Play Mendel Advanced

Summary: This module guides students through the process of investigating reference and mutant strains of Arabidopsis to learn about Mendelian genetics. Through these activities students will learn about concepts such as growth and development, anatomy, variation, segregation, inheritance, phenotypes and genotypes. An expanded version of this module is available in the *American Biology Teacher* article, Following phenotypes: An exploration of Mendelian genetics using Arabidopsis plants (Price *et al.*, 2018).

Recommended Grade Level: Middle and high school

Duration: Full module requires 30 weeks for completion of all planting procedures and assignments. For teachers looking for a shorter demonstration of Mendelian genetics, consider Play Mendel Basic, which only requires six weeks of grow time, and student activity that can be completed within a single class period.

Learning Objectives

Through this module students will:

- Define concepts and terms associated with Mendelian genetics, the growing process, and plant anatomy
- Plant, care for, and perform genetic crosses with multiple strains of Arabidopsis
- Make observations, compare phenotypes, and illustrate growth stages of mutant and reference strains of Arabidopsis
- Collect and analyze data
- Determine the inheritance of two unique traits
- Use evidence to support findings

Alignment with Next Generation Science Standards

NGSS	
Standards	-Heredity: Inheritance and Variation of Traits (MS-LS3-1, MS-LS3-2, HS-LS3-3) -Biological Evolution: Unity and Diversity (MS-LS4-4)
Science & Engineering Practices	-Developing and using models -Constructing explanations and designing solutions -Analyzing and interpreting data
Disciplinary Core Idea	-Inheritance of Traits -Variation of Traits -Natural Selection
Crosscutting Concepts	-Structure and function -Cause and effect -Scale, proportion, and quantity -Science is a human endeavor

Supporting Resources

The following supporting resources are available for download from the ABRC website:

- Play Mendel Protocol Video
- Student handout: Laboratory procedures & assignments
- Grading rubric
- Greening the Classroom Terms & Concepts
- Growing Arabidopsis in the Classroom

Materials
4 strains of Arabidopsis seeds (See seed strain details below)
Potting soil
14-14-14 fertilizer (*e.g.,* Osmocote)
64 plastic pots (Recommended size: 1 quart round pots, 4.7"d x 4.75"h)
8 solid trays (Suggested product - Hummert, Item #11-3050-1)
8 trays with holes for sub-irrigation (Suggested product - Hummert, Item #11-3000-1)
Cheesecloth or paper towels
Weighing boats
Disposable Pasteur pipettes
Labeling tape and marker
Plastic wrap
Scissors
Headband magnifier (Suggested product - Lehle Seeds, Item #DA-10)
Tweezers (Suggested product - Lehle Seeds, Item #DV-30)
Eppendorf tubes (Suggested product - Fisher Scientific, Item #05-408-138)
Plastic bouquet sleeves (Recommended: 24.6"h, 18"w at top, 6"w at bottom)
Envelopes (Recommended size: #10 envelopes)
Fine metal sieve (Suggested product - Fisher Scientific, Item #04-881-5R)
Watering can
Lab notebook
Growth space with fluorescent lights

Seed Strain Details

- **Columbia** (Col-1, Catalog # CS28169) – This laboratory strain of Arabidopsis is closely related to Col-0, whose genome has been completely sequenced. Col-1 has been used to generate many mutants, and serves as the reference strain for the *gl1-1* mutant used in this module.

- ***gl1-1*** (Catalog # CS28175) – This strain is homozygous for a mutation in the *GLABROUS1* gene which encodes for a protein involved in trichome (leaf hair) formation. The corresponding reference strain, Col-1, has trichomes on its stem and leaves. The *gl1-1* mutant is glabrous (bald), with very few or no trichomes present on the stem and leaves.

- **Landsberg erecta** (Ler-0, Catalog # CS20) – This laboratory strain contains an X-ray induced mutation in the *ERECTA* gene, which causes the plants to have a more upright growth habit. Ler-0 is widely used to generate mutants, and serves as the reference strain for the *ag-1* mutant used in this module.

- ***ag-1*** (Catalog # CS25) – This strain is heterozygous for a mutation in the *AGAMOUS* gene, which encodes a protein involved in the production of floral organs (sepals, petals, stamens and carpels). The reference strain for this mutant has flowers with all four organs present. In the *ag-1* mutants, the stamens and carpels have been replaced by petals and sepals to produce a "double" flower. The term agamous means "asexual", which represents the phenotype of the mutant plant which is sterile.

Background Information

Arabidopsis thaliana (Arabidopsis) was the first plant to have its genome completely sequenced. Although technically a weed, this plant has been transformed into an important model system for plant research, and a useful tool in teaching a variety of science concepts in K-12 and college level instruction. Arabidopsis is member of the Brassicaceae family and is related to a number of common food plants including cabbage, radish and cauliflower. It is a small, relatively easy to grow plant with a fast life cycle, going from seed to mature plant in six to eight weeks.

This module explores how you can use different strains of Arabidopsis to teach Mendelian genetics. Gregor Mendel, an Austrian monk who is generally considered to be the "father of genetics", discovered basic principles of heredity through experimentation with pea plants. Mendel's laws include:

- The Law of Segregation
    - In most cells, genes occur in pairs. Each of the two copies of the gene is called an allele. During gamete formation, the two alleles separate resulting in gametes with only one allele for each gene.

- The Law of Independent Assortment
    - Alleles for one trait separate and are passed on to offspring independent of the inheritance of alleles for other traits.

- Mendel also demonstrated that a trait can be recessive or dominant. Recessive traits are displayed only when both alleles are recessive. Only one dominant allele must be present for a dominant trait to be displayed.

Through this module, students will grow four strains of Arabidopsis and analyze the inheritance of two traits to learn about the concepts of variation, segregation and inheritance discovered by Mendel.

Schedule of Procedures and Assignments

Week	**Activity**
Week 1	Procedure 1 - Plant P generation seeds
Week 2-5	Assignment 1 – Observe growth and record phenotypes Water plants
Week 6	Assignment 1, continued - Observe growth and record phenotypes Assignment 2 - Analyze inheritance of the *ag-1* allele Procedure 2 - Perform a genetic cross
Week 7	Assignment 3 - Observe cross outcomes Water plants
Week 8-9	Do not water – Allow plants to dry for seed collection
Week 10	Procedure 3 - Collect F1 seeds
Week 11-12	No activity - Dry F1 seeds
Week 13	Procedure 4 - Plant F1 seeds
Week 14-15	Water plants
Week 16	Assignment 4 - Observe and record phenotypes Water plants
Week 17-19	Water plants
Week 20-21	Do not water - Allow plants to dry for seed collection
Week 22	Procedure 5 - Collect F2 seeds
Week 23-24	No activity – Dry F2 seeds
Week 25	Procedure 6 - Plant F2 seeds
Week 26-28	Water plants
Week 29	Assignment 5 - Analyze segregation for F2 generation plants
Week 30	Assignment 6 - Formulate next-step research questions

Laboratory Procedures & Assignments

PROCEDURE 1 – Plant P generation seeds

1. Prepare 64 pots for planting. Cut pieces of cheesecloth or paper towel to fit the bottom of a pot. Place one piece in the bottom of each pot to prevent soil from escaping during watering.

2. Place potting soil in a container and add water to moisten. The moisture level of the soil should resemble a wet sponge. Add fertilizer according to package directions. Thoroughly mix soil for even distribution of water and fertilizer. Wear gloves when handling fertilizer and fertilized soil.

3. Fill each pot loosely with soil. Do not compress the soil as you fill the pots as that will limit aeration.

4. Stack one tray with drainage holes inside a solid tray. From this point forward, this pair of stacked trays will be referred to simply as a tray.

5. Place eight pots within each tray.

6. Divide the class into two groups. Each group will plant four trays, two for each experiment. Using labeling tape and a permanent marker, label each tray with your group number, the date and the name of the experiment (see examples below).

Group 1 - Date Glabrous Experiment	Group 1 - Date Agamous Experiment

7. Label eight pots for each reference strain (Col-1 and Ler-0) and eight pots for each mutant strain (*gl1-1* and *ag-1)* (see examples below).

Group 1 Col-1	Group 1 *gl1-1*	Group 1 Ler-0	Group 1 *ag-1*

8. Seeds are planted individually on top of the soil. To start, fill a weighing dish with water. Working with one seed stock at a time, sprinkle a portion of the seeds of one stock into the water. Mix the seeds in the water by pipetting up and down slowly using a disposable Pasteur pipette. This will help to separate the seeds and make it easier to capture them individually for planting. Be sure to use a different weighing dish and pipette for each stock to prevent cross-contamination.

9. Use the pipette to draw up individual seeds and place them on the surface of the soil. Plant nine seeds, evenly spaced, in each pot (Figure 1). Do not cover the seeds with soil.

10. Once planting is complete, place four reference strain pots (Col-1 or Ler-0) and four of the corresponding mutant strain pots (*gl1-1* or *ag-1*) in each tray. Wrap each tray tightly with plastic wrap to maintain moisture levels during germination.

11. Optional – If space is available, place all of the trays inside a cold room or refrigerator at 4°C for 2-3 days. This process, known as stratification, mimics winter conditions and promotes uniform germination of the seeds. Skip this step if you do not have access to adequate refrigeration space.

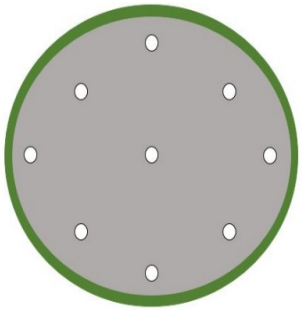

Figure 1. Placement of 9 seeds on soil surface (Price *et al*., 2018).

12. Place the trays under fluorescent lights (see Growing Arabidopsis in the Classroom for lighting suggestions). If the soil was prepared with adequate moisture, you should not need to water your pots while they are covered with plastic wrap.

13. Remove the plastic wrap once you see seedlings emerge from the soil (approximately seven days after planting).

14. Once the plastic wrap has been removed, you will begin watering the plants regularly. Do not water directly into the pots. Add water to the tray to a depth of ½ inch once or twice a week. Be careful not to overwater the pots or allow the soil to dry out.

ASSIGNMENT 1 – Observe growth and record phenotypes

Since Arabidopsis has a fast life cycle, students will be able to observe morphological changes over a relatively short period of time. After germination, students should observe plants on a weekly basis and complete the following tasks:

1. Define key terms related to plant growth and anatomy such as rosette, inflorescence, silique, stratification, trichome, germination, bolting, and senescence.

2. Make detailed drawings of the plants noting any visible differences between the four different strains.

3. Describe the Arabidopsis life cycle by identifying details about and onset of various growth stages such as the number of leaves present in the rosette, flowering, development of siliques, and senescence.

4. Identify the unique traits that differentiate each mutant from its corresponding reference strain (Figure 2). Describe the traits using drawings and notes. Make a note of when each trait was first noticeable (*e.g.*, in rosette stage or after flowering).

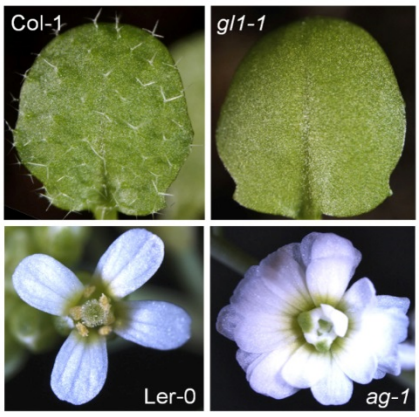

Figure 2. Phenotypes associated with Col-1, *gl1-1*, Ler-0, *ag-1* (Price *et al.*, 2018).

ASSIGNMENT 2 – Analyze the inheritance of the *ag-1* allele

For the agamous experiment, students planted a segregating (heterozygous) population of the *ag-1* mutant. In this experiment, the reference plant has a typical flower with four petals and all reproductive organs. In the mutant plant, the male reproductive organs are absent, having been replaced with a second whorl of petals creating a "double" flower. In this assignment students will analyze the inheritance of the *ag-1* allele by completing the following tasks:

1. Collect phenotypic data (Student Handout, Agamous Worksheet, Table 1) to analyze the inheritance of the *ag-1* allele. Remember, there are multiple plants in each pot and care should be taken to accurately identify each individual plant.
    a. Count the number of plants with flowers displaying the reference phenotype.
    b. Count the number of plants with flowers displaying the mutant phenotype.
    c. Calculate the ratio of plants with reference to mutant flowers for your group.

2. Make a prediction about how combining the data from both groups in the classroom may affect the ratio determined by each individual group.

3. Add the data from both groups and calculate the ratio again. Use the student worksheet to answer the following questions:
    a. Did aggregating the data from both groups cause the ratio to change?
    b. If so, explain how and why the ratio changed.
    c. Why is this important to the process of science?

4. Based on the ratio your class obtained, conclude whether the *ag-1* allele is dominant or recessive.

5. Support your finding with evidence by completing a Punnett square (Student Handout, Agamous Worksheet, Table 2) and answering the corresponding questions.
6. Define key genetic terms such as genotype, phenotype, dominant and recessive.

NOTE: At this point, the *ag-1* and Ler-0 plants can be discarded. Pots and trays can be disinfected for reuse. To disinfect materials, add ¼ cup Lysol to one gallon of water and soak for 10 minutes. Use a scrub brush to remove any soil residue or plant material then rinse and allow to air dry.

PROCEDURE 2 - Perform a genetic cross between *gl1-1* homozygous mutants and the Col-1 reference strain.
Arabidopsis is a self-pollinating plant. Due to morphological characteristics of stamens and carpels, by the time a flower is open pollination has already occurred. To avoid self-pollination, crosses must be done using a closed bud with barely visible petals at the very tip. This closed bud will become the female parent plant.

Performing a cross with Arabidopsis is a delicate procedure that requires patience. Students should start with a basic understanding of flower anatomy. If students are struggling with this concept, or the procedure overall, it may help to demonstrate the process using a species of flower with more obvious anatomy such as a lily. Before starting this procedure, have students watch the portion of the Play Mendel video that demonstrates how to perform a cross. Students will be performing two types of crosses in this procedure, first pollen from a *gl1-1* flower will be used to pollinate a Col-1 flower (Col-1 x *gl1-1*). Next, pollen from the Col-1 flower will be used to pollinate a *gl1-1* flower (*gl1-1* x Col-1). Note, when labeling a genetic cross the female parent is always listed first. These plants represent the P generation.

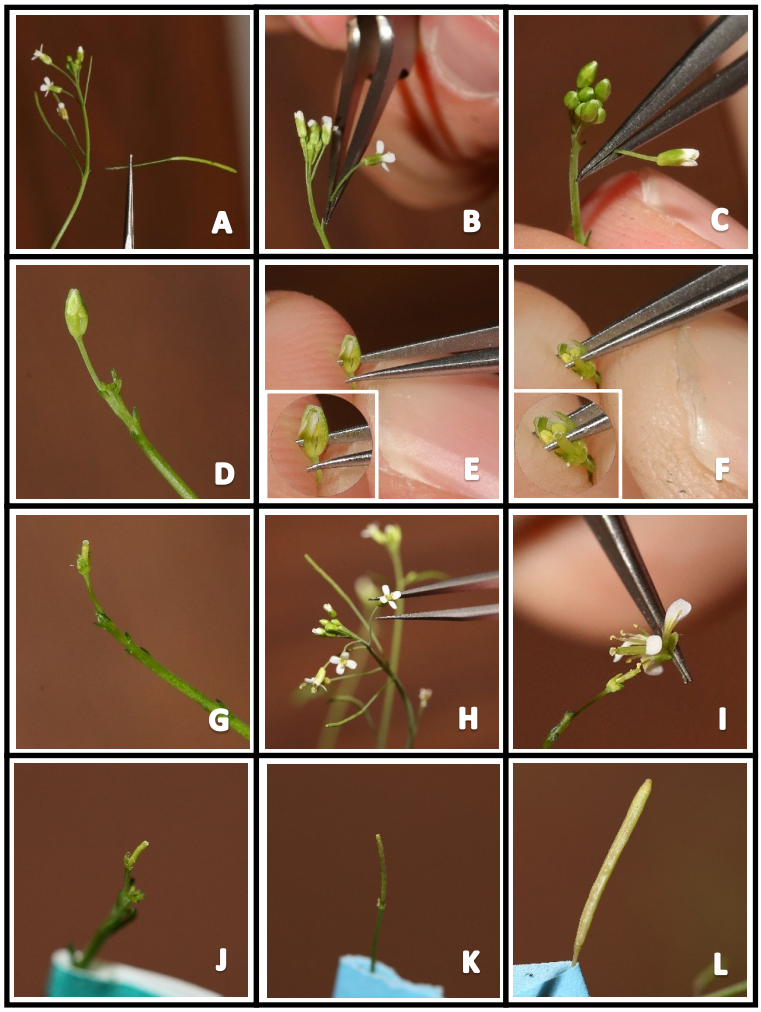

Figure 3. Steps involved in performing a genetic cross between two parent plants (Price *et al.*, 2018).

1. Working with a pot of Col-1 plants, select an inflorescence with at least two closed buds with barely visible petals at the tip (Figure 3A).

2. Carefully remove any siliques, partially open buds and flowers from the selected inflorescence (Figure 3 A-D).

3. Using tweezers and a headband magnifier, remove the sepals, petals and stamens from one of the selected buds (Figure 3E-G). Try to avoid damaging the carpel. If you do, discard that bud and start over with another bud.

4. Select a fully open flower on the male parent plant (*gl1-1*). Using tweezers, remove the flower from the plant, squeezing at the base of the flower to expose the anthers (Figure 3H).

5. Pollinate the female parent plant by brushing the anthers of the *gl1-1* flower over the carpel of the emasculated Col-1 flower (Figure 3I). Make sure to transfer some pollen to the stigma during this process.

6. Use tape to label your cross (see example below). Place tape loosely around the inflorescence below the exposed carpel (Figure 3J).

Group 1 - Date
Col-1 x *gl1-1*

7. Repeat steps 1-7 using *gl1-1* as the female parent plant. Label crosses accordingly (see example below).

Group 1 - Date
gl1-1 x Col-1

8. Observe the plants daily for signs of a successful cross. Continue to water plants for one week after performing crosses.

9. One week after performing the crosses stop watering the plants. Allow the vegetative material to dry out for two weeks.

ASSIGNMENT 3 – Observe cross outcomes

After performing the genetic crosses, students should observe the plants daily for signs of a successful cross. Students should be able to tell within 1-2 days if a cross was successful. If the cross was successful a silique will form (Figure 3K-L). During this time, students should complete the following tasks:

1. Observe plants daily and make notes on the outcomes of each of the crosses performed.

2. Draw the results of a successful and unsuccessful cross.

3. Calculate the ratio of successful to unsuccessful crosses for the class.

4. Draw the anatomy of a flower and define the role of structures involved in reproduction such as anther, filament, stamen, stigma, style, ovary, ovule, and carpel.

PROCEDURE 3 – Collect F1 seeds

After allowing the plants to dry out for two weeks the silique color should change from green to yellow-brown. It is now time to collect the F1 seeds.

1. Working with the Col-1 x *gl1-1* crosses first, locate all successful crosses.

2. Label a 2 mL Eppendorf tube with your group number, date, plant number, generation and cross type (see example below). Prepare one 2 mL Eppendorf tube for each silique to be collected.

Group 1, date, plant #
F1: Col-1 x *gl1-1*

3. Use scissors to carefully remove each silique from the plant and place in an Eppendorf tube (1 silique/tube). Gently tap the tube on a table several times to release the seeds.

4. Repeat steps 1-3 for the *gl1-1* x Col-1 crosses.

5. At this point, the P generation plants can be discarded. Pots and trays can be disinfected for reuse according to procedures outlined in the note within Assignment 2.

6. Allow the seeds to dry in the Eppendorf tubes for two weeks. This process will reduce the internal moisture content of the seeds leading to higher germination rates.

PROCEDURE 4 – Plant F1 seeds

1. Following the steps for planting outlined in Procedure 1, plant eight pots each of seeds from the Col-1 x *gl1-1* and *gl1-1* x Col-1 crosses.

2. Plant one pot each of Col-1 and *gl1-1* seeds, placing 10-20 seeds per pot, to serve as controls for phenotypic observations.

3. Follow steps 10-14 in Procedure 1 for plant care. Complete Assignment 4 three to four weeks after planting.

4. After six weeks, you will need to cover the pots with plastic sleeves to avoid cross-contamination. Prior to being placed over the pots, the plastic sleeves will need to be vented to allow for airflow. To vent the sleeves, cut two 1" x 1" openings approximately six inches up on opposite sides of the sleeve for ventilation.

5. Continue to water plants for one more week, then let plants dry-out for two additional weeks.

ASSIGNMENT 4 – Observe and record phenotypes

Through this assignment, students should complete the following tasks:

1. Draw and make detailed observations about the phenotypic differences between the Col-1 and *gl1-1* control plants and the F1 generation.

2. Compare the phenotype of the plants grown from Col-1 x *gl1-1* crosses to the plants grown from *gl1-1* x Col-1 crosses. Are there differences? Why or why not?

3. Define P, F1 and F2 generations.

PROCEDURE 5 – Collect F2 seeds

1. Carefully cut open and remove the plastic sleeve from the pots.

2. Using scissors, cut the vegetative material free from the soil and roots. Using a new envelope for each plant, place the plant material inside an envelope. Seal the envelope and press it several times to break open the siliques and release the dry seeds.

3. Cut open a corner of the envelope being careful not to lose any plant material. Holding a fine metal sieve over a clean piece of paper, pour the contents of the envelope into the sieve. Continue to run the material through the sieve until the seeds are free of soil, plant material and other contaminants.

4. Transfer the collected F2 seeds into an appropriately labeled Eppendorf tube (see example below). Allow the seeds to continue to dry for two weeks before planting.

Group 1, date, plant #
F2: Col-1 x *gl1-1*

5. Repeat steps 1-4 until all plants have been harvested. At this point, F1 plants can be discarded and pots and trays can be disinfected according to procedures included as a note in Assignment 2.

PROCEDURE 6 – Plant F2 seeds

1. Plant the F2 seeds from the Col-1 x *gl1-1* and *gl1-1* x Col-1 crosses following the steps outlined in Procedure 1. Plant eight pots for each type of cross.

2. Plant one pot each of Col-1 and *gl1-1* seeds, placing 10-20 seeds per pot, to serve as controls for phenotypic observations.

3. Follow steps 10-14 in Procedure 1 for plant care.

ASSIGNMENT 5 – Analyze segregation for F2 generation plants

In this experiment, the reference phenotype is marked by the presence of trichomes on the leaves and stem. The mutant plant shows a glabrous phenotype, with little or no trichomes present on the vegetative parts of the plant. In this assignment students will analyze the inheritance of the *gl1* mutation in the F2 generation by completing the following tasks:

1. Draw and make detailed observations about the phenotypic differences between the Col-1 and *gl1-1* control plants and the F2 generation.

2. Collect phenotypic data (Student Handout, Glabrous Worksheet, Table 1) to analyze the inheritance of the *gl1* allele. Remember, there are multiple plants in each pot and care should be taken to accurately identify each individual plant.
    a. Count the number of plants displaying the reference phenotype.
    b. Count the number of plants displaying the mutant phenotype.
    c. Calculate the ratio of reference to mutant plants for your group.

3. Make a prediction about how combining the data from both groups in the classroom may affect the ratio determined by each individual group.

4. Add the data from both groups together and calculate the ratio again. Use the student worksheet to answer the following questions:
    d. Did aggregating the data from both groups cause the ratio to change?
    e. If so, explain how and why the ratio changed.
    f. Why is this important to the process of science?

5. Based on the ratio your class obtained, conclude whether the *gl1* allele is dominant or recessive.

6. Support your finding with evidence by completing a Punnett square (Student Handout, Glabrous Worksheet, Table 2) and answering the corresponding questions.

ASSIGNMENT 6 – Formulate next-step research questions

In this assignment, students will review what they have learned about the *gl1-1* and *ag-1* mutations, the two reference strains, and the life cycle of Arabidopsis to consider future experiments. Through this assignment, students will complete the following tasks:

1. Consider and summarize what aspects of Arabidopsis they would like to investigate should the class continue working with this model system.

2. Draft a research question that could serve as the launching point for a new experiment using Arabidopsis.

3. Formulate a hypothesis to test based on the new research question.

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Additional Reading

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