The Use of C-Ferns to Study Plasmolysis and Stomata Number

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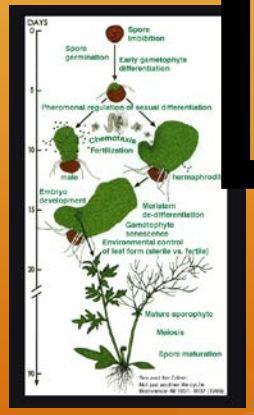
Abstract
Ceratopteris richardii **(C-ferns) have been used to routinely study genetic crosses in our genetics laboratory courses. The hermaphrodites produce a heart-shaped flat, single-cell layer gametophyte and the males produce a club-shaped structure. When a concentrated sucrose solution (10%) is added to these structures, plasmolysis can be observed in a matter of minutes. Since these plant forms are only a single-cell layer thick, they offer a clear, actually easier to view, model to observe and study plasmolysis in plants than the traditionally used *Elodea* leaves, which are two-cell layers thick. Even the next generation of C-ferns, the sporophyte, is one-cell layer thick. These sporophytes make a rosette type clump, but "leaves" can be easily plucked from the multi-leaved structure and make a neat flat sheet on a microscope slide on which plasmolysis (and/or turgor) can be observed. An additional mutant, the polka dot, "naturally" has clusters of chloroplasts in the center of the cell, so adding concentrated solutions does not affect the phenotype. Stomata are also easily observed in these plants. Students can test hypotheses about various conditions that would affect the number of open and closed stomata, for an expanded use of C-ferns in undergraduate laboratory projects.**



C-fern life cycle

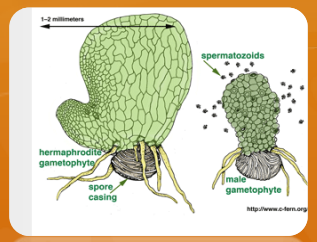
Photo credit: Joshlyn Miensah

Introduction
C-fern spores can be seeded onto a special medium that contains iron salts, and will develop into gametophyte in approximately two weeks. The gametophytes are either hermaphroditic or male. The hermaphrodites can be flooded with water and fertilization will occur. Sporophytes will then arise. The gametophyte tissue is the best to use for this experiment, which involves taking the gametophyte, observing it under the microscope, adding concentrated sucrose or saline, and then observing after ten minutes to observe plasmolysis. This study could serve as the basis for inquiry-based experiments after this initial test. Polka-dot mutants can be used as well to show the students that plasmolysis is difficult to observe in these. The polka-dot mutants have a concentration of chloroplasts in their center, and appear to not change after adding sucrose or saline. Stomata can also be observed---they are the most prominent on the sporophytes. Students could conduct inquiry-based experiments and count open and closed stomata under various conditions.

Materials and Methods
C fern kit from Carolina Biological; includes:
Meet the C-FERN® Kit
Item # 156700
C fern wild type spores
C fern polka dot spores
Small petri dishes with C fern medium
Transfer pipets
Tray with clear cover OR soft vinyl cooler that is open to light
Lamps with any type of white-light bulb
Dissection scopes
Compound scopes
Watchman forceps
Microscope slides
Cover glasses
10% saline and/or sucrose
distilled water
cameras or phones for documenting

Follow instructions with kit for growing C ferns. Usually, this will involve filling a vial of spores with 4 ml of sterile water.
Pipet up and down with transfer pipet to keep spores suspended and evenly distributed.
Put required number of drops on the small petri plate with C fern medium
Let grow to the gametophyte stage for two weeks under lamps in the windowsill. The C ferns will grow a little faster with a temperature slightly above room temperature. You can let them "overgrow" into a sporophyte stage---the leaves of these will still work for the plasmolysis experiment.

Student Outline
Take the gametophye of each type of plant, place on a microscope slide, and observe and take photo. Note that this tissue is only one cell-layer thick. Next, add a drop of distilled water to the edge of the cover glass and let it perfuse the sample. Observe and take photo. Next, add a drop of desired solution (10% sucrose or saline) at the edge of the cover glass and let it perfuse the plant. Observe and take photos. To see stomata, focus up and down---they are in a slightly different focal plane. Count stomata per field of view. What percentage are open? Closed?



Top left picture depicts hermaphrodite gametophyte with associated antheridia and archegonia plus rhizoids. The smaller male is on the right. The actual photomicrograph is depicted below, with the male on the left and the hermaphrodite on the right. The single-cell layer of the hermaphrodite is very apparent.

Selected Results
Students were able to observe plasmolysis more readily with C-fern gametophytes than with *Elodea*. The polka-dot mutants, however, yielded no change. Students were also able to count stomata in a field of view and note numbers of closed and open stomata. They were required to suggest additional experiments that they could conduct using this model in their lab reports.

Wild type C-fern before (a) and ten minutes after adding 10% saline.

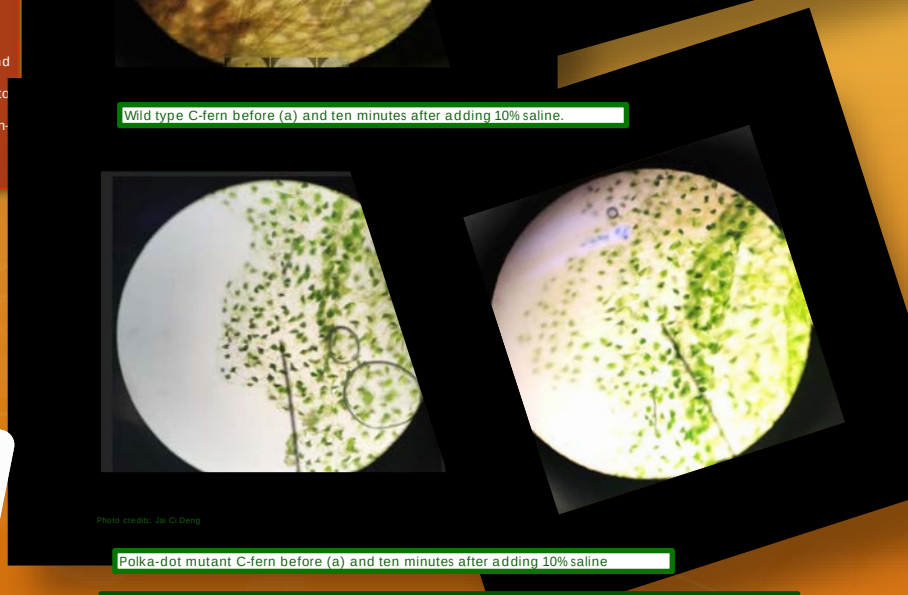


Photo credits: Jia Ci Deng

Polka-dot mutant C-fern before (a) and ten minutes after adding 10% saline

Discussion
The discovery of the C-fern as a tool to demonstrate plasmolysis was serendipitous. I was examining the gametophyte under the microscope and noticed that it was one-cell layer thick. I added some concentrated sucrose, and voila! plasmolysis occurred! With student researchers, we were able to demonstrate that this phenomenon can only easily be observed with the wild type, rather than the polka dot mutant. Both the gametophyte (preferable because it is larger) and the sporophyte can be used. We have introduced this exercise into our second semester General Biology laboratory, in which we have plenty of C-ferns that have been grown by the genetics class to study this model organism. Even though they have covered plasmolysis in the fall, they are then able to revisit it in the spring, and this principle reinforce. This is in addition to learning about the alternation of generations of plants. Stomata are also able to be observed in these plants, and conditions can be induced in which students can then take pictures and count stomata, and, moreover, note whether they are open or closed. We are devising assessments to test the efficacy of using this system to teach about plasmolysis, the importance and utility of stomata, and life cycles in plants.