carolina Biological Supply)
- Prepared 60 x 15 mm plastic Petri dishes with agar medium prepared (depends on class size and you will want to sow a few extra cultures)
- Latex (or nitrile) gloves
- 3 - Pre-packaged sterilized plastic pipettes
- 10 ml sterilized distilled H_2O (for sowing spores)
- Black Sharpie® pen
- 20 paper clips
- 80% ethanol or 80% isopropanol
- plastic sandwich baggies (does not have to be ziplock)

Evaluation Opportunity

Throughout the wet labs, gauge students' scientific abilities. Check to see if they understand or have developed a sound procedure. Notice their techniques and follow their progress through the lab. Evaluate their conclusions.

Preparing Pre-sterilized Spores: (this only takes one person)

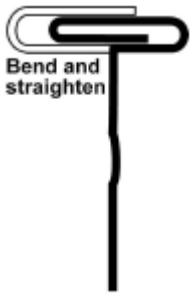
1. Wipe down work area with 80% ethanol or isopropanol.



2. Put on gloves and spray gloved hands with 80% ethanol or isopropanol.

3. Remove lid from spore vial and using one of the packaged sterile plastic pipettes, add 4 ml sterilized H_2O to the vial. Replace the lid and gently invert the vial 2 or 3 times in order to insure that the spores become completely wet.

4. Allow the spores to soak for about 15 min. This is now what we call a "spore suspension."



From Hickok and Warne, 2009

Sowing the Spores

1. First, you will need to make a sterile spore spreader. Take a paper clip and bend it into a "T" shape. Holding the straight end of the paper clip, you will wipe the "T" end with 80% ethanol or isopropanol. Let it air dry.*

2. Put gloves on. Spray hands with 80% ethanol or isopropanol then remove the lid from the spore vial. Using another sterile plastic pipette, gently suspend the spores by drawing the H_2O along with spores in and out of the pipette. Do this about 3 times.

3. Withdraw a small amount of the spore suspension into the pipette.

4. Lift the lid of the Petri dish just enough to dispense 3 drops of the spore solution onto the medium. Be careful not to touch the medium with pipette tip. Replace the lid.

5. Finish sowing the rest of your Petri dishes. You will want to re-suspend the spore solution between each sowing.

6. After you have finished sowing spores, take the **sterile spore spreader** you prepared earlier and spread the spores by allowing the spreader to rest on the agar surface, then moving it gently back and forth across the surface. Rotate the dish and go gently back and forth across the surface, again. You want try your best to distribute the spores over the entire surface of the medium. Replace the lid.

7. Using the **Black** Sharpie® pen, label the dish toward the edge with your initials and the date (mm/dd/yr). Avoid writing across the middle of the lid as this will make it difficult to observe the cultures under the dissecting microscope without removing the lid.

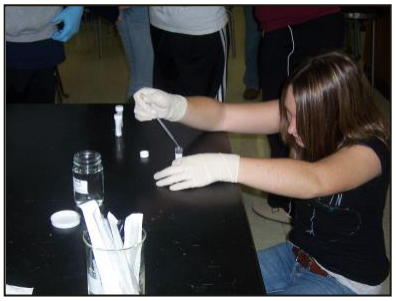
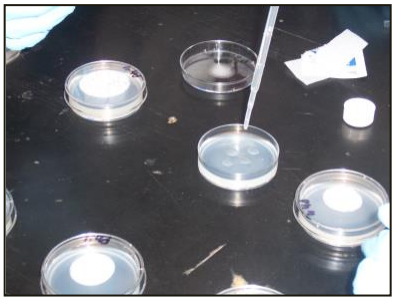
8. **Turn Petri dishes upside down. They will grow this way, with the agar side up, and lid side down.**

9. Carefully place the C-Fern® cultures into plastic sandwich baggies (no more than 2 per baggie) to maintain a moist environment. Do not seal these tight (to avoid heat build-up in bags). Place bags under lights in growing system.

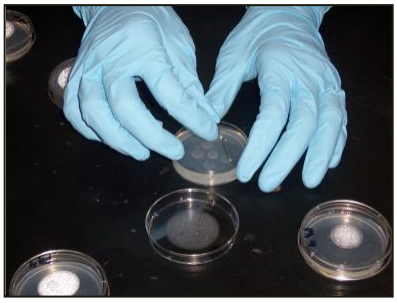
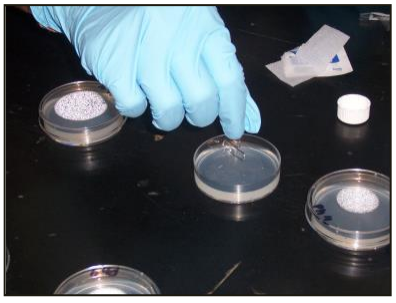
Notice

At this point we start to count the days after sowing of the spores. The day you sow spores is considered Day 0. The day after sowing is Day 1. Other events will happen a specified number of "days after sowing."

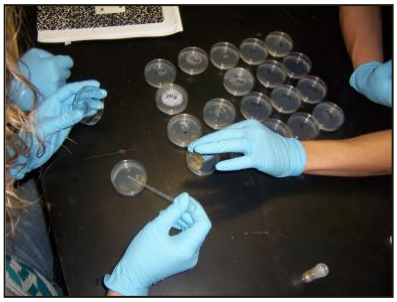
Row 1: *Left:* Withdraw spore suspension into pipette. *Right:* Dispense drops of spore suspension onto the medium.

Row 2: Spread spores with the sterile spore spreader made from a paper clip.

Row 3: *Left*: Different team members dispense drops and spread spores. *Right*: Place Petri dishes in plastic bags without sealing tight.




Row 4: Plastic bags with Petri dishes **stored upside down**, and placed under lights in growing system.


Wet Lab 3: Observing Spores

Lesson Overview
Students observe the unique morphology of C-Fern® spores under a compound microscope by making a wet mount. This lab easily follows Wet Lab 2 when students sow spores, and uses the remaining spore suspension from Wet Lab 2. If spores get squashed in making the wet mounts, the opportunity can be used to examine the contents of spores. Compound microscope skills are honed. Students also orient themselves to making drawings as a form of qualitative data.

Timeline for this lesson: 20-40 minutes. Best done on same day as Wet Lab 2. (Though spore suspension can be stored for use a few days later.)

Teacher Background
You may want to review compound microscope skills with students.

Spores are formed by the sporophyte through the process of meiosis, and are haploid (1n). They are protected by a hard coat developed around each spore while in a tetrad with three other spores. The trilete marking on the outer coat is formed by the proximity to the other three spores: 

The nucleus of the cell is frequently observable as a dark spot in the spore. Spores are also filled with lipid bodies, and if the spore wall is crushed in the making of the wet mounts (when cover slip is placed on top), students will likely see bodies of oil in the suspension under magnification.

Students will make more complex wet mounts in subsequent labs, so this is a good chance to hone these skills. Additionally, drawings will be used as a form of qualitative data collection, and setting up the lab notebook for these periodic exercises will be useful throughout the study.

Guiding Questions
- Why do you think spores are so small?
- What patterns do you see on the spore?
- What biological process could have occurred during spore development to create these markings?
- Is the spore haploid (1n) or diploid (2n)?
- Do you think there may be functions to the spore texture?
- What is contained in the spore?
- How can the spore wall be thick and tough, but still be able to detect environmental changes?
- What causes spores to germinate?
- What changes in the environment do you think the spore detects to cause it to germinate?
- Do all plants create spores?

Materials for this lesson
- Spores in suspension remaining from Wet Lab 2
- Compound microscopes
- Microscope slides and cover slips
- Latex (or nitrile) gloves
- 80% ethanol or 80% isopropanol
- Sterile pipettes

Procedure

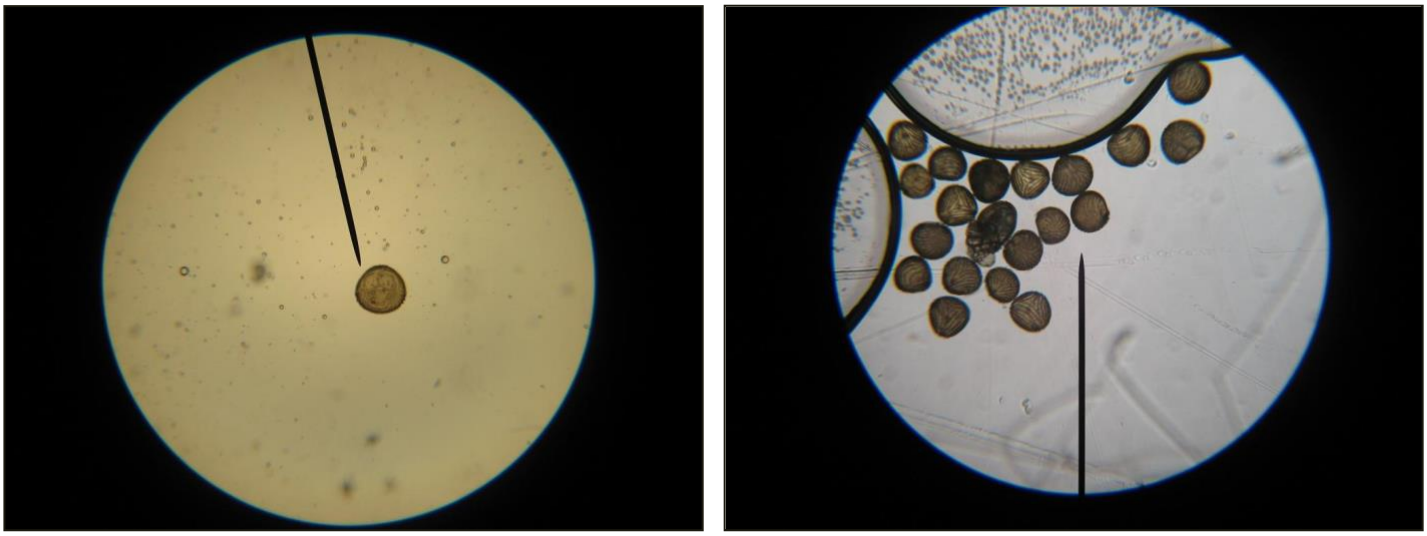
1. Set up compound microscopes
2. Make a wet mount by placing a small drop of leftover spore solution onto a microscope slide. Carefully place a cover slip over the drop. DO NOT press the cover slip over the spore with your finger because this will smash the spores.
3. Use the 10x objective lens to locate the spores, then increase magnification.
4. Sketch observations in lab notebook.
5. Make notes of what was observed, and of questions.
6. Drawings, notes and questions can be posted online for mentors.

Mentor Moment

Prompt students to contact their mentor and discuss what the spores look like.

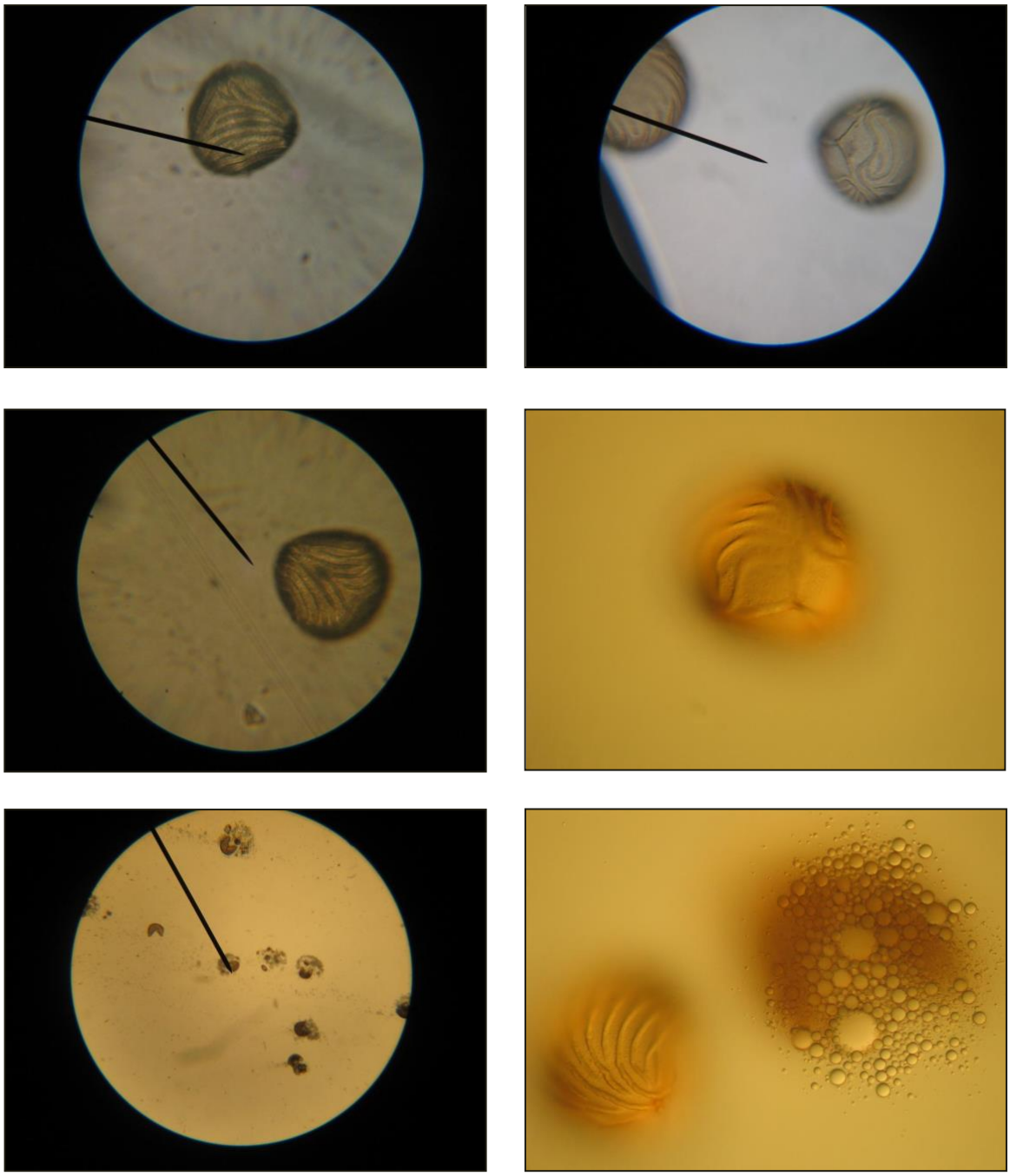
Search Images

It is often difficult to know what you're looking for under a microscope. The following images depict C-Fern® spores under magnification.



Left: A single spore among small air bubbles. *Right:* Several spores sitting against two large air bubbles.

Spores under magnification



Top and middle: Spore coat patterns. The dark somewhat fuzzy spot toward the center of the darker slides indicate the nucleus inside the spore. The orange-colored photos are from a higher-powered microscope. **Bottom:** Spores that have been squashed, revealing the lipid bodies on the inside.

Wet Lab 4: Observing Germination

Lesson Overview

This part of the C-Fern culturing involves observations of the spore as it germinates and starts its growth into a gametophyte. Wet mounts are made by lifting a spore from the Petri dish while observing under a dissecting microscope. Germinating spores are viewed under compound microscopes. Student observations are recorded, reviewed and analyzed through the use of lab notebook entries, drawings and discussions. These can be uploaded to the PlantingScience website for discussion with team mentors. Drawings and descriptions are frequently used in scientific investigation, as are photographs. Such data are carefully collected and analyzed by scientists, including some of the mentors in the PlantingScience program, to better understand how C-Ferns® grow, develop and reproduce.

Timeline for this lesson: 30 – 40 minutes
Should be conducted approximately 7 days after sowing.

Times are approximate!
You may have to proceed to the next lab earlier or later than indicated because of growing conditions (esp. temperature and light) specific to your classroom. Monitor your cultures to pick the best day to observe.

Teacher Background

Dormant spores will germinate once they are placed in proper conditions. Water is the key ingredient to initiate germination. We added the spores to water in Wet Lab 2 and made sure they were thoroughly wetted. The process of a spore (or seed) taking up water is called imbibition. After the spore imbibes water the process of germination will begin. The next important factor is light.

Germination is the sprouting of a plant after a period of dormancy such as from a seed or spore. When a spore germinates, a thin colorless filament, called a rhizoid, is the first structure to emerge in the developing gametophyte, which is haploid (1n), like the spore itself. As the gametophyte grows, cell division and growth occurs through mitosis of haploid (1n) cells, producing more haploid (1n) cells. The rhizoid has no chloroplast, and so does not photosynthesize. Rather, the mitochondria in the rhizoid turn the stored lipids in the spore into energy for growth. The oxygen needed for energy (ATP) generation from the environment. The rhizoid elongates away from the spore, absorbs water and minerals for the gametophyte, and anchors it to the substrate (in this case, the agar medium). The rhizoids are not the same as roots – roots have vascular tissue, and rhizoids do not. These rhizoids (of the gametophyte) are composed of one long thin cell with a special cell wall and plasma membrane to allow them to take up water and nutrients for the rest of the gametophyte.

Guiding Questions

- What is germination? Do only seeds germinate?
- What is the first thing that appears in germination? Is it growing in a particular direction?
- Are these cells haploid (1n) or diploid (2n)?
- How does it get energy to grow?
- Does it use light to photosynthesize? How can you tell? Dos this structure contain chloroplasts?
- Why is this filament made first and not a green leaf?
- What do you think the function of this filament is?
- How many cells thick is the rhizoid?
- Can you see vesicles in the rhizoid cells?
- Why is it not considered a root?

Materials

- Lab notebook with ink pen and # 2 pencil
- Dissecting and compound microscopes
- Probes or dissecting T-pins (the finer they are, the better – sewing needles and pins are great)
- Microscope slides and cover slips
- Latex (or nitrile) gloves
- 80% ethanol or 80% isopropanol
- Sterile distilled water
- Pipettes or eyedroppers

Advance Preparation

You might want to do a quick lab on the operation of the microscopes before students tackle their C-Fern observations. It is especially difficult to manipulate tools while looking under the dissecting microscope. **Recommended Activity:** Place a very fine dot on a piece of paper, and place it on the stage of the dissecting microscope. Have students attempt to touch this with the point of the probe while looking through the eyepieces.

If you are preparing your own sterilized water (see Appendix B and the article entitled, "Tyndallization Procedure to Produce Sterilized Water")

Mentor Moment

Prompt students to contact their mentor and discuss what to look for as the spores germinate.

Procedure

1. Set up dissecting and compound microscopes (*Recommended:* practice eye-hand coordination under the dissecting microscope)

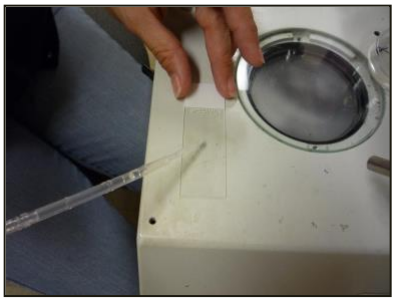
2. Put gloves on. Spray hands with 80% ethanol or isopropanol. Sterilize probe or pin by wiping thoroughly with ethanol or isopropanol and letting it air dry.

3. Carefully remove C-Fern® culture from the plastic baggie, **making sure to keep the Petri dish upside down! (This is to prevent the agar getting soaked by condensation.)**

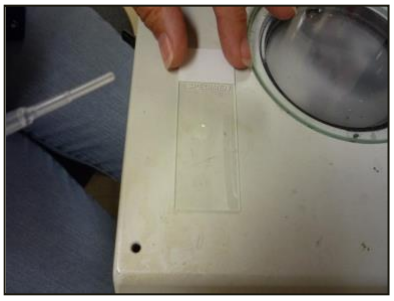
4. **Keeping the lid on the underside of the Petri dish**, remove the lid and give it a quick shake to remove the condensation **or** wipe the condensation from the inside of the lid with a sterile tissue. Either way, do this very quickly and put the lid back on the culture dish to avoid contamination. It can now be placed right side up.

5. Take out a microscope slide. Place a small drop of sterile distilled water on it with a pipette or eyedropper. Set to the side.

6. Place Petri dish right side up on the dissecting microscope stage. Remove the Petri dish lid.



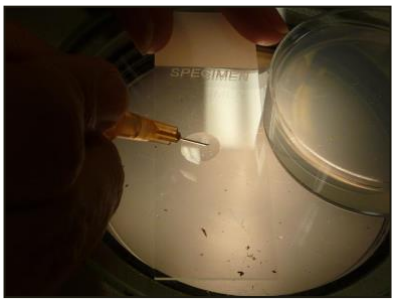
7. While looking through the dissecting microscope (and using the light from above rather than below the Petri dish), use your sterilized pin/probe to carefully lift one or two spores from the agar medium. Be careful not to disrupt the agar too much. Remove the Petri dish from the dissecting scope stage.



8. Then place the tip of your pin with the spore into the water drop on your microscope slide. Make sure the spore leaves the pin to float in the drop of water.

9. Gently lay a cover slip over the spores. DO NOT PRESS THE COVER SLIP DOWN ON SPECIMEN AS IT WILL "SQUASH" YOUR GERMINATING SPORE!!

10. Place the lid back on your Petri dish and set it upside down.



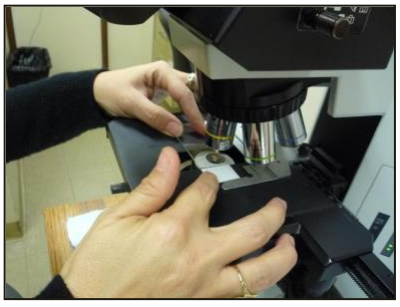
11. View your specimen under the compound microscope. Use the 10x lens to locate the gametophyte, then increase magnification.

12. Sketch your observations in your lab journal and write descriptions and comments.

13. Be sure to place Petri dishes with lids on upside down in plastic baggies, and return them to the light growing system.

Mentor Moment

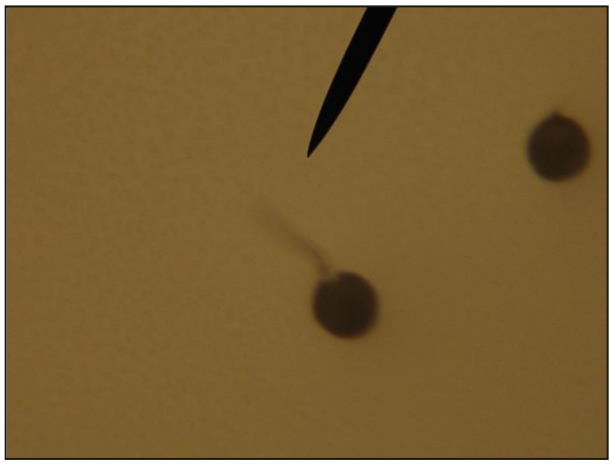
Prompt students to upload drawings on the PlantingScience website, to share with their mentors what they observed, and to ask questions.

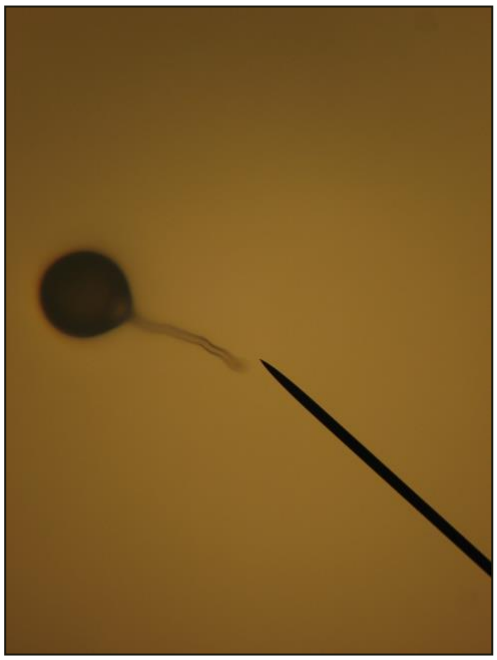


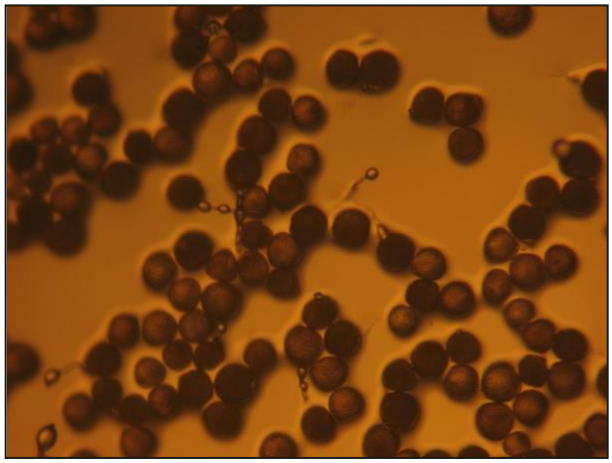
Photos *From Top:* 1) Placing drop of distilled sterile water on microscope slide with pipette. 2) Drop of water on slide. 3) Depositing spores from Petri dish into drop of water with probe needle. 4) Gently placing cover slip on to drop of water so as not to squash spores. 5) Viewing specimen under compound microscope.

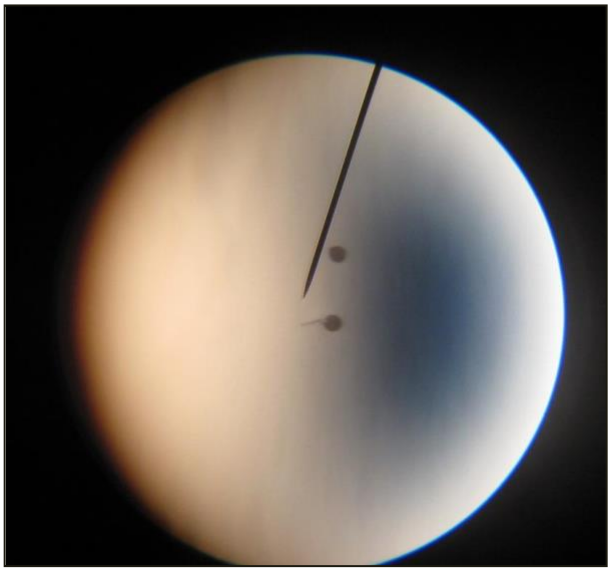
Search Images

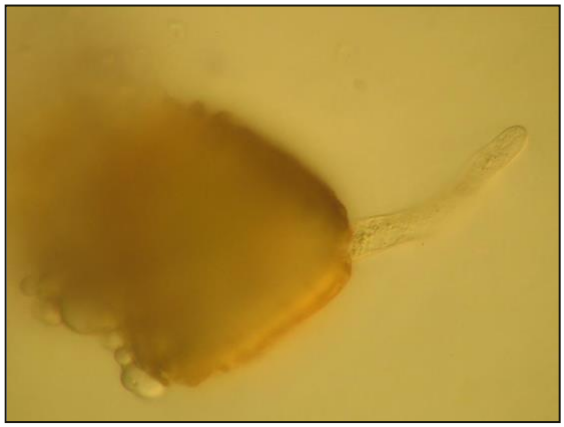
Photos of germinating spores with first filament (rhizoid)











Wet Lab 5: Observing Gametophyte Development and Swimming Sperm

Lesson Overview

After germination of the spores, gametophytes develop quickly. Gametophytes are mature when they produce gametes. Male gametophytes become sexually mature prior to female gametophytes. In this lesson, students observe the gametophytes, learn to differentiate between male and female, and may even witness swimming sperm from the male gametophytes. It is important to understand that the small gametophyte is an actual plant – one of the alternating generations of a plant. It doesn't really look like the fern plant we are familiar with, but it is a full-fledged plant. The gametophyte will have proto-roots (called rhizoids) and it will photosynthesize early on.

Students make their initial observations of the gametophytes using dissecting microscopes. Then they make wet mounts by lifting gametophytes from the Petri dish, and view them under compound microscopes. Student observations are recorded, reviewed and analyzed through the use of lab notebook entries, drawings and discussions. These can be uploaded to the PlantingScience website for discussion with team mentors.

Times are approximate!
You may have to proceed to the next lab earlier or later than indicated because of growing conditions (esp. temperature and light) specific to your classroom. Monitor your cultures to pick the best day to observe.

Timeline for this lesson: 30 – 60 minutes
Should be conducted approximately 10 – 15 days after sowing.

Teacher Background

From the germinating spore, the gametophyte body starts growing as a large mass of several green cells with chloroplasts so it can photosynthesize. Once photosynthesis begins, the gametophyte no longer needs to depend on the oil bodies from the spore for energy. Interestingly, gametophytes never develop vascular tissue, and rely on being moist for all cells to absorb water and minerals directly from the environment. Because of this, the gametophyte – green clumps of cells with attached rhizoids – never gets very thick (one to a few cells thick).

Male and hermaphrodite (functionally female) gametophytes grow into different shapes. Male gametophytes appear more like a mass of cells. Hermaphrodite gametophytes form two "wings" or laminae, the first initially appearing larger than the second, forming a "mitten" appearance. The growing point is called the apical notch, and is located between the laminar "wings." Gametophytes grow by mitosis of haploid (1n) cells, producing additional haploid (1n) cells that differentiate. Gametophytes bear the gametes, which are generated by mitosis in plants.

The male gametophytes bear several antheridia (singular = antheridium) that produce sperm. **Sperm are released from mature antheridia within a minute or so when in the presence of water** (which is why we prevent water settling on the agar medium by storing it upside down – we want to control the release of sperm!).

The hermaphrodite gametophyte is functionally female at first, as it bears 3-6 archegonia (singular = archegonium) that produce eggs. The archegonia, which bear the eggs, are located around the apical notch. The hermaphrodite gametophyte later produces antheridia at the edges of the "wings" that can produce sperm. The reasons for this hermaphroditic ability is an active area of research today.

Archegonia develop on the surface of the female gametophyte away from the agar medium. In nature, archegonia develop on the underside of the gametophyte. Whether this is due to negative geotropism, negative phototropism, or thigmotropism is unclear.

Guiding Questions

- What differences do you see in the cells forming?
- Why are some of the cells green?
- How do they get their oxygen and sugar needed for respiration?
- How do they get their necessary water and minerals?
- How many different shapes of gametophytes do you see? How would you describe these?
- Are these cells haploid (1n) or diploid (2n)?
- Where do you think new cells are growing from?
- Can you count or estimate how many chloroplasts are in one cell?
- Are there any structures on the gametophytes? If so, what do they look like and where are they located?
- Are archegonia the same as pistils in flowering plants?
- Are antheridia the same as anthers in flowering plants?
- What do the archegonia produce?
- What do the antheridia produce?
- Are the eggs and sperm haploid (1n) or diploid (2n)?
- Are fern eggs and sperm produced by mitosis or meiosis?
- On what surface are the archegonia and antheridia (in relation to the agar, light source and gravity)?
- What do you think causes them to grown on this side? How might you test that?
- Why would the hermaphrodite gametophytes produce both female and male gametes?

Materials

- Lab notebook with ink pen and # 2 pencil
- Dissecting and compound microscopes
- Probes or dissecting T-pins (the finer they are, the better – sewing needles and pins are great)
- Microscope slides and cover slips
- Latex (or nitrile) gloves
- 80% ethanol or 80% isopropanol
- Sterile distilled water
- Pipettes or eyedroppers

Advance Preparation

If you are preparing your own sterilized water (see Appendix B and the article entitled, "Tyndallization Procedure to Produce Sterilized Water")

Mentor Moment

Prompt students to contact their mentor and discuss what to look for as the gametophytes develop and mature.

Procedure for viewing gametophytes and swimming sperm

1. Set up dissecting and compound microscopes
2. Put gloves on. Spray hands with 80% ethanol or isopropanol. Sterilize probe or pin by wiping thoroughly with ethanol or isopropanol and letting it air dry.
3. Carefully remove C-Fern® culture from the plastic baggie, **making sure to keep the Petri dish upside down! (This is to prevent the agar getting soaked by condensation.)**
4. **Keeping the lid on the underside of the Petri dish**, remove the lid and give it a quick shake to remove the condensation **or** wipe the condensation from the inside of the lid with a sterile tissue. Either way, do this very quickly and put the lid back on the culture dish to avoid contamination. It can now be placed right side up.
5. Using the dissecting microscope, observe the gametophytes while they are still in the Petri dish. Make drawings and discuss what you notice.

6. Now make wet mounts. Take out a microscope slide. Place a small drop of sterile distilled water on it with a pipette or eyedropper. Set to the side.
7. Using either the unaided eye, or while looking through the dissecting microscope, use your sterilized pin/probe to carefully lift one or two gametophytes from the agar medium. Be careful not to disrupt the agar too much.

8. Then place the tip of your pin with the gametophytes into the water drop on your microscope slide. Make sure the gametophytes leave the pin to float in the drop of water.
9. Gently lay a cover slip over the spores. DO NOT PRESS THE COVER SLIP DOWN ON SPECIMEN AS IT WILL "SQUASH" YOUR GAMETOPHYTE!!

10. Place the lid back on your Petri dish and set it upside down.
11. View your specimen under the compound microscope.
12. Sketch your observations in your lab journal and write descriptions and comments.
13. Be sure to place Petri dishes with lids on upside down in plastic baggies, and return them to the light growing system.

Notes on viewing gametophytes and swimming sperm

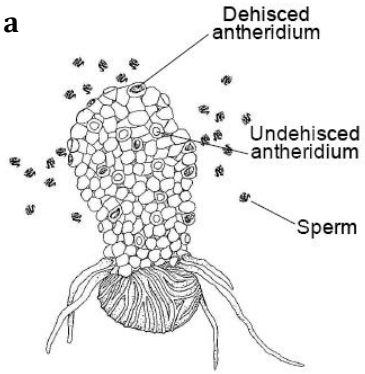
1. First try to **distinguish between the male and hermaphrodite (functionally female) gametophytes.** They have different shapes. You can do this while first looking at the Petri dish through the dissecting microscope.

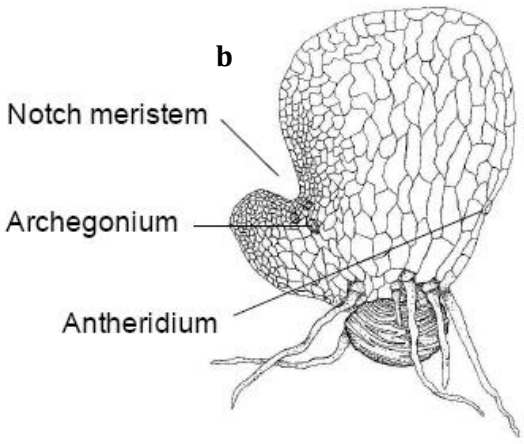
2. Make one wet mount with male gametophytes, and one with hermaphrodite (functionally female) gametophytes, and then view them under the compound microscope.

3. Try to **locate the antheridia on the male gametophyte** when viewing it under the compound microscope**.** Antheridia (singular = antheridium) are small swellings that contain the sperm. When the sperm have matured in the antheridia, they are released in the presence of water. (The term *dehiscence* refers to the opening of the antheridium to release the sperm inside.)

4. Once you are able to recognize the antheridia on the male gametophyte, try making another wet mount of another male gametophyte and, under the compound microscope, **look for the swimming sperm** that might be released from the antheridia. Once mature antheridia contact water, sperm will be released within seconds to a minute. They have multiple flagella, and swim very quickly! You might only see flashes of light as they move in the water.

5. If you are not able to see swimming sperm at this time, try looking for them in the next lab.





a. Male gametophyte
b. Hermaphrodite (functionally female) gametophyte
c. Individual sperm, highly magnified

From Hickok and Warne, 2009

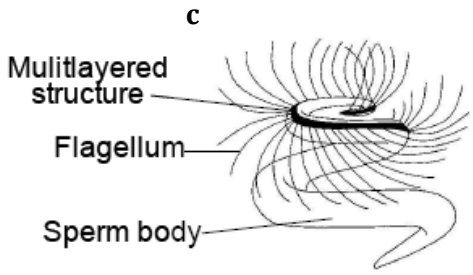


Figure 5. Individual mature sperm, ca. 8.8 × 5.5 µm.

Wet Lab 6: Fertilization

Overview of Lesson

Male gametophytes tend to mature prior to the female gametophytes. So while you might have been able to see swimming sperm in the previous lesson, you likely will have to wait a few days to attempt viewing fertilization, when female gametophytes are mature. Approximately 2 to 3 weeks after sowing spores, your cultures should be ready for fertilization. You will know the female gametophytes are ready for fertilization when you see 3-5 archegonia in the apical notch region (see "b" on diagram on previous page). You will want to monitor the gametophytes to look for this readiness.

In this lesson, students make wet mounts of female and male gametophytes together and try to see sperm swimming from the antheridia on the male gametophytes toward the archegonia on the female gametophytes. Finally, students mass fertilize the gametophytes in their Petri dishes by adding water, and then storing them upright so the water allows sperm to travel to the archegonia on the female gametophytes and fertilize the eggs therein. Students observe, record, discuss and share their findings.

Timeline for this lesson: 30-60 minutes
Should be conducted approximately 14-21 days after sowing.

Times are approximate!
You may have to proceed to the next lab earlier or later than indicated because of growing conditions (esp. temperature and light) specific to your classroom. Monitor your cultures to pick the best day to observe.

Teacher background

An important difference between most plants and animals is that plants produce gametes by mitosis. The haploid gametophyte (1n) produces haploid gametes (1n). Gametophytes are mature when they produce gametes.

The male sex organ is called the antheridium and it produces sperm, which are released from the antheridia in water. Sperm cells swim very quickly, using their multiple flagella in whip-lash fashion to propel them. Mitochondria inside the sperm cells generate ATP that provides energy for the sperm to swim.

The female sex organ is called the archegonium and it contains single celled haploid (1n) eggs. When eggs are mature and they contact water, the archegonia open their canals and release a chemical attractant (pheromone) and viscous substance that the sperm detect and swim toward (this is not well understood, and is an active area of research). Water is necessary for fertilization in ferns, as the sperm need to swim to the archegonia. Fertilization occurs in the archegonia, once a sperm cell penetrates the egg, resulting in a diploid (2n) cell.

(Interestingly, plants that produce seeds do not have swimming sperm. Rather, the male gametophyte in seed-bearing plants is the pollen. There is tremendous evidence to suggest that the earliest plants on earth had swimming sperm, similar to ferns and mosses, and that flowering plants were derived from them in more recent evolutionary time.)

Guiding Questions

- Where are sperm produced?
- Is the sperm haploid (1n) or diploid (2n)?
- What causes the sperm to be released?
- Where are the eggs located?
- Are eggs haploid (1n) or diploid (2n)?
- How do the sperm get to eggs?
- Where does sperm get its energy to swim?
- How does the sperm know where to go?
- What conditions are necessary for fertilization?
- Why do you think the petri dishes have been stored upside down while gametophytes grow?
- Should they be stored upside down now or right side up?
- Fertilization produces a cell – is this cell haploid (1n) or diploid (2n)?
- Do all plants have swimming sperm?
- How do flowering plants transport sperm?
- Do flowering plants have gametophytes?
- Speaking in terms of evolution, what do you think the first plants were more like: flowering plants or ferns?

Elaboration Inquiry
(possible extension lab)

When wetted, mature gametophytes will release sperm (from the male) and open the neck of the female gametophyte. Additionally, the female releases a chemical attractant to guide the sperm to the egg. All in a choreographed "dance." You can study this phenomenon of sperm chemotaxis in more detail with the C-Fern® Sperm Chemotaxis kit from Carolina Biological Supply Company.

Materials

- Lab notebook with ink pen and # 2 pencil
- Dissecting and compound microscopes
- Probes or dissecting T-pins (the finDer they are, the better – sewing needles and pins are great)
- Microscope slides and cover slips
- Latex (or nitrile) gloves
- 80% ethanol or 80% isopropanol
- Sterile distilled water
- Pipettes or eyedroppers

Advance Preparation

If you are preparing your own sterilized water (see Appendix B and the article entitled, "Tyndallization Procedure to Produce Sterilized Water")

Mentor Moment

Prompt students to contact their mentor and discuss what to look for as the gametophytes mature and fertilization occurs.

Evaluation Opportunity

Students' ability to extend their thinking and develop an individualized inquiry is a mark of higher understanding and a good place to evaluate.

Procedure for viewing fertilization

1. Monitor female gametophytes. When you see 3-5 archegonia on the female gametophytes (located near the apical notch), you know they are ready for fertilization.

2. Set up dissecting and compound microscopes.

3. Put gloves on. Spray hands with 80% ethanol or isopropanol. Sterilize probe or pin by wiping thoroughly with ethanol or isopropanol and letting it air dry.

4. Carefully remove C-Fern® culture from the plastic baggie, **keeping the Petri dish upside down!**

5. **Keeping the lid on the underside of the Petri dish**, remove the lid and give it a quick shake to remove the condensation **or** wipe the condensation from the inside of the lid with a sterile tissue. Either way, do this very quickly and put the lid back on the culture dish to avoid contamination. It can now be placed right side up.

6. Using the dissecting microscope, observe the gametophytes while they are still in the Petri dish. Identify the male and female gametophytes. Make drawings and discuss what you notice.

7. Now make a wet mount to observe fertilization. Take out a microscope slide. Place a small drop of sterile distilled water on it with a pipette or eyedropper. Set to the side.

8. Using either the unaided eye, or while looking through the dissecting microscope, use your sterilized pin/probe to carefully lift ***first*** a ***female*** gametophyte from the agar medium. Be careful not to disrupt the agar too much.*

9. Then place the tip of your pin with the female gametophyte into the water drop on your microscope slide. Make sure the gametophyte leaves the pin to float in the drop of water.

10. ***Then*** lift a ***male*** gametophyte from the agar medium, and place the tip of your pin with the male gametophyte into the water drop on the microscope slide. Make sure the gametophyte leaves the pin to float in the drop of water.*

11. Gently lay a cover slip over the spores. DO NOT PRESS THE COVER SLIP DOWN ON SPECIMEN AS IT WILL "SQUASH" YOUR GAMETOPHYTES!!

12. View your specimen under the compound microscope, scanning the field of view to look at all of your plants. Within a few seconds to a minute, you might see sperm released from antheridia (on the male gametophytes) and swim very quickly to the archegonia of the female gametophyte. You may even be able to see their flagella straighten out just before entering the archegonia.

13. Sketch your observations in your lab journal and write descriptions and comments.

*The sperm will be released within a few seconds to a minute after contacting water, so it is important to place the female gametophyte in the water first, *then* the male gametophyte. Work quickly after placing the male gametophyte in the water.

Procedure for mass fertilization in Petri dish cultures

1. Remove the lid from the Petri dish (only do one at a time) and add 2 mL of sterilized water. Replace the lid.

2. You can try to observe the swimming sperm as they make their way from the antheridia of the male gametophytes to the archegonia of the female gametophytes under a dissecting microscope.

3. Repeat #1 for each of the cultures.

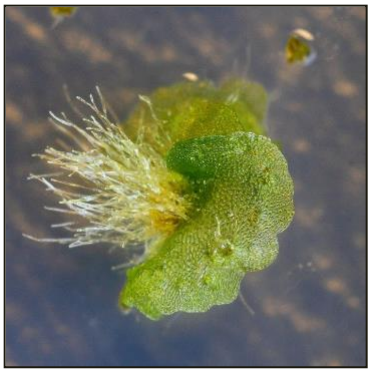
4. Once you have added water to all of your plates, put your Petri dishes back into their plastic baggies, and return them back to the light bank.

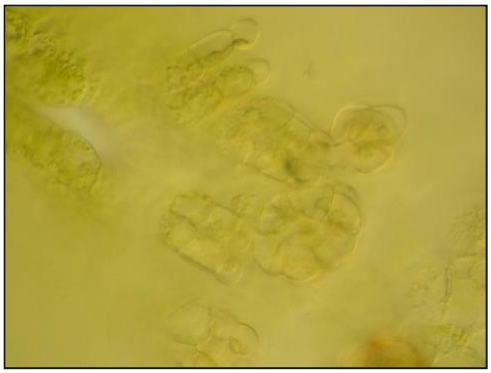
5. **Store Petri dishes right-side-up, with the lid on the top and agar on bottom (to ensure fertilization).**

6. Maintain your cultures under the same lighting and temperature conditions as you had previously.

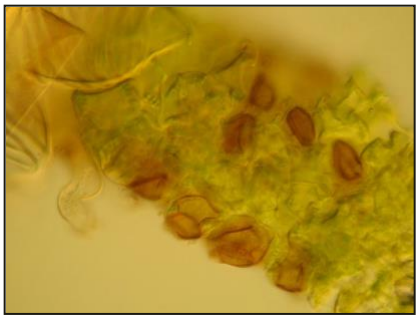
Note: Adding water to your *C-Fern*® cultures will allow antheridia to open and release sperm. Additionally, the water allows the neck canal cells of the archegonia to open and 1) discharge a chemical attractant that will help the sperm find them and 2) provide an opening for sperm to enter the archegonium and make their way to the egg.

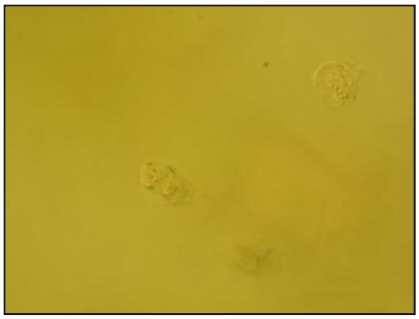
Search Images





Top *Left:* Female gametophyte as seen under a dissecting microscope. *Right:* Several necks of archegonia on the female gametophyte, under high magnification.





Bottom *Left:* Old antheridia on the male gametophyte turn dark – seen under high magnification. *Right:* Swimming sperm caught in motion under high magnification.

Wet Lab 7: Observing the Embryonic Sporophyte (2n)

Lesson Overview

In five to seven days from fertilization (which took place in Wet Lab 6), you should be able to see the swollen archegonia on the female gametophyte, indicative of the growing sporophyte embryo! You can think of it as a "pregnant" gametophyte. In this lesson, students first use the dissecting microscope, then the compound microscope, to view the new sporophyte embryo. The sporophyte will develop into the plant form that we normally recognize as a fern. Students observe, record , discuss and share their findings.

Timeline for this lesson: 30-60 minutes.
This should take place 5-7 days after fertilization.

Times are approximate!
You may have to proceed to the next lab earlier or later than indicated because of growing conditions (esp. temperature and light) specific to your classroom. Monitor your cultures to pick the best day to observe.

Teacher Background

The union of sperm (1n) and egg (1n) through fertilization produces a diploid (2n) cell which grows into the sporophyte embryo (2n). The sporophyte cells are produced by mitosis (cell division in which the daughter cells have the same number of chromosomes as the parent cell: 2n → 2n).

The first signs of the sporophyte embryo are little green swellings on the surface of the gametophyte. The young sporophyte eventually breaks out through the archegonia and grows while still connected to the gametophyte. The gametophyte initially helps nourish the sporophyte embryo, but eventually dies. The two growing points in sporophytes are the leaf tip and the root, each of which develops in opposite directions. Each sporophyte leaf develops one root (this is not typical of ferns or other plants).

Guiding Questions

- What happens to the fertilized cell in the archegonia?
- Does the sporophyte need the gametophyte? If so, indefinitely?
- What is the relationship of the gametophyte and the sporophyte?
- How is the sporophyte growing? What process produces more cells?
- Is the sporophyte haploid (1n) or diploid (2n)?

Mentor Moment

Prompt students to contact their mentor and discuss and ask questions about what they observe as the embryo begins to swell on the gametophyte.

Materials

- Lab notebook with ink pen and # 2 pencil
- Dissecting and compound microscopes
- Probes or dissecting T-pins (the finer they are, the better – sewing needles and pins are great)
- Microscope slides and cover slips
- Latex (or nitrile) gloves
- 80% ethanol or 80% isopropanol
- Sterile distilled water
- Pipettes or eyedroppers

Advance Preparation

If you are preparing your own sterilized water (see Appendix B and the article entitled, "Tyndallization Procedure to Produce Sterilized Water")

Procedure for viewing fertilization

1. Set up dissecting and compound microscopes.

2. Put gloves on. Spray hands with 80% ethanol or isopropanol. Sterilize probe or pin by wiping thoroughly with ethanol or isopropanol and letting it air dry.

3. Carefully remove C-Fern® culture from the plastic baggie. Remove the lid.

4. Using the dissecting microscope, observe the gametophytes while they are still in the Petri dish, and identify female gametophytes with swollen archegonia, which means a developing embryo. Draw and record what you observe.

5. Now make a wet mount of the developing embryo. Take out a microscope slide. Place a small drop of sterile distilled water on it with a pipette or eyedropper. Set to the side.

6. Using either the unaided eye, or while looking through the dissecting microscope, use your sterilized pin/probe to carefully lift out a "pregnant" female gametophyte with a developing embryo from the agar medium.

7. Place the tip of your pin with the female gametophyte into the water drop on your microscope slide. Make sure the gametophyte leaves the pin to float in the drop of water.

8. Gently lay a cover slip over the spores. DO NOT PRESS THE COVER SLIP DOWN ON SPECIMEN AS IT WILL "SQUASH" YOUR GAMETOPHYTES!!

9. View your specimen under the compound microscope, starting wtih 10X magnification to observe.

10. Sketch your observations in your lab journal and write descriptions and comments.

Wet Lab 8: Observing the Developing Sporophyte

Lesson Overview

Students observe the developing sporophyte in the Petri dishes. Over the next few weeks, this lesson can be repeated for students to observe the developing sporophyte regularly. You can have them stop observing once the plant becomes recognizable as the fern we are all familiar with. Or you can have them continue observing until the sporophyte reaches maturity and is able to produce spores for another generation. We have found that students usually become quite fond of their plants, so if you have the space, keep the sporophytes growing on to maturity. Observations and drawings need not take too much time. If the time required to grow the sporophytes will exceed the time your PlantingScience scientist mentors are available online, please be conscientious about having students post a project and/or concluding remarks to mentors online.

Timeline for this lesson: 20-40 minutes.

Students should make at least weekly observations of the developing sporophyte until it reaches a stage where it is recognizable as the familiar fern. You may choose to go beyond that until you have a mature sporophyte. This will take approximately 90 days after sowing.

Teacher Background

Sporophytes are diploid (2n), which grow and develop by producing additional cells by mitosis. The gametophyte initially helps nourish the sporophyte embryo, but eventually dies. The two growing points in sporophytes are the leaf tip and the root, each of which develops in opposite directions. Each sporophyte leaf develops one root (this is not typical of ferns or other plants).

Fern sporophytes develop vascular tissue, allowing water and nutrients to be carried through vessels to different parts of the plant. Vascular tissue enables a plant to grow upwards and still have access to water and nutrients.

Sporophyte leaves (or fronds) have stomata, which open and close to allow transpiration. Sporophytes have chlorophyll and photosynthesize. After 5 true leaves form on the sporophyte, it can be transplanted into soil in a terrarium so it stays moist.

Elaboration Inquiry
(possible extension lab)

The C-Fern® plant is an excellent model organism for use in investigations. In the sporophyte stage students could perform a number of experiments. Some suggestions would be:

•growth response to different light levels, temperatures, fertilizer amounts or water
•susceptibility to salt exposure, herbicide exposure, etc.

Any suggestion is usable, but students should have sound reasoning behind why they chose a particular method. We are trying to investigate biologically relevant ideas.

Mentor Moment

Prompt students to contact their mentor and discuss and ask questions about what they observe as the sporophyte grows and develops.

Guiding Questions

- What is the relationship of the gametophyte and the sporophyte?
- How is the sporophyte growing? What process produces more cells?
- Is the sporophyte haploid (1n) or diploid (2n)?
- Where are the growing points?
- What is vascular tissue?
- Why might vascular tissue be important?
- Does the sporophyte photosynthesize?
- How do you know?
- What function do the specialized stomata cells perform?

Materials

- Lab notebook with ink pen and # 2 pencil
- Dissecting microscopes
- Latex (or nitrile) gloves
- 80% ethanol or 80% isopropanol

Procedure for observing developing sporophyte

1. Set up dissecting microscopes.

2. Put gloves on. Spray hands with 80% ethanol or isopropanol. Sterilize probe or pin by wiping thoroughly with ethanol or isopropanol and letting it air dry.

3. Carefully remove C-Fern® culture from the plastic baggie. Remove the lid.

4. Using the dissecting microscope, observe the growing sporophytes while they are still in the Petri dish.

5. You can also choose young sporophytes with well developed roots and very gently wash off some of the clinging agar gel. You can observe the development of the sporophyte for several weeks and don't need a microscope to do so. Although you may want to use a microscope or magnifying glass to check out fine details. Make drawings of the sporophyte as it develops.

6. A mature sporophyte will develop around 90 days after sowing. At this point the plant will be able to produce spores (the sori containing the sporangia should be visible on the underside of some of the plant's leaves).

7. You can use these new spores to start another generation of C-Fern® plants.

8. Sketch your observations in your lab journal and write descriptions and comments

Wet Lab 9: Transplanting Sporophytes

Lesson Overview
When sporophytes have five leaves, they are hardy and large enough to transplant from the Petri dishes to a terrarium with soil. This lesson describes how to make a homemade terrarium.

Timeline for this lesson: 30-50 minutes.
This should take place between 60-70 days after sowing.

Teacher Background: See previous background.

Guiding Questions
- When you transplant the sporophyte, what conditions do you need to create?
- What is the natural environment of the C-Ferns?

Materials (for each student)
- Clear plastic 2 liter bottle
- Potting soil
- Scissors
- Clear tape (the wider, the better)
- 200 mL tap water
- C-Fern Sporophytes (having at least 5 leaves)
- Tweezers
- Dish pan

Photo of a completed C-Fern® terrarium filled with potting soil and showing the flap sealed with tape.

Procedure for transplanting sporophytes
1. Make a flap in the middle of the 2-liter bottle by cutting three sides of a rectangle. It should be wide enough for you to put your hand inside. See the photo of a completed terrarium.

2. Place potting soil into the dish pan and add the 200 ml of water. Mix the water into the soil so that the soil is nice and moist, but not soupy. If still dry, add a little more water.

3. Fill the 2-liter bottle 1/3 full with the moistened potting soil.

4. Using the tweezers, remove only one sporophyte at a time from culture. If the root is covered in agar, carefully remove it by washing it off with water.

5. Place the C-Fern sporophyte onto the soil (you do not need to make a hole in the soil and cover the roots like you would if you were planting a potted plant into the soil).

6. Once you have transplanted the sporophytes to the 2-liter bottle, tape up the opening in the bottle.

7. Place your terrarium near a window that receives indirect light during the day.

8. Once you've decided where you're going to put your fern, place a thermometer into the terrarium and monitor the temperature. Temperature should be maintained about 25 - 28°C.

What is my mental model of alternation of generations?
What evidence supports my interpretation?

Sense Making

By explaining, students gain an understanding of the concepts and can verify answers to questions or problems. Guide students to propose explanations that are consistent with their experiences. They should use evidence to reinforce their explanations. Listen carefully to be certain students aren't reinforcing misconceptions they may have started with or ones they developed during their explorations. It is also important for students respectfully listen to each other's ideas. This open sharing of ideas and hypotheses is critical in the practice of science.

As the backbone of this module is an observational study of alternation of generations, the primary data sources for students' explanations will be qualitative descriptions, recorded in a combination of drawings and notes. Students may be less familiar with using qualitative data than quantitative data as evidence to generate explanations.

Each week students should make sketches of what they observed in their cultures. They should record the date of their sketches and make careful descriptive notes about their observations, along with any thoughts or questions that have arisen. At the end of their observational study, students should refer back to their qualitative data to describe the pattern of development exhibited by C-Fern® over time.

Possible Sense Making Activities to Synthesize their Understandings and Share Them with Others

Depending on your classroom goals, here are several suggestions for activities to include as explanations of the phenomenon of alternation of generations:

1. Have student teams present to each other through PowerPoint presentations, or poster sessions, their findings and explanations.

2. Have them return to their sketches of different stages of the C-Fern® life cycle and annotate their sketches with their new understanding. Or have students create more detailed botanical drawings of particular features based on their sketches and descriptions or photographs.

3. Have the students create a compare and contrast chart to show the similarities and differences between animal and fern reproduction.

4. Have students write an essay on the evolutionary advantages of alternation of generations.

5. Have the students speculate and illustrate how they think alternation of generations occurs in flowering plants.

6. Have students explain their experimental designs if they performed any of the Elaboration Inquiries suggested throughout the unit.

7. Have the students create a C-Fern® graphic novel that illustrates concepts they have learned.

Appendix A. Student Handout

"What is C-Fern®?"

A Reading on a Model Organism

It's not "Sea-Fern" or "See-Fern." This plant doesn't live by the ocean. It's a fern that has been given a shorthand nickname, C-Fern®. The letter "C" in the nickname refers to the fern's genus, which is *Ceratopteris*. You might be familiar with this fern if you have an aquarium since it's often sold under the name 'water sprite' and can live submerged under water. There are even plastic models available for your aquarium tank made to look like *Ceratopteris*.

So it's pretty clear that *Ceratopteris* is a water-loving fern. It can be found in aquatic habitats such as ponds, rivers, rice paddies and even intermittently wet areas like drainage ditches. As we will soon see, reproduction in C-Fern® is dependent upon a watery environment. *Ceratopteris* and its relatives are found in tropical regions and areas close to the tropics. These areas provide the warm, humid environment C-Fern® needs. The C-Fern®'s full binomial classification name is *Ceratopteris richardii*.

A C-Fern® plant grown in the laboratory.

What is a Genus?

Biologists classify all organisms into various groupings based on shared characteristics. For instance, dogs and wolves are in the same genus because they are very similar to each other. But at a more general level, dogs and humans are closely related also. Organisms are grouped into broad, general categories first and then they are more specifically designated as belonging to more narrow groupings. Here's how the fern you will be using is grouped from a very broad grouping at top to a very specific grouping:

Ceratopteris is in the
Kingdom Plantae
Division Tracheophyta
Class Filicopsida
Order Filicales
Family Pteridaceae
Genus Ceratopteris

Then there can be several types or species of Ceratopteris such as the C-Fern or *Ceratopteris richardii* (when Genus and species names are put together, they are italicized.)

From the short stem are leaves (which we call fronds in ferns). It grows to a height of 10-40 cm (or about 4-16 inches). It is fleshy or herbaceous, not woody, and grows fast to complete its life cycle quickly. C-Fern® is a type of plant we call an annual. This means it completes its whole life cycle in one growing season. A particular plant specimen of C-Fern® will germinate from a spore, reproduce and then die within one season. A perennial plant on the other hand, will continue to live through not just one season, but many more into the future. Some other types of ferns are perennial.

Ferns are among the first plants to evolve on Earth and have been around for at least 360 million years.

The picture above is of *Pecopteris*, a fern fossil that is 250 million years old.

But wait! What was that word "spore" used in the previous paragraph? C-Fern® is a plant, so doesn't it use seeds? Nope! C-Fern®, like all ferns, is seedless. Other plants, such as mosses, are also seedless; however, those plants also lack a vascular system of tubes to transport water and nutrients. C-Fern® does have a vascular system, but uses spores, not seeds, in reproduction. In the big picture of plant evolutionary relationships there are 4 giant groupings. They are:

1. Seedless, non-vascular plants like mosses and liverworts.
2. Seedless, vascular plants like ferns, including *Ceratopteris*.
3. Seed bearing plants with cones like pine trees and other conifers.
4. Seed bearing plants with flowers like roses, oak trees and sunflowers.

What about those spores? Plants, and that means all of the kinds of plants in the above four groupings, produce spores. The way in which those spores participate in reproduction will be explored in this module. For now, we need to know that C-Fern® uses spores and we will study in-depth how those spores produce new plants. If you have time in your class, you will even investigate ways in which you can change how these spores behave.

An important aspect of your C-Fern® experiences is that you will be using a model organism. Such use means that you or some scientist has noticed that C-Fern® has qualities that make it a useful example organism to study. In this case, the useful characteristics are that we can see a whole life cycle from germination to new plants to death of the parent plant within a fairly short time. Also good for modeling are the two phases of growth and development in C-Fern® that allows us to make cellular and whole plant observations. The size of the plant parts are such that we can see everything with our eyes (or at least with low magnification) and without having to grow a giant plant in the classroom. There are useful genetic observations to be made as well.

Before proceeding to the next lessons, take a few minutes to write down or discuss in groups some questions you have about the life cycle of ferns or how they go about reproducing and surviving.

Appendix B. Teacher Resources

Teacher Resources

Table of Contents

For more information, visit the C-Fern® website at: www.C-Fern®.org

Of particular interest at this website may be the "C-Fern® Web Manual," which contains detailed information on many of the labs performed in this module.

Teacher Background on the C-Fern® Life Cycle

The *C-Fern 's* real name is *Ceratopteris richardii.* This fern lives in wet habitats and can be found growing in ponds, streams, rivers, wet ditches and rice paddies in moist tropical and subtropical areas around the world (Hickok and Warne, 1998). The *C-Fern®* grows as an annual which means that it completes its life cycle (i.e., it germinates, reproduces and dies) in one growing season. One spore-to-spore life cycle can take place in less than three months which is one reason why the *C-Fern®* is rapidly becoming an ideal model for students to study the life cycle of plants.

In animals, offspring are formed when a sperm (a single haploid cell) from a male parent (a multicellular diploid organism) unites with an egg (a single haploid cell) from a female parent (a multicellular diploid organism). However, the life cycle of plants differs from that of nearly all other organisms on earth because during their life cycle they produce two separate plants: a multicellular diploid (2n) plant called a sporophyte (sporo = spore; phyte = plant) undergoes meiosis to produce multicellular haploid (1n) plant called a gametophyte (gameto = gametes; phyte = plant), each giving rise to the other.

In ferns, the diploid sporophyte is the plant you are familiar with that has large green leaves called fronds. If you were to turn the fronds over and look at the underside of the leaf you will see small round dots that can be red, orange or brown in color. These dots are called sori and under the sori you will find specialized reproductive structures called sporangia. Within the sporangia are the spores. Once the spores are made (by meiosis), the sporangia dry up causing them to release the spores, which fall to the ground where they will begin to germinate and grow into a multicellular haploid gametophyte. This is the haploid generation. The gametophyte will produce gametes (by mitosis) in specialized sex organs: the male sex organ is called the antheridium and the female sex organ is called the archegonium. Many sperm cells are produced within a single antheridium but only one egg cell is produced per archegonium. There will be numerous antheridia (*plural*) and archegonia (*plural*) on each gametophyte. Antheridia are tiny round structures that look like pillows while archegonia look like vases that have round bases that hold the egg deep within the gametophyte and long neck cells that form a tunnel in which the sperm must enter and to reach the egg.

The process of fertilization in ferns takes place as follows: in the presence of water, the antheridia open to release sperm cells that will immediately begin swimming. The archegonia will also open to release a

chemical attractant that will guide swimming sperm to the egg (like in most other organisms, many sperm cells can approach the egg at once but only one sperm cell will fuse with the egg). After sperm and egg have united, a new diploid individual is formed, the sporophyte. The life cycle can now begin again.

The C-Fern, like most ferns, is homosporous which means it only produces one kind of spore. Once the spores fall to the ground the first to germinate will become female. The female gametophyte produces an invisible chemical (a certain plant hormone called a pheromone) that will cause the remaining spores surrounding it to become male gametophytes! Thus, the C-Fern® will have separate male and female gametophytes which are easy to tell apart. The male gametophytes are very tiny strap-shaped plants with numerous antheridia. On the other hand, the female gametophyte is larger and heart-shaped with six to eight archegonia, which are only found at the top of the heart.

The *C-Fern®* has two ways by which it reproduces to make a new individual: one way is sexual via gametophytes with sperm and eggs and the other way is asexual. Asexual reproduction goes like this: the sporophyte (or mother plant) produces tiny buds where the leaves are attached to the stem. These buds have a couple of small leaves and a few tiny roots so when they fall to the ground they are ready to quickly grow and develop into a new sporophyte genetically identical to the mother plant. Most plants utilize both of these reproductive methods thereby ensuring their survival as a species.

Like all homosporous ferns, *C-Fern* has two independent autotrophic phases: a structurally simple, haploid gametophyte and a vascular, diploid sporophyte. The gametophytic phase, which develops mitotically after germination of the single-celled spore, can be cultured axenically on a simple inorganic medium.

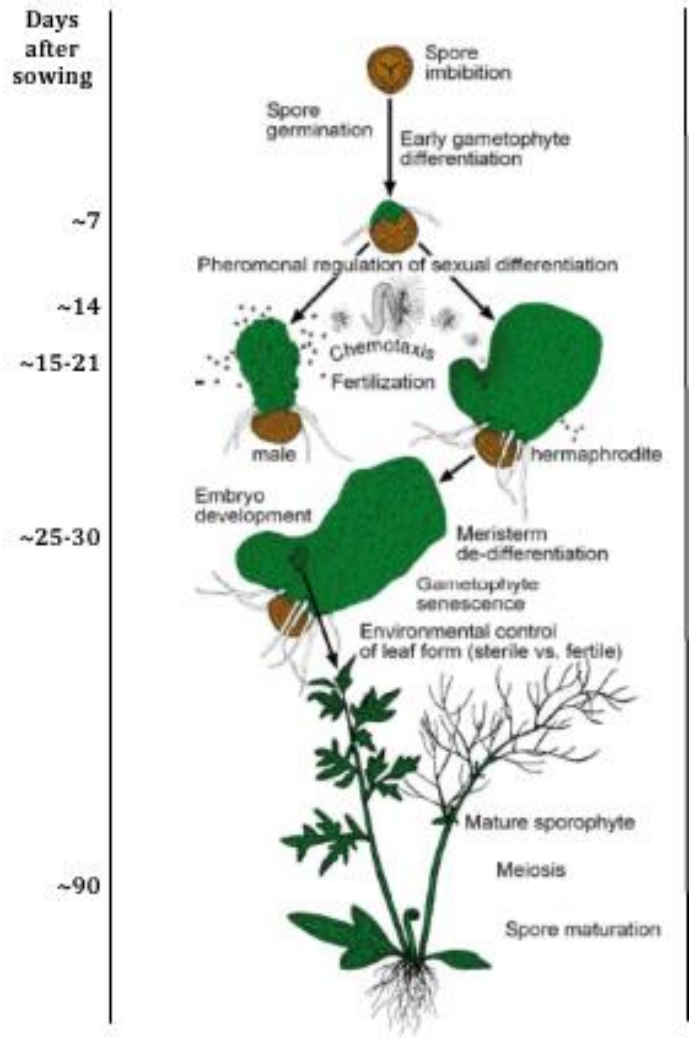
Development of this haploid phase is very rapid. Germination occurs 3–4 days following inoculation, and full sexual maturity is attained within 6–8 days from germination. At maturity, the gametophyte consists of a small (2 mm), simple, essentially two-dimensional thallus with rhizoids, vegetative cells, and sexual organs (archegonia and antheridia). The archegonium is the female organ; it contains one egg that lies at the base of a small neck sticking out from the surface of the gametophyte. The neck consists of four rows of cells, along with a few “neck canal cells” in the middle. The antheridium is the male sex organ; each contains 16 sperm. In the presence of water, the neck canal cells in mature archegonia burst open, creating a small, open canal leading to the egg. The canal cells’ contents are deposited near the top of the open neck.

Meanwhile, the antheridia are also active. In the presence of water they also burst open, discharging motile sperm (spermatozoids). The rapidly swimming sperm are irresistibly attracted by the discharge from the archegonium. In a few minutes, hundreds of sperm can be seen swarming around the neck of the archegonium, and one of them eventually wiggles its way down the neck and fertilizes the egg. After fertilization of the egg, the resulting diploid zygote develops rapidly by mitotic cell division, forming an embryo. Embryos are clearly visible after a few days, and in only 1–2 weeks roots and leaves can be seen on the small diploid sporophytes. The gametophyte soon dies and the sporophyte grows to maturity. It undergoes meiosis and produces spores to continue the life cycle.

The pheromone-like substance, antheridiogen (A_{Ce}), secreted by developing gametophytes controls differentiation of two distinct sexual forms of gametophytes. A_{Ce} is likely biosynthetically related to gibberellins and is effective at extremely low concentrations (Warne and Hickok 1989). In the absence of A_{Ce}, gametophytes develop initially as heart-shaped (cordate) females with archegonia and subsequently

as hermaphrodites with both archegonia and a few antheridia. In hermaphrodites, a defined meristematic region (notch meristem) is present, and growth is indeterminate until fertilization of an egg occurs. Meristematic activity ceases shortly after fertilization. In contrast to hermaphrodites, gametophytes that mature in the presence of A_{Ce} develop into tongue-shaped males that are small, determinate, lack a meristem, and produce large numbers of antheridia. At the vascular sporophyte stage, a *C-Fern* consists of a short upright stem (rhizome) with roots and leaves (fronds) and reaches a height of 10–40+cm. In contrast to many ferns, the *Ceratopteris* sporophyte is not woody and grows rapidly as an annual. Spore production via meiosis occurs within sporangia that are located on the margins of fertile leaves. Upon maturity, spores are produced continually and are unlimited in number. Compared to many ferns, spores are quite large (ca.120 µm) and relatively easy to handle. Because individual haploid gametophytes can be self-fertilized, sporophytes completely homozygous across all genetic loci can be produced in one generation of selfing. Such sporophytes produce an unlimited number of genetically identical spores. If kept dry, spores remain viable for many years.

The following illustrations may be helpful references.
The life cycle. p. 17 C-Fern® Web Manual. Hickock and Warne, 2009.



Spore (1n)

Germination

Gametophytes (1n)
produce gametes by mitosis

Fertilization
Gamete (1n) + Gamete (1n) = Embryo (2n)

Sporophyte (2n)
produce spores by meiosis

Spores. Gametophytes.

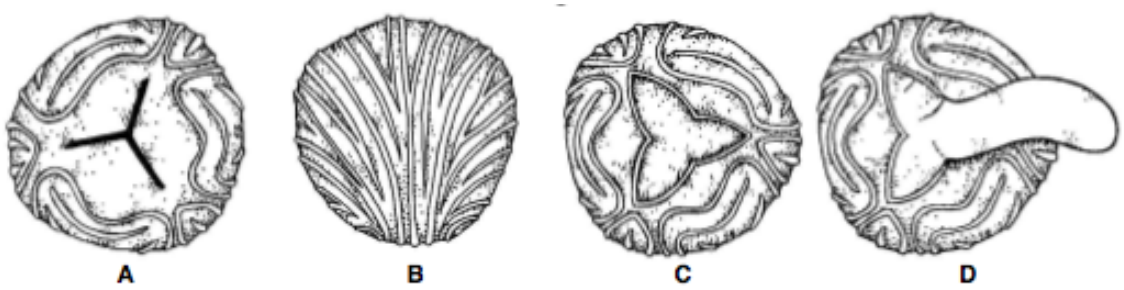


Figure 1. C-Fern spores. A,B — ungerminated, proximal (A) and distal (B) views; C,D — germinated, showing splitting along trilete mark (C) and emergence of primary rhizoid (D). Spore diameter ca. 120 µm.

Figure 2. Young C-Fern gametophyte, 5 days from start (DFS) of culture. Spore coat diameter ca. 120 µm.

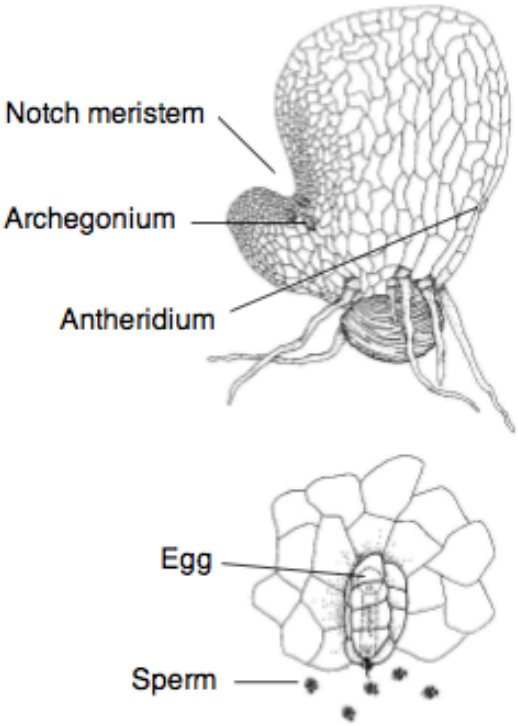


Figure 3. Mature hermaphroditic C-Fern gametophyte with archegonia behind the notch meristem and a single antheridium on the margin, ca. 10 DFS. Spore coat diameter ca. 120 µm. Close-up: view of mature archegonium during fertilization. Sperm enter the open neck canal, uncoil, and move toward the egg.

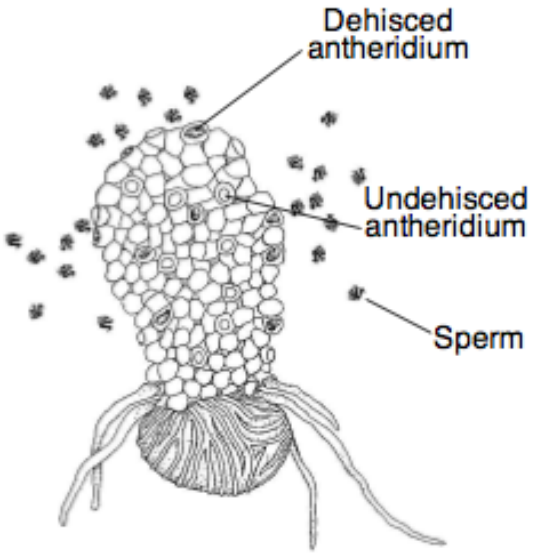


Figure 4. Mature male C-Fern gametophyte, ca. 10 DFS. Sperm are released from the numerous antheridia on the surface of the male gametophyte. Spore coat diameter, ca. 120 µm.

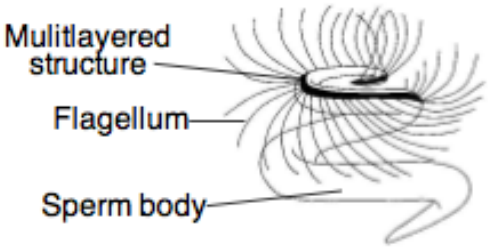


Figure 5. Individual mature sperm, ca. 8.8 × 5.5 µm.

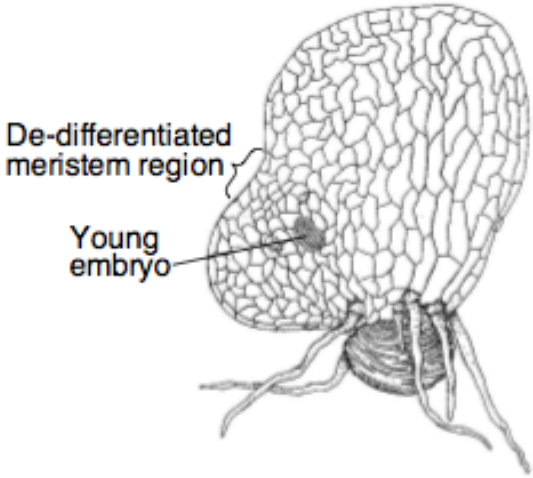


Figure 6. Hermaphroditic C-Fern gametophyte, ca. 3 days after fertilization, showing a young sporophyte embryo. The embryo is covered by proliferated archegonial tissue. Following fertilization, cell division ceases in the notch meristem region of the gametophyte and cells enlarge. As the sporophyte continues to develop, the gametophyte eventually dies. Spore coat diameter, ca. 120 µm.

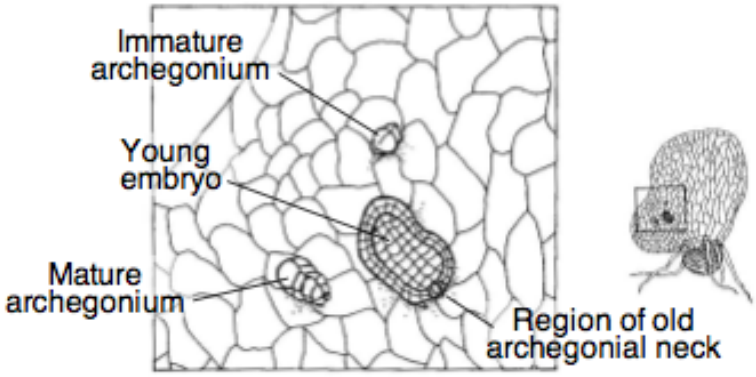


Figure 7. Close-up of hermaphroditic C-Fern gametophyte, ca. 3 days after fertilization, showing unfertilized (immature and mature) archegonia and young embryo developing within proliferated archegonial tissue. The remains of the old archegonial neck can be seen at the distal end of the embryo.

Prepare a Growing System / Monitoring Cultures

A. Growing System:
Three growing system options for C-Ferns are described below. **All require:** a light source, a thermometer, a container/shelf to hold tray of petri dishes, and regular monitoring of temperature.

1. A florescent light bank and bench

2. Growth Pod or Culture Dome (from Carolina Biological)

3. Constructed Light Box (inexpensive materials from office or big box store)

3. Materials and Procedures for Constructing Light Boxes: need 2 boxes per class

- 4 Light Housings: Home Series Incandescent Trouble Light. 15 ft. 18/2 gauge (cord thickness) – unscrew protective head. $6 ea. X 2 = $24
- 4 Plastic File Crates: Sterilite Officeware Legal.Letter 1693 Black (or other color). $5 ea. X 2 = $20
- 4 Fluorescent Screw-In Light Bulbs 15-watt: $2 ea. X 2 = $8
- 1 Roll Aluminum Foil. $3
- Double Sided Scotch Tape

To make the light boxL

1. Line the 3 long sides of each crate with foil using double sided Scotch tape
2. Turn plastic crates on ends, long sides vertical
3. Unscrew protective head of light housings
4. Place light housing on outside of crate, at hole in the middle of "top" of a crate as it stands upended
5. Screw the fluorescent light bulb from the *inside* of the crate into the housing. The plastic crate should be in between the housing and the light.
6. The inside space will be where the C-Fern trays will be placed.
7. Do this for each crate. Plug in the light housings, and you're good to go!

B. Maintaining *C- Fern*® cultures:
For optimal growth, *C-Fern*® requires continuous lighting (24 hours/day, 7 days/week). If using the *C-Fern*® Growth Pod™, the light can be set directly on top of the acrylic lid. If using the Culture Dome, suspend the light over the top of the dome. You can also use a bank of fluorescent lights, or light boxes. The height should be adjusted to achieve a 25-28˚C internal temperature. Use a small, inexpensive thermometer to keep track of the temperature. (Hint: if necessary, a small electric heater can be placed near the cultures to help maintain optimal temperature). It is important to monitor temperature regularly, as temperatures can rise once the Petri dishes are placed in the growing system.

Tyndallization Procedure to Prepare Sterilized Water

1. Fill a 250 mL Erlenmyer flask with 100 mL of tap water and cover the brim with a 5 cm x 5 cm square of aluminum foil to prevent contaminates from the air from entering the vessel.

2. Place flask on a hot plate and bring to a rolling boil; as soon as boiling is reached, turn down heat slightly so that the water is gently boiling. Allow water to boil for 30 minutes. About 30 mL of water evaporates. This kills all vegetative bacterial cells.

3. After 30 minutes of boiling, remove flask from the hot plate and allow the flask to cool until tepid. Place flask in the light bulb heated growth chamber pre-warmed to 30°C. Allow water to incubate at 30°C for 24 hours so that all heat resistant bacterial endospores germinate and become heat-labile vegetative cells.

4. Repeat step 2; another 30 mL of water should evaporate. Incubate the water at 30°C for an additional 24 hours to allow late germinating endospores to form vegetative cells.

5. Repeat step 2 for a third time; after this boiling there should only be approximately 10 mL of water remaining. After 30 minutes of boiling, remove the water from the heat and allow it to come to room temperature before using.

"Sex and the Single Fern"

Conversion of Male to Hermaphrodite Gametophytes in the *C-Fern*®

Description: Students will observe and document the haploid phase of the *C-Fern*® life cycle as spores develop into sexually mature gametophytes. Students will also experiment by altering the chemical environment in their cultures in order to examine certain aspects of sexual differentiation and reproduction in *C-Fern*® gametophytes.

Goals and Objectives: This lab is designed to expose students to sexual reproductive processes in a non-flowering plant. At the end of this study, students will have engaged in scientific inquiry and experimentation and an opportunity to improve data collection and analysis skills. This study allows students to make simple observations and collect manageable data that can be analyzed using simple statistics. More advanced students should be able to make connections between populations and ecosystems and how that might relate to the sex ratios and sexual determination

Time necessary: 4 weeks
Supplies:
Presterilized *C-Fern*® spores (1 vial of wild type strain, RNW*T1*)
30-60 mm plastic petri dishes
Basic *C-Fern*® Medium (large, 400 ml bottle)
Clean cotton swabs
Growth Pod or Culture Dome (or any type of growth chamber where optimal light and temperature can be maintained)

Teacher's Instructions:

- This lab requires some preparation prior to its presentation in class. The medium should be poured into the plastic petri dishes as follows: plates should be poured ¾ full and allowed to cool, completely undisturbed, until the medium solidified. Store at room temperature making sure that the plates remain undisturbed to prevent contamination.

- Students should sketch and note their weekly observations in their lab journals. It's very important that the dates of their observations are recorded as this information is pertinent data analysis.

- Maintaining *C- Fern*® cultures: For optimal growth conditions, C-Fern requires continuous lighting. This can be achieved using a simple utility light with a 15-watt screw in fluorescent bulb. If using the Growth Pod, the light can be set directly on top of the acrylic lid. If using the Culture Dome, suspend the light over the top of the dome. The height should be adjusted to achieve a 28-30°C (82-86°F) internal temperature. Use a small, inexpensive thermometer to keep track of the temperature. (Hint: if necessary, a small electric heater can be placed near the cultures to help maintain optimal temperature).

"The Effects of Moisture on Spore Germination in the *C-Fern*®"

Description: Students will observe and document the effect of moisture on *C-Fern*® spore germination. Students will experiment by sowing spores on sterilized filter paper wetted with different levels of moisture. Germination rates will be counted after 7 d, but the cultures will be allowed to develop to the tenth day for observation and measurement.

Goals and Objectives: This lab is designed to expose students to how living things respond to their environment. At the end of this study, students will have engaged in scientific inquiry and experimentation and will have had an opportunity to collect, organize and analyze data collection and apply statistical methods to the data to reach and support their conclusions.

Time necessary: 4 weeks
Supplies:
Presterilized *C-Fern*® spores (1 vial of wild type strain, RNW*T1*)
30-60 x 15 mm plastic petri dishes
Basic *C-Fern*® Powdered Nutrients (without agar)
Sterilized filter paper (Whatman #1, 5.5 cm)

Teacher's Instructions:

- This lab requires some preparation prior to its presentation in class. The Basic *C-Fern*® Powdered Nutrients should be mixed according to directions but do not add agar, you will want a liquid medium.

- Maintaining *C- Fern*® cultures: For optimal growth conditions, C-Fern requires continuous lighting. This can be achieved using a simple utility light with a 15-watt screw in fluorescent bulb. If using the Growth Pod, the light can be set directly on top of the acrylic lid. If using the Culture Dome, suspend the light over the top of the dome. The height should be adjusted to achieve a 28-30˚C (82-86˚F) internal temperature. Use a small, inexpensive thermometer to keep track of the temperature. (Hint: if necessary, a small electric heater can be placed near the cultures to help maintain optimal temperature).

- Students should sketch and note their weekly observations in their lab journals. It's very important that the dates of their observations are recorded as this information is pertinent data analysis.

"The Effect of pH on Spore Germination in the *C-Fern*®"

Description: Students will observe and document the effect of pH on *C-Fern*® spore germination. Students will experiment by sowing spores on sterilized medium of four different pH levels. Germination rates will be counted days 4 and 7.

Goals and Objectives: This lab is designed to expose students to how living things respond to their environment. At the end of this study, students will have engaged in scientific inquiry and experimentation and will have had an opportunity to collect, organize and analyze data collection and apply statistical methods to the data to reach and support their conclusions.

Keywords and Vocabulary: germination, spore, gametophyte, pH.

Time necessary: 4 weeks
Supplies:
Presterilized *C-Fern*® spores (1 vial of wild type strain, RNW*T1*)
30-60 x 15 mm plastic petri dishes
Basic C-Fern® medium (4 different pH levels: 4.5, 5.5, 6.5, and 8.5)
I-1000 ml flask
4- 400 ml flasks
HCl
NaOH

Teacher's Instructions:

- This lab requires some preparation prior to its presentation in class. Prepare 1000 ml Basic *C-Fern*® medium according to standard protocol. Before adding the agar, the medium will need to be brought to the varying pH levels. Pour equal amounts of media into 4 smaller flasks. Using a pH indicator, adjust the pH of the medium in each flask by adding drops of HCl to make the media more acidic and NaOH to make the media more alkaline.

- Maintaining *C- Fern*® cultures: For optimal growth conditions, C-Fern requires continuous lighting. This can be achieved using a simple utility light with a 15-watt screw in fluorescent bulb. If using the Growth Pod, the light can be set directly on top of the acrylic lid. If using the Culture Dome, suspend the light over the top of the dome. The height should be adjusted to achieve a 28-30°C (82-86°F) internal temperature. Use a small, inexpensive thermometer to keep track of the temperature. (Hint: if necessary, a small electric heater can be placed near the cultures to help maintain optimal temperature).

- Students should sketch and note their weekly observations in their lab journals. It's very important that the dates of their observations are recorded as this information is pertinent data analysis.

"The Effects of Temperature on Spore Germination in the C-Fern®"

Description: Students will observe and document the effect of temperature on *C-Fern®* spore germination. Students will experiment by sowing spores on sterilized filter paper wetted with different levels of moisture. Germination rates will be counted after 7 d, but the cultures will be allowed to develop to the tenth day for observation and measurement.

Goals and Objectives: This lab is designed to expose students to how living things respond to their environment. At the end of this study, students will have engaged in scientific inquiry and experimentation and will have had an opportunity to collect, organize and analyze data collection and apply statistical methods to the data to reach and support their conclusions.

Time necessary: 4 weeks
Supplies:
Presterilized *C-Fern®* spores (1 vial of wild type strain, RNW*T1*)
30-60 x 15 mm plastic petri dishes
Basic *C-Fern®* Medium (large bottle 400 ml)
Teacher's Instructions:

- This lab requires some preparation prior to its presentation in class. The Basic *C-Fern®* Powdered Nutrients should be mixed according to directions but do not add agar, you will want a liquid medium.

- Maintaining *C- Fern®* cultures: For optimal growth conditions, C-Fern requires continuous lighting. This can be achieved using a simple utility light with a 15-watt screw in fluorescent bulb. If using the Growth Pod, the light can be set directly on top of the acrylic lid. If using the Culture Dome, suspend the light over the top of the dome. The height should be adjusted to achieve a 28-30°C (82-86°F) internal temperature. Use a small, inexpensive thermometer to keep track of the temperature. (Hint: if necessary, a small electric heater can be placed near the cultures to help maintain optimal temperature).

- Students should sketch and note their weekly observations in their lab journals. It's very important that the dates of their observations are recorded as this information is pertinent data analysis.