Appendix B
Prep Notes for C-fern Lab

Timeline

1. At least 14 days before the first lab, prepare C-fern medium and pour plates.
2. Pre-germinate 1 vial each of wild type (WT) and *her1* spores 2-3 days before sowing the spores.
3. Sow the spores for population density experiment 10-12 days before the first lab.
4. Prepare the WT and *her1* cultures for the conditioned filtrate 11-13 days before the first lab.
5. Sow the spores for A_{CE} experiment 3-4 days before the first lab.
6. Prepare the conditioned filtrate 1 day before the first lab.

Supplies

C-fern powdered medium, basic (Carolina Biological 15-6782). Each packet of medium makes 1 L. 1 L makes about 80 plates.
Bacto-agar (Sigma-Aldrich A5306)
C-fern basic wildtype spores, presterilized vial (Carolina Biological 15-6728). One vial will be pre-soaked; other vial(s) will have 4 mL sterile distilled water added at time of sowing. We use 110 µL of diluted spore suspension per plate.
C-fern maleless (*her1*) spores, presterilized vial (Carolina Biological 15-6744). One vial will be pre-soaked; other vial(s) will have 4 mL sterile distilled water added at time of sowing. We use 110 µL of diluted spore suspension per plate.
C-fern culture domes (Carolina Biological 15-6792)
Fluorescent light bank (Carolina Biological 97-1944)
BD Falcon cell strainer, 70 µm pore size (VWR 21008-952)
BD 20 mL syringe with Luer-Lok tip (VWR BD309661)
Millex GP filter unit 0.22 µm (Millepore SLGP033RS)
60x15 mm Petri plates. We figure 4 plates/student for both weeks.
Sterile distilled water in 125-mL Pyrex bottles (half full)
1 100-1000 µL pipettor
1-2 Boxes of sterile 1000-µL pipette tips
Sterile 15-mL disposable centrifuge tubes
Sharpie
1 propane burner and striker or matches
1 widemouth plastic jar containing 95% ethanol (to sterilize spreader)
1 L-shaped glass rod (spreader)

Preparing C-fern medium

1. Measure about 900 mL of distilled water into a 2 L beaker.
2. Add contents of C-fern medium packet to the distilled water and dissolve completely.
3. Adjust the pH to 6.0 with freshly made 1.0 N NaOH.
4. Bring volume to 990 mL with distilled water and aliquot 495 mL into two 1000-mL Erlenmeyer flasks (larger flasks are too heavy to pour).
5. Add 5 g of Bacto-Agar to each flask, plug with foam plug and cover with aluminum foil.
6. Autoclave along with the bottles of distilled water and boxes of pipet tips.

Pouring plates

1. The liquid medium should be at about 50°C when pouring the plates (not too hot to hold the flask comfortably). If you have a lot of flasks, it's helpful to keep the flasks in a 50°C water bath so that the medium doesn't solidify while you're pouring the plates.
2. Working in a tissue culture hood, pour plates so that they are ½ to ¾ full.
3. Leave lids off to prevent condensate from forming as media solidifies.
4. When the agar has solidified, plate the spores or put the petri plates back in their sleeves and store them at room temperature.

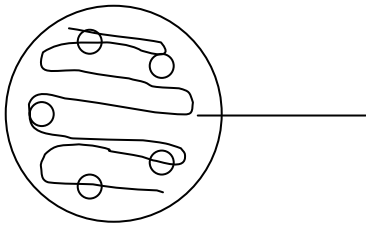
Pre-germinating spores

A portion of the pre-soaked spores will be mixed with the rest of spores (see "Sowing spores") so that there will be hermaphrodites to send out the signal.

1. Working in a tissue culture hood, tap vial to knock spores to the bottom.
2. Add 4 mL of sterile distilled water to each vial. Invert the vial several times to wet spores.
3. Wrap vial in foil to keep spores in the dark.

Sowing spores for population density experiment

1. Working in a tissue culture hood, add 4 mL of sterile distilled water to a vial of WT spores. Mix well to ensure even distribution of spores. 110 µL of this suspension contains approx. 300 spores.
2. To make the 1:2 dilution (approx. 150 spores per 110 µL), transfer these 4 mL plus 1 mL of the pre-soaked WT spore suspension to a 15 mL sterile disposable centrifuge tube and add 5 mL sterile distilled water.
3. Repeat steps 1 and 2 for the *her1* spores.
4. For the population density experiment, make a serial dilution of each spore suspension to prepare 1:4, 1:8, 1:16, and 1:32 dilutions.
5. Plate the spore suspensions on the agar plates as follows.
   a. Using a 1000 µL micropipettor (the spores don't pass through smaller pipette tips), add 110 µL of spore suspension over approx. five spots on the periphery of the petri dish (see diagram below). Make sure to mix spores thoroughly with the pipette right before removing volume since the spores settle very fast.



   b. Flame sterilize a glass spreader (L-shaped rod) and after it has cooled, use it to spread the spores in a zigzag pattern. Spores should be spread as evenly as possible across the plate.
6. Place Petri plates unstacked and unsealed in culture domes under fluorescent light bank. The height of the fluorescent light should be adjusted so that the temperature inside the dome is 28-30°C (about 5 cm above the top of the dome). Cultures should be 10-12 days old at time of experiment.

Preparing cultures for conditioned filtrate

1. Make 1 L of liquid C-fern medium (without the agar)
2. Aliquot approx. 90 mL of medium into each of 4 250-mL Erlenmeyer flasks. Stopper with foam plug, cover with aluminum foil, and autoclave.
3. Add 4 mL of sterile distilled water to 2 WT and 2 *her1* spore vials.
4. After autoclaved flasks have cooled down, add the contents (4 mL) of each WT and *her1* vial to each of the 4 flasks.
5. Place flasks in shaking incubator at 28-30°C with continuous light for 11-12 days.

Sowing spores for A_{CE} experiment

1. Working in a tissue culture hood, add 4 mL of sterile distilled water to a vial of WT spores and a vial of *her1* spores. Mix well to ensure even distribution of spores. 110 µL of this suspension contains approx. 300 spores. It is not necessary to use pre-soaked spores.
2. Make a 1:8 dilution of each spore suspension and add 110 µL to each Petri plate as described previously.
3. Spread spores evenly across agar as described previously.
4. Place Petri plates in culture dome under fluorescent light bank so that temperature inside dome is approx. 28-30°C. Cultures should be 3-4 days old at time of experiment.

Preparing conditioned filtrate for A_{CE} experiment

1. Collect gametophytes from each culture flask (see "Preparing cultures for conditioned filtrate") on 70 µm sieve and filtrate in 50 mL sterile tubes.
2. Working in a tissue culture hood, filter-sterilize filtrate through 0.22 µm sterile syringe filter.
3. Using sterile water, serially dilute the 100% conditioned filtrate to make 33% (1:3), 11% (1:9), 3.7% (1:27), and 1.2% (1:81) filtrate. Store filtrates at 4°C.