

Lab 5: Genetics in Action: *Ceratopteris*

I. Framing the Investigation:

Background:

During the late 1800s, Gregor Mendel, spent many years investigating the inheritance of traits in pea plants. For Mendel, even though he could raise two generations of peas in a growing season, the experiments conducted in his garden plot in a monastery at Brünn (now Brno in the Czech Republic) often lasted for several years. His success was dependent upon careful recordkeeping, painstaking analysis, and a keen eye for detail. As a result of his efforts, the basic rules of what is now known as Mendelian inheritance were formed. Fortunately, we can now look at various aspects of Mendelian inheritance in organisms that allow a more rapid progression through their life cycle. In this exercise, C-Fern will be used to investigate some of the basic principles of inheritance that Mendel studied with peas.

Purpose:

- Use a sampling procedure to determine segregation ratios of a visible trait in gametophytes produced from spores of an F1 hybrid.
- Describe mutant and wild-type phenotypes.
- Observe sperm release and fertilization events that lead to formation of an F2 sporophyte population.
- Form hypotheses about the genotypic and phenotypic ratios in the F2 sporophyte generation.
- Test the hypotheses by analyzing ratios using the chi-square statistic.
- Develop skills with stereo- and/or compound microscopes.

Hypothesis:

Using variable assigned, develop a hypothesis, using the **If**, and, **then** concerning the observed and predicted phenotypic ratios for the gametophyte and sporophyte generations.

II. Designing the Investigation:

Materials: Below make a list of materials used.

Procedure:

- **First Period (Week 1)**

Prior to this class, petri dishes containing Basic C-Fern Medium were inoculated with spores from an F1 hybrid C-Fern plant. (If this has not been done for you, your instructor will tell you how to sow your own plates.) Label the plates provided to you with your name and the inoculation date. Observe the cultures using the highest magnification on a stereomicroscope with transmitted light (i.e., from below). Answer the questions in the **Analyzing and Interpreting Results** section.

Step 1: Answer question 1-3

When you are finished with your observations, place the cultures back in the culture dome under the lights.

- **Second Period (Week 2)**

Observe the cultures under a stereomicroscope. Answer the questions in the **Analyzing and Interpreting Results** section. Record your data in Table 1 in **Collecting and Presenting Data** section.

Step 1: Answer question 4

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 from antheridia and their attempts to find and fertilize mature eggs within archegonia.

For this experiment we will focus only on the larger, heart-shaped hermaphroditic gametophytes. The smaller, tongue-shaped ones are males.

Step 2: Answer question 5

Take a random sample of the gametophyte population in each dish by counting up to 50 individuals and identifying their phenotype. Record your data in Table 1. 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.

Step 3: Answer question 6

Step 4: Answer question 7

- **Third Period (Week 3 or 4)**

Observe your cultures under the stereomicroscope.

Step 1: Answer questions 8-9

Take a random sample of the sporophyte population in each dish by counting 5 up to 50 individuals and identifying their phenotype. For this part of the experiment the lid may be removed from the culture. It may be easier to score the phenotype after gently and randomly pulling up individual sporophytes with a dissecting needle or toothpick and laying them out in a row on empty areas of the culture plate. Observe the largest leaf on each sporophyte, using a stereomicroscope and reflected light from the side or top. Examine the differences carefully before recording data. The small leaves on each sporophyte should not be scored. These may appear to be uniformly green regardless of their genotype. This is because the polka-dot phenotype is not clearly visible in leaves until they have fully expanded. Record your data in Table 2. Following scoring of phenotypes, the lid may be placed back on the plate and the culture returned to the culture dome or taken home for observations over the next several weeks to determine if the phenotype is apparent on older sporophytes without the use of a microscope.

III. Collecting and Presenting Data:
Results:

Date Data Recorded	Age of Cultures:					
Description of Phenotypes	Number of Gametophytes					% of ALL Classes
	Quad 1	Quad 2	Quad 3	Quad 4	TOTAL	
Wild Type +						
Polka Dot						

Table 1: Gametophyte phenotypes

Date Data Recorded	Age of Cultures:					
Description of Phenotypes	Number of Sporophytes					% of ALL Classes
	Quad 1	Quad 2	Quad 3	Quad 4	TOTAL	
Wild Type +						
Polka Dot						

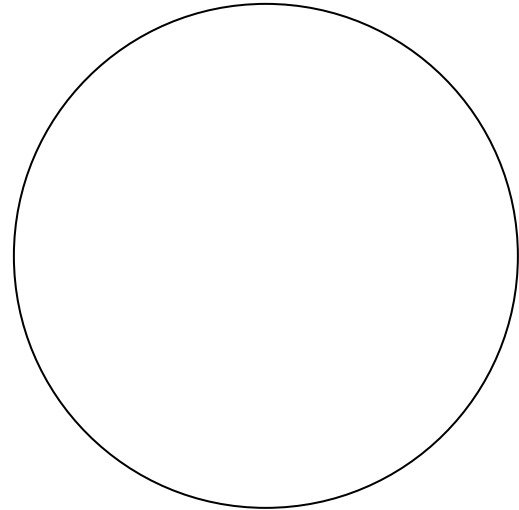
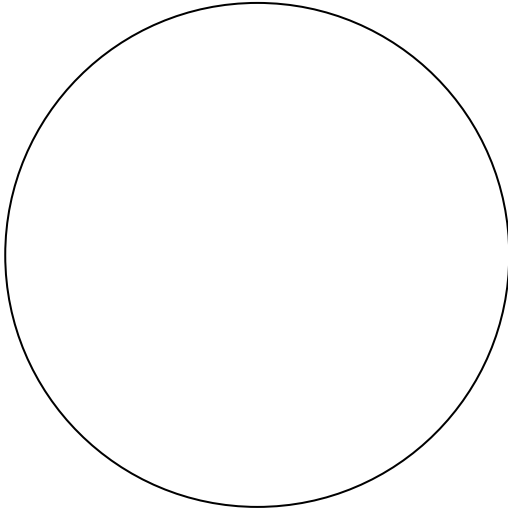
Table 2: Sporophyte phenotypes

Graph Data for Table 1 and 2:

For these graphs a pie graph is best!

Graph 1 Title: _____

Graph 2 Title: _____



Legend:

Legend:

IV. Analyzing and Interpreting Results:

Analysis Questions for each Period:

• **First Period (Week 1)**

Q1. Do some of the spores show signs of germination? What is the first visible structure in germinated spores?

Q2. Are the spores haploid or diploid?

Q3. If the F1 hybrid sporophyte were heterozygous for a single mutant trait, what genotypes would be present in the spores? What would be the expected ratio of genotypes?

- **Second Period (Week 2)**

Q4. Do all the gametophytes have the same phenotype? Describe any differences you observe.

Q5. Which of the phenotypes would you designate as a mutant? Why?

Q6. Are the plants in the culture haploid or diploid?

Q7. When and how could your hypothesis be tested?

Third Period (Week 3 or 4)

Q8. What things are different from last time? Can you observe any new structures showing the mutant and wild-type phenotypes?

Q9. Are the young sporophytes haploid or diploid? Why?

Conclusion:

1. The purpose of this exercise was to demonstrate some basic principles of inheritance. One of the principles derived from Mendel's work was the Law of Segregation. Did you observe segregation in this experiment? At what stage did it occur?

2. Most genetic predictions are based on the expectation that gametes unite at random to form zygotes. Did you observe what appeared to be random fertilization? When did it occur?

3. Mendel also observed dominance and recessiveness of characters. Were these observed in your experiment? Were they observed equally in both gametophytes and sporophytes? How did you determine which character was dominant and which was recessive?

4. If gametophytes had not expressed the phenotype, would you have been able to form a hypothesis from observations of the gametophyte generation? Why or why not?

V. References:

C-Fern: A Plant for Teaching and Research, <http://www.c-fern.org/>

VI. TEKS/TAKS:

TEKS: 1A, 1B, 2A, 2B, 2C, 2D, 3A, 3F, 5A, 5B, 6A, 6D, 6E, 11B, 13A, 13B

TAKS: 1,2, 3