ABRC: Greening the Classroom Module

Play Mendel 2.0 – Adapted for Blended and eLearning

Summary: This version of the Play Mendel Advanced module has been adapted for virtual instruction. Students are able to complete simulated experiments on paper that mimic the results obtained through the laboratory version of Play Mendel. Both the original and this adapted module guide students through the process of investigating reference and mutant strains of Arabidopsis to learn about Mendelian genetics. Through these activities, students learn about genetic variation, segregation, inheritance, phenotypes, genotypes and plant anatomy.

Recommended Grade Level: Middle and high school

Duration: This adapted version of Play Mendel includes six assignments that can be paced at the instructor's discretion. Optional pre/post-activity suggestions have been included to extend student learning.

Suggestions for Blended Learning: ABRC suggests that the agamous experiment from the original Play Mendel Basic module be completed in-class to reinforce the concepts highlighted in this adapted version. The original Play Mendel Basic lab protocols require six weeks of grow time to bring the plants to maturity. Planting and plant care can be completed by the teacher outside of class, or in class by students if time allows. The student assignment for the original agamous experiment can be completed within a single class period.

Prerequisite Knowledge

Before beginning this activity, students should have a basic understanding of:

- DNA
- Genes and alleles
- The concept of dominant and recessive
- Mendel's Laws

Learning Objectives

Through this module students will:

- Define concepts and terms associated with Mendelian genetics and plant anatomy
- Understand how genetic factors influence the growth of organisms
- Investigate how traits are passed from parents to offspring
- Use results from simulated experiments as evidence to determine the inheritance of two unique traits and better understand Mendelian genetics
- Use Arabidopsis as a model system to understand how genetic changes can result in structural and functional changes in an organism
- Understand how a plant's phenotype can affect the plant's ability to reproduce.

Alignment with Next Generation Science Standards

NGSS	
Standards	From Molecules to Organisms: Structures and Processes (MS) Heredity: Inheritance and Variation of Traits (MS, HS) Biological Evolution: Unity and Diversity (MS)
Science & Engineering Practices	Developing and using models (MS) Constructing explanations and designing solutions (MS) Analyzing and interpreting data (HS)

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

- Student Worksheet

Additional ABRC resources that may be of interest to support teacher instruction and student learning:

- Play Mendel Advanced Module
  - Play Mendel Module Video
  - Play Mendel Advanced Protocol

Materials
Printed Student Worksheet (One per student with appendices printed single-sided)
Scissors
Two small bowls or bags
Pencil or pen

Seed Strain Details

- **Columbia** (Col-1) – 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*** – 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.

- ***ag-1*** – This segregating strain is the result of a cross between two heterozygotes that contain a mutation in the *AGAMOUS* gene. The AGAMOUS gene 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 a 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.

Student Assignments
Students will complete two simulated experiments to analyze the inheritance of two unique traits. Through these activities, students will learn about the concepts of variation, segregation and inheritance discovered by Mendel.

Assignment 1	General terms and phenotypes for the glabrous mutation
Assignment 2	Complete the F1 generation
Assignment 3	Determine the genotypes of the F2 generation
Assignment 4	Phenotypes for the agamous mutation
Assignment 5	Analyze the inheritance of the *ag-1* allele
Assignment 6	Punnett Square for *ag-1*

Assignment Details & Answer Guide
NOTE: Text in blue is included as a guide for assessing student responses.

ASSIGNMENT 1 - Terms and genotypes for the glabrous mutation
In this simulated experiment, students will follow the outcome of a genetic cross between two different parent plants through to the F2 generation to determine the inheritance of the *gl1* mutation. The homozygous strains that make up the parent generation are Columbia and *gl1-1.* 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. This assignment provides foundational knowledge that will put the rest of the virtual experiment in context. Assignment tasks are outlined below.

1. Define the following genetic and anatomy terms:

Term	**Definition**
P generation	Parent generation, two distinct individuals that are crossed to produce offspring
F1 generation	First filial generation, the offspring that results from the crossing of the P generation
F2 generation	Second filial generation, offspring that results from the crossing of two distinct individuals in the F1 generation
Allele	A copy of a gene. There can be multiple alleles possible for a single gene. In sexual reproduction, offspring receive one allele from each parent for each gene in the genome.
Genotype	The genetic makeup of an individual, genotype can refer to a single gene or of an individual's entire genome
Genome	The complete DNA of an individual
Phenotype	The physical characteristics that an individual displays. An individual's phenotype is directly influenced by its genotype, as well as by other factors such has environment.
Homozygous	Term that describes a gene in which both alleles are the same
Heterozygous	Term that describes a gene in which the alleles are different
Mutation	A change in the DNA of an organism
Dominant	The presence of one dominant allele in a gene pair determines the phenotype of the organism for a specific trait.
Recessive	The presence of one recessive allele in a gene pair is masked by the presence of a dominant allele. In order for a recessive trait to be expressed, an individual must be homozygous for the recessive allele.
Sequence	The process of analyzing and identifying all of the genes in an organism's genome
Trichome	A hair-like structure on a plant, often found on the leaves and stems
Sepal	The leaf-like structures that surround an unopened flower, and are positioned outside of the petals near where the petals meet the stem on an open flower
Petal	Modified leaves that are part of a plant's reproductive structures
Stamen	The male reproductive structure of a flower that is made up of a filament and anther. The anther is the structure that produces pollen.
Carpel	The female reproductive structure of a flower made up of a stigma, style and ovary

2. Explain what the term 'reference strain' means.
   In an experiment, the reference strain is the specific strain of an organism that is used as the benchmark against which other strains are compared. In many cases, this strain does not possess a mutation for a specific gene or does not display a specific phenotype.

   In some experiments, the reference strain is a strain of organism that was collected in the wild, as opposed to generated in a laboratory setting. In this case the term *wildtype* is sometimes used instead of *reference strain*.

3. Read the seed strain details (provided in previous section and the student worksheet) to learn more about the two strains of Arabidopsis that make up the P generation in this experiment.

4. Describe the reference and mutant phenotypes in this experiment.
   Plants with the reference phenotype in this experiment have trichomes (plant hairs) on the leaves and stems. Plants with the mutant phenotype in this experiment have few or no trichomes present.

ASSIGNMENT 2 - Complete the F1 generation
The genes of interest in this simulated experiment are annotated as GL1-1 and gl1-1. For ease, these annotations are shortened to G and g for this activity. In this assignment, students will investigate the outcome of the genetic cross of the two parent strains to determine the genotypes and phenotypes of the F1 generation.

1. Review the image below that represents the genetic cross between the two parent strains, Columbia and *gl1-1*. Complete the Punnett square to determine the genotypes of the resulting F1 offspring.

P

G/G

X

g/g

F1

	G	G
g	Gg	Gg
g	Gg	Gg

2. List the F1 genotypes identified in your Punnett square. Predict the phenotype of each genotype.
   The genotype of all of the offspring in the F1 generation are Gg. An accurate prediction would be that all of the offspring would have trichomes present on the leaves and stems.

ASSIGNMENT 3 Determine the genotypes of the F2 generation
In this assignment, students will simulate the results of a genetic cross of two F1 generation plants to obtain the genotypes of the F2 generation. Students will complete the activity multiple times to understand the importance of sample size for accurate results.

1. Print Appendix 1 (Play Mendel: *gl1-1* Genotype Activity) and Appendix 2 (Play Mendel: Play Mendel: F2 Genotypes).

2. Cut out each square on Appendix 1. This activity represents the genetic cross of two F1 plants (the plants that resulted from the crossing of the P generation). The grey squares represent the alleles of one F1 plant in the cross. The white squares represent the alleles of the other F1 plant in the cross.

3. Place all of the grey squares in a bowl or bag. Place all of the white squares in a different bowl or bag. Mix the squares in each bowl or bag.

4. Set up your workspace so that you have the Appendix 2 worksheet in front of you, along with the two bowls or bags with the white and grey squares (alleles). Without looking, select and place one white allele in each of the white spaces on Appendix 2. Then, select and place one grey allele in each of the grey spaces. Fill all boxes on the worksheet for a total of 12 pairs.

5. The resulting allele combinations represent the genotypes of the F2 generation. Record the number of each possible genotype for round one in datasheet provided in the student worksheet.

6. Repeat steps three through five four more times. Add together the total number of plants in each column and note that sum in the final row.

7. Circle the genotypes that display the reference phenotype: G/G G/g g/G g/g

8. Circle the genotypes that display the mutant phenotype: G/G G/g g/G g/g

9. Based on your answers above, determine the number of reference and mutant phenotypes in each round and for all rounds combined. Next, determine the ratio of reference to mutant phenotypes for each round and for all rounds combined.
   The expected ratio for reference to mutant phenotypes in this experiment is 3:1. Students will likely obtain a variety of ratios for individual rounds.

10. Did the ratio from each individual round differ from the ratio obtained from the combined totals?
    It is expected that the ratio obtained during individual rounds will differ from the ratio obtained when the totals from all rounds are combined.

11. If so, how did the ratio change? Is this what you expected? Explain.
    The more rounds that are completed and combined, the closer the result will get to the expected 3:1 ratio. A smaller sample size (represented by a single round) is more likely to stray from the expected ratio. A larger sample size is a more accurate representation of a population.

    As an example, imagine you traveled to a different country but only went to one house during your visit, and all four of the individuals living in the house had red hair. You may conclude from this experience that every person living in that country had red hair. If you had stayed longer and met more people you would have realized that the country's population was made up of individuals with blonde, brown, red and black hair. The more people you meet the better idea of the distribution of different hair colors you would have. By the end of your visit, you may even realize that people with red hair are the minority.

12. Based on the combined ratio you obtained, is the *gl1-1* allele dominant or recessive?
    The *gl1-1* allele is recessive.

ASSIGNMENT 4 – Terms & phenotypes for the agamous mutation

In the agamous experiment, student investigate a segregating 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. This assignment focuses on background information that will put future assignments in context. Assignment tasks are outlined below.

1. Read the seed strain details (provided in previous section and the student worksheet) to learn more about the specific strain of Arabidopsis you are investigating.

2. This strain produces plants with two unique phenotypes, a single flower phenotype and a double flower phenotype. Which phenotype is the reference and which is the mutant?
   Reference phenotype = single flower
   Mutant phenotype = double flower

ASSIGNMENT 5 – Analyze the inheritance of the *ag-1* allele
In this assignment students will analyze the inheritance of the *ag-1* allele by simulating the results of planting a segregating population of *ag-1* plants.

1. Print Appendix 3 (Play Mendel: *ag-1* Phenotype Activity). Cut out each square. Place squares in a bowl or bag. Thoroughly mix the paper squares.

2. Without looking, select and place one paper square on the table so that the flower image is visible. Repeat this step until have 25 squares.

3. Count the number of squares with the reference phenotype and the number of squares with the mutant phenotype. Record that number in the datasheet below. Determine the ratio of reference to mutant phenotypes for this round. Record the ratio on the datasheet included in the student worksheet.

4. Repeat steps two and three for fourteen more rounds.

5. Add the number of reference phenotypes from each round together and record this total on the datasheet. Do the same for the mutant phenotypes. Determine the ratio of reference to mutant phenotypes for all rounds combined.
   The expected ratio is 3:1 reference to mutant phenotypes. Students will likely obtain a variety of ratios for individual rounds.

6. Did combining the data from all 15 rounds cause the ratio to change?
   It is expected that the ratio obtained during individual rounds will differ from the ratio obtained when the totals from all rounds are combined.

7. If so, explain how and why the ratio changed.
   See response to Assignment 3, Question 11.

8. Based on the ratio you obtained, is the *ag-1* allele dominant or recessive?
   The *ag-1* allele is recessive.

ASSIGNMENT 6 – Punnett Square for *ag-1*
1. Complete the Punnett square below. This will serve as evidence to support your findings in Assignment 5, Question 5.

	A	a
A	AA	Aa
a	Aa	aa

2. Which offspring will display the reference phenotype?
   AA & Aa

3. Which offspring will display the mutant phenotype?
   aa

Optional Extension Assignments

Pre-Activity: Getting to know Gregor Mendel
Have students research the life and work of Gregor Mendel (on the internet or other source of your choosing). Students should write a summary of Mendel's experiments with pea plants, and explain the impact this work had on the field of genetics.

Post-Activity: Understanding flower anatomy and pollination
In this assignment, students will learn basic flower anatomy, and the function of the different flower structures. Through these activities, students will have a better understanding of how genetic crosses are performed using Arabidopsis.
Ask students to observe a flower and draw the different structures they see. Depending on the season, students can observe flowers growing outdoors, look at a cut bouquet, or search for flower pictures on the internet. Once the drawing is complete, students should research flower anatomy (online or using a source of your choosing) to identify and label each of the structures in their drawing. Students should research the function of each flower structure and identify those that play a key role in reproduction. If cut flowers are available students can use fine tip tweezers, small scissors or their fingernails to gently pull apart the flower and identify each different structure.

Next, have students watch the Play Mendel Module Video from 4:35-6:00 to see how to perform a genetic cross using Arabidopsis. Explain that Arabidopsis is a self-pollinating plant. Ask students to consider why when performing a genetic cross with Arabidopsis, it is important to use a flower bud that has not opened for the female parent.

Post-Activity: Ornamental plants
The agamous mutation investigated in this module has been used in flower breeding to develop showy varieties of a number of ornamental plant species. In this assignment, students should investigate 'double flowers' and identify common backyard flowers that are bred to have this mutation. Ask students to consider if this mutation is beneficial, neutral or harmful in nature. Have students explore other mutations that are used to produce interesting varieties of ornamental plants.

References

Arabidopsis Biological Resource Center [ABRC]. (2016). Education and Outreach. Available online at https://abrcoutreach.osu.edu/

Price, C., Knee, E., Miller, J., Shin, D., Mann, J., Crist, D., Grotewold, E., & Brkljacic, J. (2018). Following phenotypes: An exploration of Mendelian genetics using Arabidopsis plants. *The American Biology Teacher*.

Robinson, T. R. (2010). *Genetics for Dummies, 2nd Edition*. Hoboken: John Wiley & Sons.

Additional Reading

Ausubel, F.M. (2000). Arabidopsis genome: A milestone in plant biology. *Plant Physiology, 124,* 1451-1454.

Knee, E.M., Rivero, L., Crist, D., Grotewold, E., and Scholl, R. (2009). Germplasm and molecular resources. In: Genetics and Genomics of the Brassicaceae. Jinnie Kim, Senior Editor in revision.

Koornneef, M. & Meinke, D. (2010). The development of Arabidopsis as a model plant. *The Plant Journal*, *61(6),* 909-921.

Pang, P.P. & Meyerowitz, E.M. (1987). Arabidopsis thaliana: A model system for plant molecular biology. *Nature Biotechnology, 5(11),* 1177-1181.

Provart, N.J., Alonso, J., Assmann, S.M., Bergmann, D., Brady, S.M., Brkljacic, J. *et al.* (2016). 50 years of Arabidopsis research: Highlights and future directions. *New Phytologist*, *209(3),* 921-944.

Rivero, L., Scholl, R., Holomuzki N., Crist, D., Grotewold, E., & Brkljacic, J. (2014). Handling Arabidopsis plants: Growth, preservation of seeds, transformation, and genetic crosses. *Methods in Molecular Biology, 1062,* 3-25.

Somerville, C. & Koornneef, M. (2002). A fortunate choice: The history of Arabidopsis as a model plant. *Nature Reviews Genetics, 3(11)*, 883-889.

Wyatt, S. & Ballard, H.E. (2007). Arabidopsis ecotypes: A model for course projects in organismal plant biology and evolution. *American Biology Teacher, 69(8),* 477-481.

Zhang, Z.-L. (2006). Use of the *gl1* Mutant & the *CA-rop2* Transgenic Plants of *Arabidopsis thaliana* in the Biology Laboratory Course. The American Biology Teacher online publication November/December: 148-153.