C-Fern® Lab Part 3

BIOL 202 LAB 7: C-Fern® Investigations: Genetics in Action Mendelian Genetics
(Note that this document contains instructions and information for observing the C-Fern® spores, which were sown in the previous lab.)

Equipment, Supplies & Materials:
- Petri dishes containing nutrient agar upon which presterilized C-Fern® spores of an F₁ hybrid were sown
- Sterile distilled water, 1-2 mL per Petri dish
- Sterile pipets
- Clean dry Petri dish lids, if available (for use in scoring gametophytes as described in the procedure section).
- C-Fern® culture instructions and background information (Hickok & Warne, 1998a)—instructor's copy
- C-Fern® Investigations: Genetics in Action Mendelian Genetics Student Instructions (Hickok & Warne, 1998b)—one copy per student
- Compound and dissecting stereomicroscopes

Activities:
- Overview of Lab 7: Visualization of basic principles of Mendelian inheritance in C-Fern® by following the segregation of a visible marker, polka dot, in both the F₁ gametophyte and F₂ sporophyte generations. Students sow spores of an F₁ hybrid (wild type x polka dot) to produce F₁ gametophytes. Sporophytes also may be observed.
- YouTube video presentation on the life cycle of mutant (Polka Dot) and wild type (normal) C-Ferns from spore to sporophyte (Casale, 2012)
- Follow procedure as described in lab instructions (and as indicated by instructor)

Assignment:
- Lab Report #7 (final version to be submitted to instructor the Friday following conclusion of this lab at 5:00 pm, following editorial review by the Science Writing Mentor)
**C-Fern® Lab Part 3**

**Procedure** (Adapted from Hickok & Warne, 1998b, pp. H-4-H-5.):

(Note: This procedure describes the addition of sterile water to your cultures in order to achieve fertilization of eggs by sperm. It also includes directions for observing your cultures prior to and after adding the sterile water. Please read through the instructions carefully before proceeding, and determine whether you may wish to do all your observations and counting prior to adding the sterile water, with the exception of observing fertilization events following the addition of water.)

1. **Observation of C-Fern® Cultures**—Observe the cultures using both dissecting and compound microscopes at various levels of magnification as necessary.

2. **Draw and Label Your Observations**—Draw and label each different thing that you can observe in your Petri dish, including any new developments in your cultures. Based on your observations, labelled drawings, and the lecture content associated with this lab, please answer the following questions:
   - **Q1:** What things are different from last week?
   - **Q2:** What type of cell division took place during the growth of gametophytes?

3. **Addition of Water to Cultures**—While you are observing the culture, tilt the lid up and use a sterile pipet to add 1-2 mL sterile distilled water. Lower the lid and tilt the plate back and forth to cover all of the gametophytes with water. Observe the release of swimming sperm (or spermatozooids) from antheridia and their attempts to find and fertilize mature eggs within archegonia (Figures 1 and 2).
   - **Q3:** Do all the gametophytes have the same phenotype? Describe any differences you observe.
   - **Q4:** Did you observe differences in sexual phenotypes as well as in the other visible phenotype?

4. **Gametophyte Types**—For this experiment we will focus only on the larger, heart-shaped hermaphroditic gametophytes. The smaller, tongue-shaped ones are males (Figure 3).
   - **Q5:** Which of the phenotypes would you designate as a mutant (Figure 4)? Why?

5. **Sampling and Counting**—Take a random sample of the gametophyte population in each dish by counting up to 50 individuals and identifying their phenotypes. Record your data in Table 1 below. During this procedure the lid should be left on the Petri dish if possible. If it becomes fogged, a "clean" lid from an unused dish may be quickly exchanged for the old one. After scoring, replace the old lid over the culture.

Because of variations in technique during inoculation, some dishes may have fewer than 50 scorable gametophytes. Data should still be collected from the available gametophytes and used individually and as part of the pooled class data.

<table>
<thead>
<tr>
<th>Date Data Recorded:</th>
<th>Age of Cultures:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description of Phenotypes</td>
<td>Number of Gametophytes</td>
</tr>
<tr>
<td></td>
<td>Dish 1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Q6:** Why is it important to take a random sample from the cultures?
Q7: What would be a suitable method of taking data that would insure a random sample?
Q8: Can you determine anything about the pattern of inheritance from the data in Table 1?
Q9: Are the plants in the culture haploid or diploid? (See Figure 5.)
Q10: Can you identify the reproductive structures that are present?

6. **Draw and Label Any Additional Observed Structures**—Using your existing drawings that you made earlier in this lab, use these terms to label structures that you observe in the cultures: *antheridium, archegonium, meristem, rhizoid, and spore coat.* (Note: You may have already provided some of these labels based on your earlier observations. If not, please take this opportunity to further develop and label your drawings. Also note that Figures 5-8 may be of assistance in this step.)

7. **After Observation of C-Fern® Cultures**—When you are finished with your observations, remove any excess water from the culture by lifting the lid slightly and pouring off the excess. When you are finished with the culture, place it back into the culture dome under the lights. Be sure the Petri dish lid is in place.

Q11: What products will result from the fertilization events that you observed? Will they be haploid or diploid?

**Prediction**—Predict the genetic outcome of the fertilizations by formulating a hypothesis to explain the inheritance of the trait. Use the symbols *d* and *D* for the mutant and wild-type states, respectively (as indicated below under C-Fern® Polka Dot Mutation Notes). Based on your hypothesis, diagram the *F*₁ and *F*₂ generations, showing all genotypes and phenotypes. Indicate expected ratios for both the gametophyte and *F*₂ sporophyte generations.
Q12: When and how could your hypothesis be tested?
Figures:

Figure 1. C-Fern® gametophytes—hermaphrodite and male—showing release of sperm (spermatozooids) (Fern_babies, n.d.).

Figure 2. Fern sperm (spermatozooids) (Houseman & Ford, 2014).

Figure 3. C-Fern® gametophytes, male (left) and hermaphrodite (right) (Gametophytehermandmalesstandard, n.d.).

Figure 4. C-Fern® gametophytes, wild (above) and mutant (below) (Gametophytestumbletypeandpolkadot, n.d.).
Figure 5. C-Fern® life cycle (reproduced as Figure 10.I.1 from Hickok & Warne, 1998c).
Figure 6. C-Fern® reproductive details (Hickok, L., Warne, T., & Duncan, n.d.).
Figure 7. C-Fern® hermaphroditic gametophyte, showing antheridia and archegonia (Fern Gametophyte, n.d.).

Figure 8. C-Fern® hermaphroditic gametophyte, showing apical notch (meristem) (Hermaphroditic Gametophyte, n.d.).
C-Fern® Lab Part 3

C-Fern® Polka Dot Mutation Notes:

1. **C-Fern® Polka Dot Mutant Gene Alleles**—The strain of C-Fern® used in this laboratory has both normal and mutant alleles associated with the Polka Dot mutant as follows ("Are There Different Kinds," n.d.; "You Will Be Following," n.d.):

<table>
<thead>
<tr>
<th>Allele</th>
<th>Contribution to Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal: D</td>
<td>normal distribution of chloroplasts – dominant phenotype</td>
</tr>
<tr>
<td>mutant: d</td>
<td>chloroplasts clumped (polka dot) – recessive phenotype</td>
</tr>
</tbody>
</table>

The Polka Dot phenotype is visible in both haploid and diploid forms of the fern (except in the spores, eggs, and sperms). As a result:

<table>
<thead>
<tr>
<th>For haploids: (F₁ gametophytes)</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>polka dot</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>For diploids: (F₂ sporophytes)</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>Dd</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>dd</td>
<td>polka dot</td>
<td></td>
</tr>
</tbody>
</table>

8. **Phenotype Considerations**—Based on the normal (wild type) and mutant (Polka Dot) alleles in the strain of C-Fern® used in this laboratory, and given what is know about dominant and recessive alleles as related to basic Mendelian genetics, we may expect the following: The F₁ sporophyte will produce gametophytes in a 1:1 ratio of Polka Dot mutants to normal (wild) type. The recessive mutation results in a 3:1 ratio of the F₂ sporophyte generation ("C-FERN Spores, F1 Polka," 2016). Based on your observations and the lecture content associated with this lab, please answer the following questions:

a. If you were to count 50 individual F₁ gametophytes, how many normal and how many mutant individuals would you expect to count? Why?

b. If you were to count 50 individual F₁ sporophytes, how many normal and how many mutant individuals would you expect to count? Why?
References

You will be following the development of a fern. (n.d.). Retrieved from Course Hero, Inc. website: https://www.coursehero.com/file/p2jtddj/You-will-be-following-the-development-of-a-fern-Ceratopteris-richardii-c-fern/