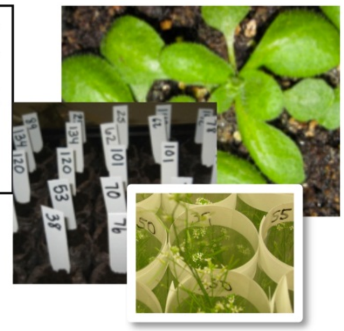
PlantingScience Genetics in inbred lines of Arabidopsis thaliana



Module Summary

Targeted grade levels: Upper high school courses such as Genetics, Biotechnology, Honors or AP Biology; also well suited for undergraduate courses

Module length: 4-8 weeks

Prior student background: Basic biology and genetics

Abstract: Designed as a whole-class experiment, students work in teams to understand genotypic variation among recombinant inbred lines (RILs) of the model organism *Arabidopsis thaliana*. Seeds are used from two parent lines, Columbia and Landsberg, as well as numerous recombinant inbred lines (RILs) developed by crossing the parent lines. In a guided inquiry investigation, students sow and cultivate all the plants in the same controlled environments (to minimize variation due to environment), and monitor traits to observe genetic variation among the RILs. Protocols are provided to investigate whether given traits are continuous (quantitative) traits – the result of multiple genes, or if they are discrete traits – the result of a single gene. In addition to this guided inquiry, students may also choose other traits to investigate. Students can even propose whether a continuous trait is linked to one of the discrete traits or not. Student teams pool data with other teams in the class to analyze trends and patterns, such as identifying continuous versus discrete traits, and determining potential genetic linkages among traits. Students communicate with scientist mentors online to discuss their experiment, from generating questions to constructing evidence-based conclusions.

Concepts

- The inheritance of traits from parents to offspring involves the transmission of genes through reproduction.
- Traits within a population vary. This naturally occurring variation is due to genetic and environmental factors.
- Phenotypic markers are used to understand the inheritance of traits.
- Discrete traits are the result of a single gene, and follow Mendelian patterns of inheritance, which can be analyzed through Punnett squares.
- Continuous traits are the result of multiple genes interacting, with trait expression varying along a graded continuum.
- Some traits only appear at particular moments in development.
- The tendency for genes to be linked depends on how close two genes are on a chromosome.
- To experimentally study the factors affecting variation, tight control must be maintained on the environment as well as the hereditary lines.
- Science is an active process of curiosity, inquiry, investigation, and communication.

Collaboration and Support: This module was developed for the PlantingScience program of the Botanical Society of America in collaboration with Larry Griffing at Texas A&M University in College Station, Texas; Allison Landry at the Louisiana School for Math, Science, and the Arts in Natchitoches, Louisiana; and Randy Dix at Olathe North High School in Olathe, Kansas. Additional funding has been provided by the National Science Foundation, and the Monsanto Foundation.

Module Information and Use: A Word to Teachers

We provide tools and instructions for Arabidopsis Genetics on the PlantingScience website to help you develop your class investigation using the model organism *Arabidopsis thaliana*. The module consists of a **Teacher's Guide** and **Student Sheets**. The Teacher's Guide suggests a sequence of lessons, supports for student investigation skills and helpful resources. A list of references and resources is also found on the last page.

Student Sheets can be found in a separate document. They are designed for easy printing / duplication to distribute to students. They contain a great deal of background information, and they will be valuable whether you use them with students or just for your own background. Additional material such as database templates can also be found on the PlantingScience website.

Arabidopsis thaliana is one of the most used model organisms in plant research, and it is increasingly used in classroom laboratories. The Arabidopsis Biological Resource Center, a stock center for Arabidopsis, makes seeds and information freely available for education purposes (http://abrcoutreach.osu.edu/).

The seed lines used in this module have been specially cultivated by scientists to investigate variation in discrete traits and continuous traits. Two parent lines, Columbia and Landsberg, have been crossed, and from that F1 generation and subsequent inbreeding, several recombinant inbred lines (RILs) have been developed (see Student Sheets pages 3-4). A main take-home message about the genetic implications for the investigation is that **given the high homozygosity in the RILS, and given that the different RILs and parent lines are grown under the same environmental conditions, phenotypic variation observed among the lines is likely due to genetic variation among these lines.**

The parent lines, Columbia (COL) and Landsberg (LER), are two of several ecotypes that have been accessioned by scientists to study. Ecotypes are distinct groups of the same species that are separated by geography and have unique traits. Many of these traits are assumed to have developed as adaptations to the local conditions (such as cold tolerance or flowering time), yet some traits may simply be those linked to adaptive traits but are not necessarily adaptive themselves.

Scientists are cataloguing the different traits of each accession (ecotype). They then use the RILs formed from crossing different accessions to observe the traits in each RIL. What can they learn from this? They learn which traits tend to be paired together, suggesting they are genetically linked. Since linkage suggests that genes are in close proximity to each other on a chromosome, geneticists can then map genetic traits onto the genome. Using molecular techniques, they also can identify specific genes related to certain traits.

This module can be used as a **guided inquiry** for students to investigate traits already known in the parent lines. Three traits (trichome number, flowering time, and growth form) are described in the module material. Although they may be known in the parent lines, students can contribute new knowledge to the field of research by documenting the variation of these traits among the RILs. This is an active field of research!

This module can also be used as a more **open inquiry** for students to investigate other traits, some of them not so well known, in the parent lines. Some suggested traits to investigate are listed on page 13 of the Teacher's Guide (this document). Students can also think of their own traits to observe. These student investigations can also contribute new knowledge to this active field of research!

A fundamental inquiry question that this module allows students to explore is *whether specific traits are continuous or discrete traits*. Every population of organisms exhibits some variation in phenotypes. By analyzing this variation within the population of *Arabidopsis thaliana* students grow in class, they can find evidence that a specific trait is a continuous or a discrete trait. The labs offer guidance for investigating this question for three given traits (trichome number, flowering time and growth form). If students choose their own traits, *the methods of data analysis will be similar as those given in this module.*

An additional inquiry question that this module allows students to explore is *whether different traits are genetically linked*. Students need to be able to analyze data from both continuous traits and discrete traits in order to analyze data for linked genes. The instructions given in this module for analyzing linkage do so by comparing data from a continuous trait with that of a discrete trait. (Note: the discrete trait is seen about 5 weeks after sowing seeds.) While it is possible to analyze linkage between two continuous traits, the methods are advanced and not given here. By understanding genetic linkage and how scientists explore it, students can gain insight into how geneticists map genomes of organisms.

In addition, **biotechnology classes** can integrate molecular markers as discrete traits to analyze.

Frequently Asked Questions

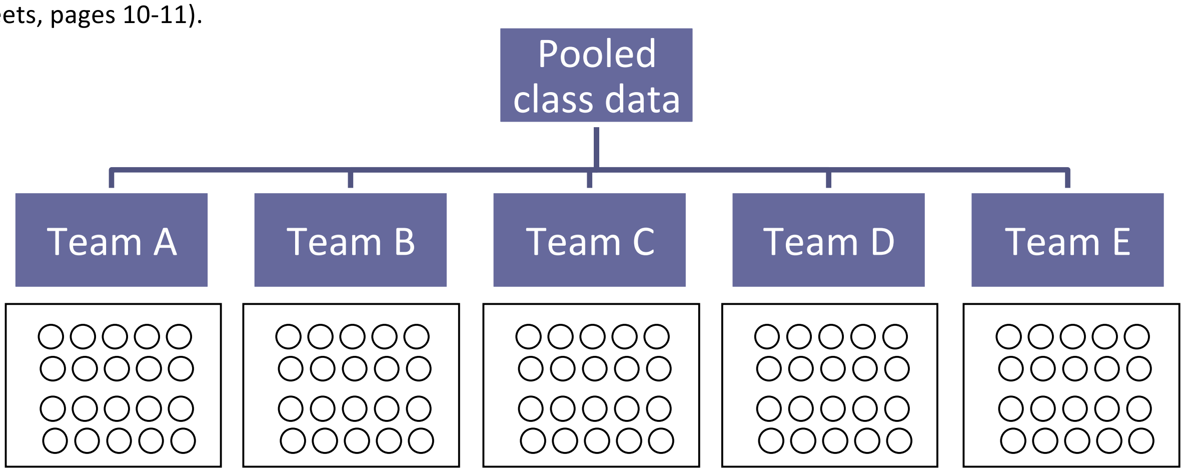
Do we *really* need so many RILs, soil pellets and plants? Yes, the genetic investigations conducted in this module rely on large data sets for analysis. As many RILs as possible should be included (~48 RILs). If one to a few RILs are lost for some reason, that's OK – it is still possible to analyze the data. Additionally, it is essential to plant and grow the parent lines (COL and LER), so that traits in the RILs can be compared to each parent line. You might plant some additional parent seeds in extra pellets just to be sure you have good samples from the parent lines.

Can we adjust the length of the module? Yes! The specific traits that are described in detail in the module will require growing the plants for 4-6 weeks. However, if you want to focus on other traits that can be observed earlier or later, please consult the list of observable traits on page 13 of the Teacher's Guide. In addition, the module is divided into **Basic** and **Extended** versions that can help adjust for time constraints (See "Notes on Timing" on page 10 of the Teacher's Guide).

How do open inquiry investigations integrate with the guided inquiry? Student teams can choose their own traits to monitor, or the class can identify other unique traits – a suggested list of additional traits is provided on page 13. We suggest you lead a discussion to see if they think of these traits on their own.

Module Design

Guided Inquiry and Pooling Class Data: Designed as an all-class experiment, living plants are used in a guided hands-on inquiry to explore aspects of genetics. The planting and growing conditions of the plants will be guided for the students, as will some of the activities. This module depends on pooling data from all teams in a class in order to analyze patterns of variation in a population. For instance, in the diagram below, each little circle represents a soil pellet with approximately 5 plants in it. Each team will be responsible for monitoring a number of pellets. There are 100 pellets per class. (See "Experimental Design" in Student Sheets, pages 10-11).



Student Teams: Students will be grouped into small teams. Activities such as planting, watering, observing and collecting data can be conducted in teams. Each team will be assigned one scientist mentor to communicate with on the PlantingScience website. Please allow time for this important and rewarding communication. The data from student teams will be pooled with other teams within the class for analyzing the RILs in comparison to each other and to the parents (COL and LER).

Learning Goals:

Students will:

1. Understand and establish research questions and experimental design to investigate genetic phenomena in the model organism, *Arabidopsis thaliana*.
2. Sow, cultivate and grow a population of *Arabidopsis thaliana* plants from seed to flower in the classroom in order to investigate the research question(s).
3. Follow good lab techniques of note-taking, labeling, frequent monitoring and troubleshooting.
4. Monitor traits in the plants during the course of their development.
5. Collect appropriate data on traits.
6. Analyze data to find trends and/or patterns.
    a. Pool data from all class members to analyze.
    b. Represent data graphically to look for trends and patterns.
    c. Understand the difference between continuous and discrete traits.
    d. Explore possibilities of linked traits relating linkage to chromosome structure.
7. Develop logical conclusions based on evidence of experiment.
8. Communicate with scientist mentors throughout the experiment, including establishment of research questions, plant cultivation, trait data collection, data analysis, and development of conclusions.

Alignment of Learning Goals and Activities

Days after sowing	Life Cycle Stage	Learning Goals	Learning Activities	Pages of Teacher's Guide
Prepare		8	• *Recommended:* sow a demonstration set of plants 3-4 weeks in advance • Prepare growing system for students • Register online and take pre-test • Introductions to mentors	
0	Sowing seeds	1,2,3,8	**Basic Hands-On Lab:** • **Lab 1:** Sowing, cold-treating and germinating seeds	pp. 18-19
1-5	Germination	1,8	**Engage Options:** • **Lesson Option 1:** Are traits determined more by genes or environment? • **Lesson Option 2:** What is a model organism and why is *Arabidopsis thaliana* one? • **Lesson Option 3:** When do traits appear in *Arabidopsis thaliana*? • **Lesson Option 4:** What are linked genes and how might we look for them?	pp. 14-17
6-20	Early development of rosette	1,2,3,8	**Basic Labs:** • **Lab 2:** Observing traits – variation in a population • **Lab 3:** Developing the research question • **Lab 4:** Understanding the experimental design • **Lab 5:** Counting trichomes • **Lab 6:** Analyzing trichome data	pp. 18-26
21-42	Flowering and bolting	1,2,3,4,5,6,8	**Extended Labs:** • **Lab 7:** Recording and analyzing flowering time • **Lab 8:** Bolting plants and the erecta phenotype • **Lab 9:** Analyzing data to investigate linked traits	pp. 27-29
42-56	Fruiting	1,2,3,5	• **Lab 10:** Collecting seeds	p. 30
Throughout	Throughout	1,2,3,4,5,6,8	**Sense-Making Activities** • Suggestions for capstone or culminating activities to deepen student understanding of the experiment	p. 31

Alignment with National Science Education Standards

The PlantingScience Arabidopsis genetics module addresses the following **National Science Education Standards*** for grades 9-12.

<table>
<tr><th>Content Standard A:
Science as Inquiry</th><th>Content Standard C:
Life Science</th><th>Content Standard G:
History and Nature of Science</th></tr>
<tr><td>As a result of activities in grades 9-12, all students should develop:
▪ Abilities to do scientific inquiry
○ Identify questions and concepts that guide scientific investigations
○ Design and conduct scientific investigation
○ Use technology and mathematics to improve investigations and communications
○ Formulate and revise scientific explanations and models using logic and evidence
○ Recognize and analyze alternative explanations and models
○ Communicate and defend a scientific argument
▪ Understandings about scientific inquiry</td><td>As a result of their activities in grades 9-12, all students should develop under-standing of:
▪ The molecular basis of heredity
▪ Biological evolution
▪ Matter, energy and organization in living systems</td><td>As a result of activities in grades 9-12, all students should develop under-standing of:
▪ Science as a human endeavor
▪ Nature of scientific concepts
▪ Historical perspectives</td></tr>
</table>

*National Research Council (1996). *National Science Education Standards*. The National Academies Press, Washington, DC.

This module also aligns with the **Conceptual Framework for New K-12 Science Education Standards**†. The Framework identifies three dimensions: Practices, Crosscutting Concepts, and Disciplinary Core Ideas.

<table>
<tr><th>Scientific and Engineering Practices Addressed</th><th>Crosscutting Concepts Addressed</th><th>Disciplinary Core Ideas in Life Sciences Addressed</th></tr>
<tr><td>1. Asking questions (for science) and defining problems (for engineering)
2. Developing and using models
3. Planning and carrying out investigations
4. Analyzing and interpreting data
5. Using mathematics and computational thinking
6. Constructing explanations (for science) and designing solutions (for engineering)
7. Engaging in argument from evidence
8. Obtaining, evaluating, and communicating information</td><td>1. Patterns
2. Cause and effect: Mechanism and explanation
4. Systems and system models
5. Energy and matter: Flows, cycles, and conservation
6. Structure and function
7. Stability and change</td><td>LS1. From Molecules to Organisms: Structures and Processes
A. Structure and Function
B. Growth and Development of Organisms
C. Organization for Matter and Energy Flow in Organisms
LS2. Ecosystems: Interactions, Energy, and Dynamics
A. Interdependent Relations in Ecosystems
B. Cycles of Matter and Energy Transfer in Ecosystems
LS3. Heredity: Inheritance and Variation of Traits
A. Inheritance of Traits
B. Variation of Traits
LS4. Biological Evolution: Unity and Diversity
A. Evidence of Common Ancestry and Diversity
B. Natural Selection
C. Adaptation
D. Biodiversity and Humans</td></tr>
</table>

†National Research Council (2012). *A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas*. The National Academies Press, Washington, D.C. www.nap.edu

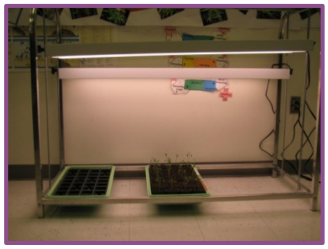
Materials

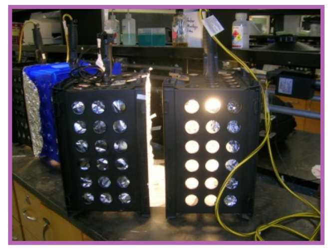
Overall cost:

Approximately $25, not including light system. Light system can be made for $60.*

Materials per class:

- *Arabidopsis thaliana* seeds from parent lines Columbia (COL) and Landsberg (LER) and 48 RILs. See details for ordering seeds on the PlantingScience website (www.plantingscience.org) on the *Genetics in Inbred Arabidopsis* plant theme page.





- Light System
    - Fluorescent light banks (above left), such as available from Carolina Biological Supply, **OR**
    - Home-made screw-in fluorescent bulb in 2 constructed light boxes (above right) – Directions for Light Box Construction on page 36 of Teacher's Guide (this document).

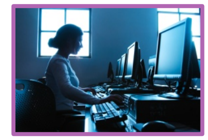
- Planting System
    - Recommended: Jiffy-7 Peat Pellets (42 mm) with tray, 100 pellets total (alternate methods can work, such as 2" pots with germinating soil mix)
    - Osmocote Smart-Release Plant Food (granules)
    - Miracle Gro Liquid All Purpose Plant Food 8-7-6 (if growing longer than 3 weeks)
    - Water dispenser (can, beaker, etc.)
    - Roll of aluminum foil
    - Access to refrigeration (for cold treatment)
    - Labels to indicate which seed lines are planted in each soil pellet (can be plastic, or flags made from wood sticks and tape, or something similar)
    - Sharpies for labeling
    - Small 3" x 3" pieces of paper
    - Toothpicks or wooden applicator sticks to manipulate the tiny seeds
    - 100 plastic transparent sheets (one per pellet) for keeping the Arabidopsis plants separate when seeds fall (if growing plants to the flowering / seed set stage)

- Lab equipment and tools
    - Wet lab or table space that can get dirty
    - Dissecting microscopes (or hand lenses)
    - Measuring tools – rulers, calipers, etc. (for measuring plants, leaves, etc.)
    - Dissecting needles
    - Graph paper and/or database to record data
    - Pipettes for measuring 2 ml fertilizer (if growing longer than 3 weeks)
    - 50 vials in which to collect seed

Practical Considerations

You'll Need:

- **Access to computers**



  - Necessary for online communication with scientist mentors
  - Helpful for entering, pooling and analyzing data (such as in Microsoft Excel)
  - Optional: Access to scanner and image software (such as Photoshop) for collecting data on some traits
- **Time for dialogue with mentors***

  - Scheduled time for students to communicate with mentors online
  - Time for teachers to monitor dialogues
  - Time for teachers to assess and award credit for online participation
- **Lab notebooks**

  - Important for ongoing record of observations, data collection, sketches, notes, concept maps and ideas
  - Optional: selection of pens, pencils and/or paints for illustrating
  - Optional: digital camera for taking pictures and/or videos of plants and student work

* **Include mentors in your planning:** Consider requiring students to post online to mentors 2-5 times a week for credit. PlantingScience evaluations indicate that teachers who *require* students (for credit) to post to scientist-mentors have greater student engagement and meaningful dialogue with online mentors.

A note from our scientist author: One of the most powerful approaches incorporated into PlantingScience is the fact that different classrooms will provide slightly different environmental conditions. Within each classroom, each individual plant within a single RIL should show very little variation in comparison with the other individuals in the same RIL because, presumably, the environment that they are all growing in should be the same and their genetics are the same. However, in different classrooms, although the genetics within a RIL are the same, the environmental conditions might vary somewhat. In the best possible scenario, all the classes will closely monitor light and temperature (as well as watering and fertilization). We expect that a classroom in the northern latitudes will be colder than a classroom in the southern latitudes. We expect that most classrooms will have constant lighting (24 h constant, long-day light), but that the actual intensity of illumination might be different, if different classes get different bulbs. This will give us a handle on experimental conditions that produce a range of variation, a range of variation that is probably less than that occurring in nature, but will nonetheless produce some variation in expression. Nailing this down by comparing data between classes will therefore be quite interesting.

~Larry Griffing

Recording and Sharing

Keeping a Lab Notebook – Sharing with Mentors

What goes into a lab notebook? How is it organized?

At the heart of every scientific investigation is a sense of curiosity and wonder. How does something work? What is going on here? Why does this happen? Why is this thing not behaving like I think it should?

Science is also fundamentally based on evidence. As we try to answer questions, we use evidence to put the puzzle pieces together to make sense out of them. Scientists develop explanations based on evidence. That is one reason it is important to keep careful notes and records – sometimes important pieces of evidence slip past us when we don't even know they're important at the time!

Scientists vary in how they use their notebooks, but in all cases it's where the ongoing aspects of the investigation are noted. Experiments are described, drawings made, and comments noted (often filling the margins!). When plants are watered, how much water was added? How is each group of plants progressing? All observations are noted. It's so easy to forget little details that turn out to be important, so we write them down in one place. You might come in one day and find the temperature in the classroom changed dramatically because a window had been left open. Write that down! It may help explain phenomena we find later. Drawings or sketches often speak volumes, and they help develop the skill of observation. Lab notebooks are also where predictions can be made, thoughts worked out, questions asked.

If you have a scanner or can take photos of student journals and upload them, the scientist mentors would delight in seeing these so they can comment and ask questions!

Sharing and Presenting

Every scientist shares her or his investigations and results.

By reading and listening to what other scientists have learned, new questions are generated. Scientists share with others to get their minds turning over new perspectives and to learn from each other.

Usually it is an interesting exercise full of curiosity: How did you do that? Why were you interested in that? How did you think to design it that way?

Scientists are also open to challenges. Sometimes challenges from other people make us think more clearly about what we did. We might think we are even more correct and defend our methods (or conclusion or question, etc.). Sometimes we have overlooked something or discover something that never occurred to us before. The most interesting scientists are those open to learning new things from other people.

Lesson Plans and Activities

Lessons and activities are organized to:

A) Engage student interest
B) Explore and explain in labs and data analysis
C) Make sense of the learning through sharing
D) Continually communicate with scientist mentors

Please adapt these to your own needs, and feel free to experiment with new ways of investigating! Inquiry is at the heart of what we all do in both teaching and science, and we are open to your input and expertise!

NOTES ON TIMING

Planting Seeds: Because seeds should be planted about 2 weeks prior to data collection, the planting of seeds (Lab 1) may take place *before* students have deeply understood the experimental design. That is, we recommend conducting Lab 1 (Sowing, cold-treating and germinating seeds) before completing the Engage Lessons that appear first. After planting, you will have about 1-2 weeks in which lessons can focus on the conceptual foundations of phenotypic and genotypic variation in Arabidopsis and RILs, and how they relate to the investigation.

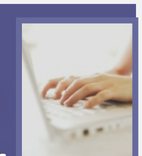
Basic vs. Extended: The full length of this module (6 – 8 weeks) may be difficult to implement completely. It is possible to shorten the module by limiting the traits students observe to those that appear early in development. For instance, data on trichomes can be collected 2 weeks after sowing seeds. You could focus on the concept of continuous traits, and simply collect trichome data. This is considered the *Basic* version. To collect data on plant growth form and flowering time, plants will need to grow to maturity, about 5 weeks after planting.

Investigating Genetic Linkage: The guided investigation here into linked traits uses the discrete trait of the erecta growth form. This can be observed about 5 weeks after planting.

SEE SUGGESTED CALENDAR, p. 11 of Teacher's Guide

We recommend a demonstration class set of Arabidopsis plants! It will be very valuable to have a set of plants YOU sow weeks early, and ideally staggered in sowing time, for several reasons. Prior to student plant sets being ready, students can observe and handle Arabidopsis, become familiar with its traits, and explore its life cycle. In addition, a demonstration set can give students practice in collecting and recording plant data before they do so with their own plants. **This will require that you plan ahead and plant the demonstration set a few weeks in advance of students sowing their seeds.**

Suggested Activity and Lesson Planner

	Monday	Tuesday	Wednesday	Thursday	Friday
Week 1	Prepare growing system Student and mentor introductions	LAB 1 Planting Cold treat seeds	Engage activities →	→ 	→ *Scientists & students explore phenotypic variation*
Week 2	Engage activities	LABS 2, 3, 4 Observing traits, developing research question, understanding experimental design	→ 	→ *Scientists & students engage in questions of genetics, using a model organism, formulating a research question and experimental design*	
Week 3	LAB 5 Collect trichome data →	→	→ 	*Scientists and students explore differences between discrete and continuous traits*	
Week 4	Analyze data on trichome number →	→	*Share data with scientists* *Scientists – talk about trichome function* *Connections to relevant real world topics*		
Week 5	EXTENDED VERSION: LABS 7, 8 →	Collect data on flowering time and growth form →		*Share data with scientists* *Scientists – talk about why different flowering times and/or growth forms might be adaptive*	
Week 6	EXTENDED VERSION: LABS 7, 8, 9 Analyze data on flowering time, growth form, and linkage →	SENSE-MAKING ACTIVITIES: Discuss, share and form conclusions →	*Discuss inquiry with scientists, develop models for inheritance* →	→	

Teams are encouraged to post online 2-3 times per week. Teams can blog from school or home.

General Life Cycle of *Arabidopsis thaliana*

The following table describes the developmental stages of the life cycle of *Arabidopsis thaliana*. This is meant as a general guide. Different accessions (ecotypes) show variation in the timing of some stages. Studying this variation is very much an active area of research (for instance, the average day of first flowering appears to be different in some of the accessions). The day that seeds are sown is Day 0.

Days After Sowing	Stage	Cultivation, Plant Care and Data Tasks
0	Seeds	Plant and Cold Treat
2	Imbibed seeds	Pull out of cold treatment, place under lights with lid on
~7	Germination, both cotyledons visible	Tilt lid ajar, water **with fertilizer** (use fertilizer if growing for more than 3 weeks)
~10	Cotyledon expands, production of first primary leaf	
~12-14	Appearance of up to 6 rosette leaves	Remove plastic lid, water again without fertilizer Data collection: Trichome counting on first primary leaves larger than 1 mm long
18	Production and growth of rosette leaves	Water if necessary (without fertilizer)
20	Buds form on very short stem (prepare for bolting)	Water if necessary **with fertilizer** Data collection: Begin monitoring for first flowering.
26	Stem elongates (bolts) and flowers open	Water (without fertilizer) Data collection: Continue monitoring for first flowering.
29	Rosette begins to reach maximum width, siliques become visible	Water if necessary Data collection: Continue monitoring for first flowering
33	Stem growing and adding cauline leaves	Water if necessary **with fertilizer,** place plastic transparency around each pellet Data collection: Record growth form.
42	First siliques begin to yellow	
49	Yellow siliques turn brown, seeds are visible through the pod	
56	Seeds mature	Start harvesting seeds from brown siliques – do not harvest from any yellow or green siliques

Arabidopsis life cycle links:

http://www.prep.biochem.vt.edu/timeline/

http://www.arabidopsis.org/portals/education/aboutarabidopsis.jsp

Arabidopsis general morphology link:

http://www.prep.biochem.vt.edu/expinfo/expinfo_anatomy.html

Suggested Traits to Observe

This module specifically describes background, data collection, and analysis procedures for three traits that can be observed within six weeks of sowing (shown in bold in the table below): 1) trichome number; 2) flowering time; and 3) plant growth form.

Below we suggest additional traits based on the research literature to observe if you would like to shorten the module for your class or encourage teams to investigate traits of their own choosing. To help you plan, the table includes the time the traits can be observed in days after sowing (DAS) reported from the current literature, and means for analysis. Students can identify and monitor traits other than those listed here.

Observable traits in COL and LER accessions of *Arabidopsis thaliana* and RILs formed from their crosses

Trait	Days After Sowing (DAS)	Data Collection Options
Seed size	0	Photoshop on scanned seeds (at high dpi)
Seed color	0	Photoshop on scanned seeds
Seed weight	0	Mass
Days to first true leaf	6-10	Date that 1st leaf is 1mm long
Number of trichomes	**12-14**	**See module document**
Epidermal cell density 4	18-20	#cells / area viewed (could use field of vision)
Epidermal cell # per leaf 4	18-20	Count under microscope
Rosette leaf surface area at budding	20	Use leaf #6; Photoshop video – tutorial*
Flowering time	**21-32**	**See module document**
Rosette leaf number at flowering	21-32	Count
Rosette leaf area at flowering	21-32	Photoshop video – tutorial
Number cauline leaves at flowering	21-32	Count
Plant height at flowering time	21-32	Measure
Plant growth form: erecta vs. floppy	**33-42**	**See module document – Score by sight**
Plant height at Day 33	33	Measure
Number cauline leaves at Day 34	34	Count
Plant height at Day 40	40	Measure
Number cauline leaves at Day 41	41	Count
Plant height at Day 47	47	Measure
Silique number at Day 49	49	Count
Average silique length	49	Measure/average

*See video tutorial by Zach Jarou on measuring leaf area: http://youtu.be/E3O-V6WLw0g

ENGAGE

How much is an organism determined by genes, and how much by environment?
What is a model organism?
Why is *Arabidopsis thaliana* a good model organism for studying genetics?
What is a *genotype* and what is a *phenotype*?
What are recombinant inbred lines (RILs)?
What are linked genes?

Engaging Student Interest

Primary goals of these lessons are for students to gain conceptual background, specifically about genetics, *Arabidopsis thaliana* as a model organism, and the use of RILs for investigating genetic questions. These can also be used as formative assessments.

Lesson Option 1:
Are traits determined more by genes or by environment?

Learning Goals: • Students ask questions about genetics • Students understand that expressed traits (phenotype) are influenced by genotype interacting with the environment • Students engage in dialogue in classroom and with online mentors	**Materials:** • **Student Sheets page 1 – Traits and Phenotype** • **Optional – Student Sheets page 2 – Variation of Traits** • Lab notebooks, pencils • Computers for posting to mentors **Procedure:** • Introduce the topic in an open discussion • Break students into their teams and have them discuss the topic. • **Assign Writing or Discussion Activity – Nature or Nurture (Student Sheets page 1)** • Share in class and discuss. • Have students post to mentors what they learned and any questions they have. **Optional:** Discuss **Student Sheets page 2 – Variation of Traits** • Sheet gives background on how scientists can determine if differences in plants are based on genetic differences or environmental differences.
Timeline: 10 to 30 minutes, possible homework assignment	**Advance Preparation:** • Secure computers
Teacher Background: Understanding of phenotype, and the role of environment and genotype in the expression of traits	**Resources:** • Stanford Encyclopedia of Philosophy, The Genotype/Phenotype Distinction, Richard Lewontin **http://plato.stanford.edu/entries/genotype-phenotype/** • Gene, Organism and Environment, Richard Lewontin – YouTube **http://www.youtube.com/watch?v=we4ZzjKxFHM** • **http://www.nature.com/scitable/topicpage/Phenotypic-Range-of-Gene-Expression-Environmental-Influence-581**

Lesson Option 2:
What is a model organism and why is *Arabidopsis thaliana* one?

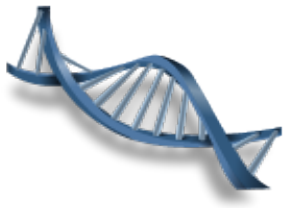
<table>
<tr><td>Learning Goals:
• Students understand the use of model organisms
• Students understand benefits and limitations of using model organisms
• Students engage in dialogue in classroom and with online mentors</td><td>Materials:
• Student Sheets page 3 – Model Organism, Ecotypes and RILs
• Web sites and/or articles on model organisms (see resources below)
• Lab notebooks, pencils
• Computers for posting to mentors

Procedure:
• Introduce the topic in an open discussion
• Break students into their teams and have them research the topics.
• Students read Model Organism, Ecotypes and RILs – Student Sheets page 3
• Optional: Students read Inheritance and RILs in Arabidopsis – Student Sheet Page 4 and/or online resources listed below
• Use Study Questions on Student Sheets page 5
• Have students write a paragraph (or more) on what they have learned.
• Share in class and discuss.
• Have students post to mentors what they learned and any questions they have. Document files or scanned images can be uploaded on student team pages.</td></tr>
<tr><td>Timeline: 20 minutes to 2 lessons, possible homework assignment</td><td>Advance Preparation:
• Secure computers and/or print articles</td></tr>
<tr><td>Teacher Background:
Familiarity with use of model organisms for research and teaching, of ecotypes, and of RILs (see background material in student sheets pages 3 - 5) and resources to the right.</td><td>Resources:
• National Institute of Health, Model Organisms http://www.nih.gov/science/models/
• National Center for Biotechnology Information, Model Organisms http://www.ncbi.nlm.nih.gov/About/model/index.html
• Howard Hughes Medical Institute, A Brief Guide to Model Organisms http://www.hhmi.org/genesweshare/e300.html
• The Arabidopsis Information Resource (TAIR), Education and Outreach, About Arabidopsis http://www.arabidopsis.org/portals/education/aboutarabidopsis.jsp
• Partnership for Research and Education in Plants (PREP) Online Lab Notebook http://prep.biochem.vt.edu/timeline/
• Video introduction to Arabidopsis as model research plant by PREP http://www.youtube.com/watch?v=foHiKrlY9Qc&list=UUQgD6eojgUpAe4x3vmO4hOw&index=10&feature=plcp
• Wyatt, S. and Ballard, H. 2007. Arabidopsis ecotypes: A model for course projects in organismal plant biology and evolution. American Biology Teacher.69: 477-481.</td></tr>
</table>

Lesson Option 3: When do traits appear in *Arabidopsis thaliana*?

Learning Goals:	**Materials:**
• Students observe phenotypic traits of Arabidopsis and understand when they appear during the life cycle • Students engage in dialogue in classroom and with online mentors	• **Demonstration class set of Arabidopsis with plants representing seedling, early vegetative growth and flowering stages** • Lab notebooks, pencils • Computers for posting to mentors **Procedure:** • Set selection of plants in random order for students to consider • Break students into their teams and have them agree on how they will organize the plants to represent stages of a life cycle • As large class discuss teams' ideas and reconcile ideas • Have students carefully observe one trait of interest to them. Encourage drawing, sketching or photographing of the trait. • Have students discuss and write in their journals questions they may have about the morphology of *Arabidopsis thaliana*, or about how scientists work. • Have students post questions to mentors to get feedback and input.
Timeline: 20 - 50 minutes	**Advance Preparation:** • Sow seeds for class demonstration set in intervals prior to time needed
Teacher Background:	**Resources:** • Partnership for Research and Education in Plants (PREP) Online Lab Notebook **http://prep.biochem.vt.edu/timeline/** • Plants in Motion time lapse video of early leaf development **http://plantsinmotion.bio.indiana.edu/plantmotion/vegetative/veg.html**

We recommend a demonstration class set of Arabidopsis plants! It will be very valuable to have a set of plants YOU sow weeks early, and ideally staggered in sowing time, for several reasons. Prior to student plant sets being ready, students can observe and handle Arabidopsis, become familiar with its traits, and explore its life cycle. In addition, a demonstration set can give students practice in collecting and recording plant data before they do so with their own plants. **This will require that you plan ahead and plant the demonstration set a few weeks in advance of students sowing their seeds.**



Lesson Option 4: What are linked genes and how might we look for them?

Learning Goals:

- Students understand that linked genes are those that are located on the same chromosome
- Students understand that genes located closer together on the same chromosome have a greater degree of linkage
- Students understand that traits (phenotypes) of linked genes tend to be inherited together
- Students understand that looking for traits that tend to appear together are clues to linked genes
- Students apply the concept of linkage to the experimental design of the module (including the traits they monitor)

Materials:

- **Student Sheet pages 6-9 – Genetic Linkage, Recombination, and RILs**
- Web sites and/or articles on genetic linkage (see resources below)
- Lab notebooks, pencils
- Computers for posting to mentors

Procedure:

- Introduce the topic in an open discussion, possibly reviewing meiosis and independent assortment of homologous chromosomes, and recombination during crossing-over events.
- Students read **Genetic Linkage, Recombination and RILs – Student Sheet pages 6-9** (could be homework assignment)
- Optional: Students read **Inheritance and RILs in Arabidopsis – Student Sheet page 4**
- Split students into teams and ask them to propose a method to investigate whether phenotypic traits are linked or not.
- Share in class and discuss.
- Have students post on website their proposed method to investigate linkage, and ask mentors any questions they have.

Timeline: 30-50 minutes, possible homework assignment

Advance Preparation:

- Secure computers and/or print articles

Teacher Background:

Familiarity with linked genes. See Student Sheets pages 4, 6-9, and resources to the right.

This module provides data analysis protocols (Lab 9: Student Sheets pp. 36-37) for exploring genes linked to the discrete trait of the erecta growth form (observable at 5 weeks). It is advisable to review these in advance to make sure you have time to explore linked genes.

Resources:

- Meiosis (short animated video): **http://youtu.be/D1_-mQS_FZ0**
- Griffiths AJF, Miller JH, Suzuki DT, et al. An Introduction to Genetic Analysis. 7th edition. New York: W. H. Freeman; 2000. Sex chromosomes and sex-linked inheritance: **http://www.ncbi.nlm.nih.gov/books/NBK22079/**
- Griffiths AJF, Miller JH, Suzuki DT, et al. An Introduction to Genetic Analysis. 7th edition. New York: W. H. Freeman; 2000. The discovery of linkage: **http://www.ncbi.nlm.nih.gov/books/NBK22076/**
- Griffiths AJF, Miller JH, Suzuki DT, et al. An Introduction to Genetic Analysis. 7th edition. New York: W. H. Freeman; 2000. Linkage maps. **http://www.ncbi.nlm.nih.gov/books/NBK21827/**
- Scitable article on genetic linkage: **http://www.nature.com/scitable/topicpage/thomas-hunt-morgan-genetic-recombination-and-gene-496**

EXPLORE AND EXPLAIN IN LABS

What does Arabidopsis need to grow?
What are trichomes, and what are their functions?
Why might flowers open at different times on different plants?
Why do Arabidopsis plants have different growth forms?
Are these traits discrete or continuous traits, and how can I tell?
How can I tell if traits are linked?
How do I analyze and represent data so they make sense and I can form conclusions?

Exploring *Arabidopsis thaliana* in Labs

Labs are organized into BASIC and EXTENDED sections. BASIC lessons can be completed within 4 weeks. EXTENDED lessons will require 6-8 weeks to complete.

Lab 1 is intended to be completed as early as possible, perhaps prior to the Engage lessons that establish conceptual background. Although not ideal, the students can still establish research questions and experimental design after sowing the seeds. If you introduce all the background material prior to sowing seeds, please allow extra time for the module.

NOTES ON TIMING

Planting Seeds: Since seeds should be planted approximately 2 weeks prior to data collection, the planting of seeds (Lab 1) may take place *before* students have deeply understood the experimental design. That is, we recommend conducting Lab 1 (Sowing, cold-treating and germinating seeds) before completing the Engage Lessons that appear above. After planting, you will have about 2 weeks in which lessons can focus on the conceptual background of Arabidopsis and RILs, and developing research questions

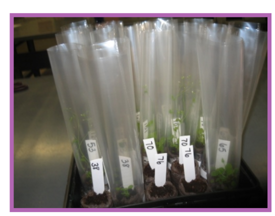
Basic vs. Extended: The full length of this module (6 – 8 weeks) may be difficult to implement completely. It is possible to shorten the module by limiting the traits students observe. For instance, data on trichomes can be collected ~2 weeks after sowing seeds. You could focus on the concept of continuous traits, and simply collect trichome data. This is considered the *Basic* version. To collect data on plant growth form and flowering time, plants will need to grow to maturity, about 5 weeks after planting.

SEE SUGGESTED CALENDAR, P. 11 of TEACHER'S GUIDE

Basic – Lab 1:
Sowing, cold-treating and germinating seeds

Learning Goals: • Students successfully plant, cold-treat and germinate seeds • Students understand plants require water, light, air, nutrients to grow • Students practice skills of good lab techniques: working with tiny seeds, measuring, labeling • Students engage in dialogue in classroom and with online mentors	**Materials:** • Lab Materials – **See page 7 of Teacher's Guide (this document); also Student Sheets page 12** • **Student Sheets pages 12 – 14 –Lab 1** • **Student Sheets pages 10-11 – Experimental Design** • Lab notebooks, pencils • Computers for posting to mentors
	Procedure: • Prepare students to work with tiny seeds • Review – or Preview – **Experimental Design, Student Sheets – pages 11-12** • Preview planting instructions **Student Sheets pages 13-14** • Split students into teams • Divide materials equally among teams • Follow planting and cold treating directions • Clean up • Post to mentors what they did and what they found interesting about planting
Timeline: 50 - 90 minutes, possibly two periods (one to prep, the second to plant) Ongoing care of plants throughout module	**Advance Preparation:** • Collect materials • Print student pages • Prepare classroom lab • Secure computers
Teacher Background: • Read Student Sheets pp. 10-11 – Experimental Design • Read Student Sheets pp. 12-14, Lab 1	**Resources:** • Partnership for Research and Education in Plants (PREP) Online Lab Notebook **http://prep.biochem.vt.edu/timeline/** • TAIR time lapse video of seed germination **http://www.arabidopsis.org/portals/education/movies/germination.mov**



Photos courtesy of students at Monroe Technology Center in Leesburg, VA

Basic – Lab 2:
Observing traits – Variation in a population

Learning Goals: • Students define a population • Students recognize differences in phenotypes among individuals in a population • Students become familiar with phenotypic traits of Arabidopsis • Students practice collecting data on plant traits • Students construct graphs to represent data in meaningful ways • Students read and interpret graphs • Students engage in dialogue in classroom and with online mentors	**Materials:** • Demonstration class set of Arabidopsis with plants representing seedling, early vegetative growth and flowering stages (with individuals labeled) • **Student Sheets page 15 – Lab 2: Observing Traits** • Lab notebooks, graph paper, pencils • Computers for posting to mentors **Procedure:** • Use **Student Sheets page 15** as a guide for group discussion, either in teams or as a whole class • Have students observe and discuss various traits in the plants, emphasizing variation of the trait among individuals • Ask each team to choose one trait, and to decide how they will record their observation of that trait (count, measurement, color according to a scale, presence vs. absence, etc.) • Have students record data on their chosen trait on numerous individuals • Graph data either as a class or as a team so that variation of that trait among individuals is apparent • Discuss what the variation might be due to (differences in genetics, age of plant, environment it was grown in, etc.) • Have students post to mentors their activities and any questions.
Timeline: 50-90 minutes	**Advance Preparation:** • Sow seeds for class demonstration set in intervals prior to the time needed (**Note: this requires a few weeks of advance sowing)**
Teacher Background: • Read Teacher' Guide pages 32-35 – Variation in a Population, and page 13 • Read Student Sheets page 15	**Resources:**

We recommend a demonstration class set of Arabidopsis plants! It will be very valuable to have a set of plants YOU sow weeks early, and ideally staggered in sowing time, for several reasons. Prior to student plant sets being ready, students can observe and handle Arabidopsis, become familiar with its traits, and explore its life cycle. In addition, a demonstration set can give students practice in collecting and recording plant data before they do so with their own plants. **This will require that you plan ahead and plant the demonstration set a few weeks in advance of students sowing their seeds.**

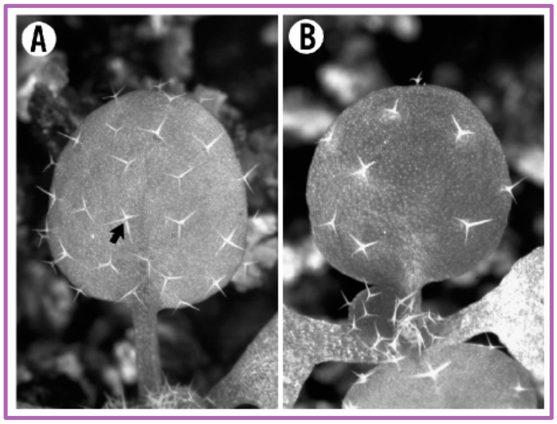
Basic – Lab 3:
Developing the research question(s)

<table>
<tr>
<td rowspan="2">Learning Goals:
• Students build on trait observation lab (Lab 2) and develop a research question(s) in relation to the Arabidopsis thaliana genetics module
• Students engage in dialogue in classroom and with online mentors</td>
<td>Materials:
• Student Sheets pages 16-18 – Lab 3: Developing the Research Question
• Student Sheets page 19 – Observations, Data and Pooling Data
• Lab notebooks, pencils
• Computers for posting to mentors</td>
</tr>
<tr>
<td>Procedure:
• Have students read Student Sheets pages 16-18, OR guide students through an interactive dialogue about the material.
• Break students into teams, and brainstorm research questions, using Student Sheets page 18 as a guide.
• Come back together as a class, and decide on what questions the whole class will investigate (with pooled data from all teams), and if individual teams will investigate their own questions (possibly also with pooled data).
• Refer to the teacher information on pages 21-22, which gives you more information about the traits than the students are given. Share with students at your discretion.
• Have students post their research questions on their PlantingScience web page to get feedback and input from mentors.
• TIP: Consider having students propose a research question to their mentors before solidifying it.</td>
</tr>
<tr>
<td>Timeline: 30 to 50 minutes (possibly combine with Lab 4), possible homework assignment (reading material)</td>
<td>Advance Preparation:
• Secure computers
• Print student pages</td>
</tr>
<tr>
<td>Teacher Background: Read Student Sheets pages 16-19. Also pages 21-22 in Teacher Guide. Also Linked Genes pages if relevant to student questions.</td>
<td>Resources:
• Gene Inheritance and Transmission: http://www.nature.com/scitable/topic/Gene-Inheritance-and-Transmission-23</td>
</tr>
</table>

Potential inquiry questions (not an exhaustive list): Are certain traits continuous (quantitative) traits, or discrete traits, and how would we tell the difference? Are certain traits in the RILs more similar to COL, to LER, or to neither? Is there evidence that any of the traits may be linked? Is there evidence that any of the traits are related to other traits not described here (such as leaf number, stem height, etc.)? Scientists have learned that a number of traits are linked to the erecta phenotype. They have also learned that flowering time appears to be linked to the number of rosette leaves. Can students find evidence for these linkages?

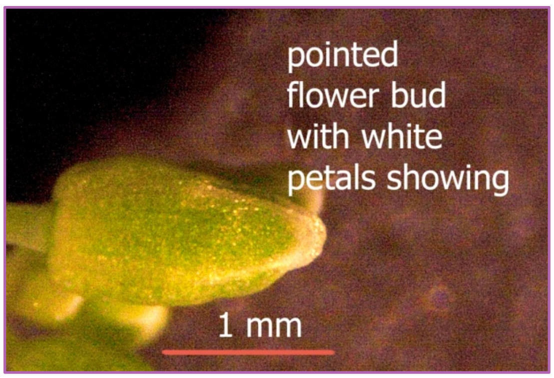
Continuous and Discrete Traits: The traits described on the following page have additional information about which traits are continuous and which are discrete. **Student sheets do not have this information – share with them at your discretion** (i.e., if they are investigating this question, let them discover the answers through their research).

Trichome (leaf hair) number: One of the *continuous* (*quantitative, polygenic*) traits is **trichome number**, and will show variation among individuals in a continuous pattern. This trait can best be quantified in the first true leaf at about 2 weeks after sowing the seeds.



Two naturally-occurring variants for leaf hairs: (A) Columbia and (B) Landsberg. The "cactus-like" 3-pronged hair on the surface of leaves vary in number. Columbia and Landsberg are naturally-occurring accessions of Arabidopsis. Accessions can be thought of as ecotypes, though Arabidopsis accessions may not follow the strict definition of ecotype. There are over 750 Arabidopsis accessions, which have been discovered all over the world (see map – http://www.arabidopsis.org/images/geo_distribution.png-)

Flowering Time: Another *continuous (quantitative, polygenic)* trait is **flowering time**, and will show variation among individuals in a continuous pattern. Flowering may begin about 21 DAS. This trait is also related to number of rosette leaves, a trait for which students may also wish to collect data.



The day that white petals are seen between the outer green sepals of the bud is considered the day of flowering. Advantages and disadvantages exist for early and late flowering time, and students can communicate with mentors to explore this idea.

Erecta plant architecture: The **"erecta" growth form** is a *discrete (qualitative, discontinuous)* trait, and shows Mendelian patterns of inheritance. This trait can be observed about 5 weeks after sowing the seeds. A number of traits have been found to be linked to the erecta phenotype.

University of Arizona Biotech Project
http://biotech.biology.arizona.edu/Scientific_Method/method_plants.html

The plant on the left shows the wildtype plant architecture where the stem-like structure that produces flowers is elongated. The flowers make this somewhat top heavy and the plants tend to droop and even fall over. The plant on the right shows the *erecta* phenotype, with a squat, sturdy architecture with less spacing between the flowers and mature seed capsule. The flowers are held upright in an erect fashion.

Basic – Lab 4: Understanding the experimental design

Learning Goals: • Students understand and explain the experimental design • Students understand the importance of controlling environmental variables • Students identify independent and dependent variables • Students understand the importance of replicates • Students understand how data analysis and experimental design are related • Students engage in dialogue in classroom and with online mentors	**Materials:** • **Student Sheets pages 10-11: Experimental Design** • Lab notebooks, pencils • Computers for posting to mentors **Procedure:** • Review the research question(s) • Have students read **Student Sheets pages 11-12**, **OR** guide the class in an active dialogue to establish the experimental design. • Check to make sure research question(s) can be potentially answered with this experimental design • Break students into teams, and have them write the experimental design in their lab notebooks. • Have students post experimental design onto their PlantingScience web page for discussion with mentors.
Timeline: 20 – 40 minutes, possible homework assignment (reading material)	**Advance Preparation:** • Secure computers • Print student pages (if needed)
Teacher Background: Read Student Sheets pages 10-11 – Experimental Design	**Resources:**

Note: The experimental design is essentially given to students in this guided inquiry. It is fruitful, however, that students understand the importance of the experimental design. Specifically, in order to investigate genetic influences on plants, certain conditions are controlled.

- The environmental conditions are controlled so that all plants ideally grow in the same environment. This would mean that differences in traits among the individual plants can be attributed to genes.
- The genetic make-up of the different seed lines have also been controlled in order for certain inferences to be made. For instance, the recombinant inbred lines (RILs) have been inbred in a way to try to ensure homozygosity of genes.

Basic – Lab 5:
Counting trichomes

About two weeks after planting and cold treatment, the plants will be ready for counting trichomes. The first structures you will see when the plants germinate are the two cotyledons. Cotyledons are not true leaves. The first true leaves will appear as a pair, and one of these will be the leaf on which students will count trichomes.

Learning Goals: • Students explore properties of continuous traits • Students recognize, identify, and understand the difference between cotyledons and first true leaves • Students recognize, identify and count trichomes • Students collect and record data accurately • Students practice good lab skills of data collection: repeating counts, relying on team members, and critical thinking • Students engage in dialogue in classroom and with online mentors	**Materials:** • Well labeled Arabidopsis plants in pots or soil pellets (divide total number of lines equally among the teams) • Dissecting microscopes (or hand lenses) • **Student Sheets pages 20-25 – Lab 5: Counting Trichomes** • Optional: Computers with data base software (such as Excel) for data entry **– Database template can be found on the PlantingScience website** **Procedure:** • Introduce subject of trichomes on Arabidopsis plants – see **Student Sheets page 20** • Clarify difference between cotyledon and first true leaf (see **Student Sheets page 20**) • Split students into teams • Divide plants equally among teams, keeping both pots of each RIL in the same team • Prior to counting trichomes, practice finding trichomes, stressing that *although each trichome has three prongs on it, they count each as only one trichome* • Hand out Data Collection Sheets and Instructions (**Student Sheets pages 22-23),** and help explain • Students count trichomes and record data • Clean up • Post to mentors about what they experienced
Timeline: Two 50 minute periods, possibly more depending on how many plants each student needs to count	**Advance Preparation:** • Collect materials • Print student pages – Multiple copies of Data Collection Sheet (**Student Sheets page 23**) may be necessary • Prepare classroom lab with dissection microscopes • Secure computers
Teacher Background: • Read Student Sheets pages 20-25 – Lab 5 • Read Teacher Guide page 25	**Resources:** • Partnership for Research and Education in Plants (PREP) Online Lab Notebook **http://prep.biochem.vt.edu/timeline/**

Teacher Notes for Counting Trichomes

Discussion questions

- What advantage or disadvantage would trichomes have for a plant?
- What advantage or disadvantage would greater or fewer trichomes have for a plant?
- What is the difference between cotyledons and true leaves?
- If you want a good count of something (e.g., trichome number) is one count enough? How many repetitions of counting is enough?
- How do scientists believe and have confidence in each others' methods?
- How can you represent data so it makes sense?

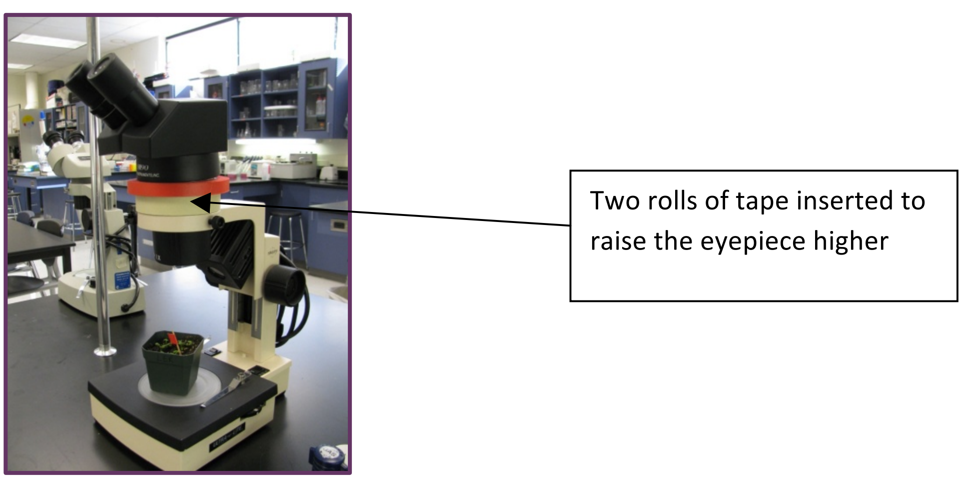
Function of trichomes

Trichomes, or plant hairs, are fine structures on the epidermis of plants. Trichomes are different from prickles and thorns. They have multiple functions in different types of plants, such as trapping moisture, deflecting light in desert environments, defending against insects, and secreting oils and other chemicals. In *Arabidopsis thaliana*, trichomes seem to function as deterrents against insects and other herbivores, though much still has to be learned. Geneticists have been able to determine certain genes that are involved in trichome development in Arabidopsis, and many of them are the same genes involved in root hair development.

Students may be inspired to do additional experiments to test the functions of trichomes in Arabidopsis.

Dissecting microscope problem and solution

One teacher testing this module used 2" plastic pots with germinating soil to grow Arabidopsis in his classroom. When it came time, however, for students to count trichomes using dissecting microscopes, they had trouble focusing so high in the field of view. The soil level was not close to the stage, but rather 2-3" higher than the stage, and thus higher than the natural focal point of the microscope. He solved the problem by placing rolls of masking and labeling tape between the eyepiece and the base of the scope, as shown in the photo below.



Basic – Lab 6: Analyzing trichome data

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Learning Goals:

- Students organize and analyze data
- Students are able to read and understand graphs
- Students construct graphs to represent data in meaningful ways
- Students interpret graphs
- Students explore patterns in data
- Students recognize trichome data as having properties of continuous traits (expression along a gradient)
- Students engage in dialogue in classroom and with online mentors

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Materials:

- Completed Trichome Data Collection Sheets
- White board, poster paper or other means for pooling class data (see database options below)
- Graph paper and Lab Notebooks
- **Student Sheets page 24 – Analyzing Data and Looking for Patterns**
- **Student Sheets pages 25-29 – Lab 3: Analyzing Trichome Data**
- Optional: Computers with data base software (such as Excel) for graphing **– Database template can be found at PlantingScience website**
    - Template contains two worksheets – tabs are found at the bottom of the worksheets
    - "Team Data" worksheet is the same as the student Data Collection Sheets
    - "Pooled Class Data" is a template for pooling class data
- Computers for posting to mentors

Procedure:

- Introduce subject of analyzing data – **Student Sheet page 24**
- Pool data from class together and distribute to students
- Clarify difference between categorical data and continuous data
- Have students represent their data using graphs of their choice on graph paper. Discuss their graphs.
- Introduce means of analysis as described in **Student Sheets pages 25-29 – Lab 6: Analyzing Trichome Data**
- Have students construct frequency distribution graphs
- Discuss results
- Based on results, discuss whether trichome number is a discrete or a continuous trait (it is a continuous trait)
- Compare data from RILs to the two parent lines (COL and LER)
- Optional: Discuss concepts from **Teacher Guide pages 32-35 – Background Information: Patterns of Inheritance**
- Post to mentors – upload files (database files with graphs, or photos of hand-made graphs)

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Timeline: One to two 50 minute periods, possibly more depending on whether you include the Optional discussion

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Advance Preparation:

- Print student pages
- Have students bring data sheets
- Prepare materials
- Secure computers

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Teacher Background:
Read Student Sheets pages 25-29. Also Teacher's Guide pages 32-35.

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Resources:

- Genetics and Statistical Analysis: **http://www.nature.com/scitable/topicpage/Genetics-and-Statistical-Analysis-34592**

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Extended – Lab 7: Recording and analyzing flowering time

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Learning Goals:

- Students explore properties of continuous traits
- Students recognize, identify and record flowering time as outlined in the protocol
- Students collect and record data accurately
- Students organize and analyze data
- Students are able to read and understand graphs
- Students construct graphs to represent data in meaningful ways
- Students interpret graphs
- Students explore patterns
- Students recognize flowering time data as having properties of continuous traits (expression along a gradient)
- Students engage in dialogue in classroom and with online mentors

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Materials:

- Well labeled Arabidopsis plants in pots or soil pellets (divide total number of lines equally among the teams to monitor)
- Lab Notebooks
- **Student Sheets page 30-33 – Lab 7: Recording Flowering Time**
- **Video** – made from time-lapse photos of Arabidopsis plant growth and flowering (with time since sowing labeled on the images) – **can be found at PlantingScience website**
- Optional: Computers with database software (such as Excel) for data entry **– Database template can be found at PlantingScience website**

Procedure:

- Introduce subject of flowering time in Arabidopsis plants – see **Background on Student Sheets pages 32-33**
- Go over protocols **Lab 7 on Student Sheets pages 30-31**
- Show video of time-lapse **(found at PlantingScience website)** to help in estimating
- Set up daily (or twice daily) monitoring schedule and assign plants to student teams
- Students monitor flowering time record data over 7-10 days
- Students analyze data preparing graphs, looking for patterns. For guidance to graphing options, refer to **Student Sheets pages 25-29 – Analyzing Trichome Data,** and **Student Sheets pages 32-33 – Variation in Flowering Time as a Continuous Trait**
- Based on results, discuss whether flowering time is a discrete or a continuous trait (it is a continuous trait)
- Compare data from RILs to the two parent lines (COL and LER)
- Post to mentors – upload files (database files with graphs, or photos of hand-made graphs)

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Timeline:

- 30-50 minutes to introduce theme and protocol
- Daily monitoring of plants once they begin to flower for 7-10 days
- 50-90 minute period to analyze data and post to mentors

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Advance Preparation:

- Collect materials
- Print student pages – Multiple copies of Data Collection Sheet (Page 31) may be necessary
- Prepare classroom lab
- Secure computers
- Download video of time lapse photos of Arabidopsis growth and development from PlantingScience website

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Teacher Background:

- Read Student Sheet pages 30-33, and review Lab 6 Student Sheets pages 25-29

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Resources:

- Partnership for Research and Education in Plants (PREP) Online Lab Notebook **http://prep.biochem.vt.edu/timeline/**

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Extended – Lab 8:
Bolting plants and the erecta phenotype

This section explores a phenotype that appears to be distinct and discrete, coded by one specific gene. The erecta plant architecture (see photo at right) is a trait that only shows up after the plant bolts (sends up the stem for flowering), and so plant development is also a part of this exploration. **The activity assumes students already have been introduced to discrete traits and the use of Punnett Squares.**

Learning Goals: • Students explore and understand properties of discrete traits • Students predict discrete trait ratios of an F2 generation assuming homozygous parents using Punnett squares • Students recognize, identify, and understand the growth forms of *Arabidopsis thaliana* • Students collect and record categorical data accurately • Students compare their predictions to actual data, and make sense of similarities and differences • Students represent data in table, graph, diagram so results are clear • Students engage in dialogue in classroom and with online mentors	**Materials:** • Well labeled Arabidopsis plants in pots or soil pellets (divide total number of lines equally among the teams) • Lab Notebooks • 100 clear transparency sheets (one for each soil pellet or pot) • **Student Sheets pages 34-35 – Lab 5: Bolting Plants and the Erecta Phenotype** • Optional: Blank monohybrid cross Punnett square worksheets (or have students draw these simple charts) **Procedure:** • Introduce subject of bolting developmental stage, and the erecta phenotype in Arabidopsis plants – see **Student Sheet page 34** • Split students into teams • Have students work through **Student Sheet pages 34-35** o Make predictions about erecta trait using Punnett squares, assuming homozygous parents (even though homozygosity not necessarily true in COL and LER) o Score plants as having erecta or wildtype phenotype o Pool class data o Compare predictions to results o Represent data in table, graph, etc. • Clean up • Post to mentors about what they experienced
Timeline: Two 50 minute periods, or one 90 minute period.	**Advance Preparation:** • Collect materials • Print student pages • Prepare classroom lab • Secure computers
Teacher Background: • Read Student Sheets pages 34-35 – Lab 5: Bolting Plants and the Erecta Phenotype	**Resources:** • Partnership for Research and Education in Plants (PREP) Online Lab Notebook **http://www.prep.biochem.vt.edu/expinfo/expinfo_anatomy.html** • Test Crosses: **http://www.nature.com/scitable/topicpage/Test-Crosses-585** • University of Arizona Biotech Project **http://biotech.biology.arizona.edu/Scientific_Method/method_plants.html**

Extended – Lab 9: Analyzing data to investigate linked traits

<table>
<tr>
<td rowspan="2">Learning Goals:
• Students organize and analyze data
• Students are able to read and understand graphs
• Students construct graphs to represent data in meaningful ways
• Students interpret graphs
• Students explore patterns in data
• Students recognize data on two traits (one continuous and one discrete) as being linked or not
• Students engage in dialogue in classroom and with online mentors</td>
<td>Materials:
• Completed pooled class data for a continuous trait
• Complete pooled class data for a discrete trait (e.g., erecta vs. floppy growth form)
• Graph paper and Lab Notebooks
• Optional: Student Sheets page 24 – Analyzing Data and Looking for Patterns
• Student Sheets Pages 36-37 – Lab 9: Analyzing Data to Investigate Linked Traits
• Optional: Computers with data base software (such as Excel) for graphing
• Computers for posting to mentors</td>
</tr>
<tr>
<td>Procedure:
• Review (or introduce if not done yet) concepts of linked genes (see Engage Lesson Option 4, page 17 of Teacher Guide; and Student Sheet Pages 6-9)
• Introduce subject of analyzing data – Student Sheet page 24
• Make sure each team has tables of the continuous trait (e.g., trichome data or flowering time) and the discrete trait (e.g., erecta vs. floppy)
• Clarify difference between continuous data (continuous trait) and categorical data (discrete trait)
• Follow the steps outlined in Student Sheets pages 36-37
• Have students construct graphs
• Discuss results
• Based on results, discuss whether there is evidence that the traits are linked or not linked
• Post conclusions on website
• Post to mentors – upload files (database files with graphs, or photos of hand-made graphs)</td>
</tr>
<tr>
<td>Timeline: One to two 50 minute periods</td>
<td>Advance Preparation:
• Print student pages
• Have students bring data sheets
• Prepare materials
• Secure computers</td>
</tr>
<tr>
<td>Teacher Background:
• Read Student Sheets Lab 9: pages 36-37
• Read background to genetic linkage Student Sheets pages 6-9</td>
<td>Resources:
• Gene Inheritance and Transmission: http://www.nature.com/scitable/topic/Gene-Inheritance-and-Transmission-23</td>
</tr>
</table>

Extended – Lab 10: Collecting seeds

Learning Goals: • Students recognize, identify, and understand the growth stages of *Arabidopsis thaliana* from seed to seed • Students collect and label seeds • Students engage in dialogue in classroom and with online mentors	**Materials:** • Well labeled Arabidopsis plants in pots or soil pellets (divide total number of lines equally among the teams) • Lab Notebooks • **Student Sheets page 38 – Lab 10: Collecting Seed** • 50 vials to place collected seeds in • Sharpies to label vials • Clear white paper (1 per 4 students) **Procedure:** • Introduce subject of collecting seeds from Arabidopsis plants – see **Student Sheet page 38** • Split students into teams • Have students work through **Student Sheet page 38** • Clean up • Post to mentors about what they experienced
Timeline: One 50 minute periods.	**Advance Preparation:** • Collect materials • Print student pages • Prepare classroom lab • Secure computers
Teacher Background: • Read Student Sheets page 38 – Lab 10: Collecting Seeds	**Resources:** • Partnership for Research and Education in Plants (PREP) Online Lab Notebook **http://www.prep.biochem.vt.edu/expinfo/expinfo_anatomy.html** • Handling Arabidopsis plants and seeds, Arabidopsis Biological Resource Center, Ohio State University: **http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/handling.htm**

Photo from Creative Commons, author *User:Roepers at nl.wikipedia*

SENSE-MAKING ACTIVITIES

What does your evidence show?
Does it answer your research question(s) and prediction(s)?
How can you tell the difference between a continuous and a discrete trait?
How did the traits in the RILs compare with the parent lines?
Did you find evidence that any of the traits might be linked?
How do I represent my findings so they make sense?
How do I share my findings so that I can get good feedback from others?
What new questions has my research generated?

Allow time for sense-making and sharing

A critical element to scientific practice is sharing results, contemplating the results and conclusions, and soliciting feedback from others. In the context of a busy classroom, these important steps can be easily minimized. However, this is where student learning can be most profound. We encourage you to allow time for students to share, collect feedback, and reflect on their findings and conclusions during the experiments and at the end. The PlantingScience scientist-mentors cherish the opportunity to comment on student results and conclusions! Here are some ways teachers use to highlight these important elements of science.

- Students (or teams) prepare PowerPoint presentations to share in class.
- Students (or teams) prepare posters to share in class.
- Students (or teams) create drawings of the *Arabidopsis thaliana* life cycle, with a timeline of when specific traits are measured and/or scored. For each trait, have students draw different phenotypic expressions as seen in the RILs and parent lines.
- Students create a chart for each trait they examined. On each chart, students diagram how closely related the traits of each RIL are in expression to each parent (COL and LER). Do they see evidence of linked traits?
- Students write and share a reflection paper on what they learned, what was surprising, what new questions this project generated, and how they might test these questions.

Please allow for these assignments to be uploaded to scientist-mentors, *and* for mentors to have time to give feedback to students. Files such as documents or PowerPoints can be uploaded directly to the PlantingScience student team pages. Photos of posters or charts can also be uploaded.

Background Information: Patterns of Inheritance

Traditionally, genetics is introduced to students by reviewing meiosis in the formation of gametes through **discrete traits** and the use of Punnett Squares. This makes sense because discrete traits, which are the result of a single gene, are so tractable. Most traits, however, are the result of several genes interacting, and are termed **continuous** (or quantitative) **traits.** Genes involved in these traits often work in concert in an additive manner.

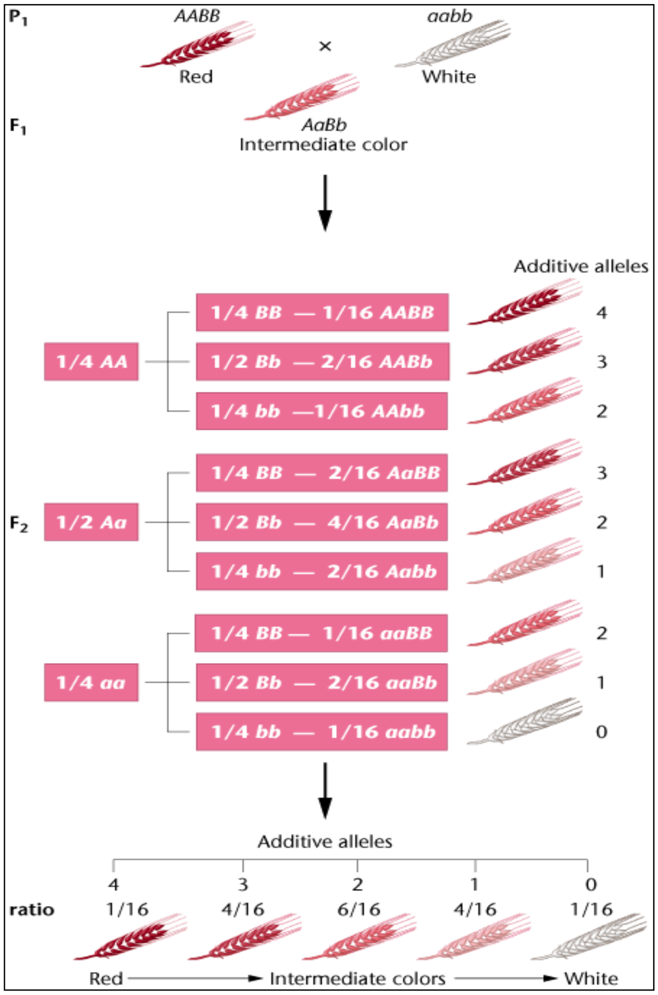
You can use Punnett Squares to predict the expected ratios of discrete traits in offspring of homozygous parents. You can hypothesize the nature of a particular trait you notice in the Arabidopsis plants, or one that we will be specifically investigating (trichome number, erecta plant architecture). If you predict that a trait is discrete, you can use Punnett Squares to predict the ratios you would expect to see among the RILs, assuming one parent (Columbia or Landsberg) is homozygous for the trait. You can later compare your actual data with your predictions to decide if you have evidence supporting or not supporting their hypothesis.

Relationship of Discrete and Continuous Traits

After understanding the inheritance of discrete traits, it may be helpful to understand that continuous traits are inherited in a similar manner. The difference is that multiple genes interact to produce a single trait.

Consider the following hypothetical case where two genes (and four alleles) are co-dominant for seed color (see figure on following page). **Given a heterozygous parent with *AaBb***, where *A* and *B* are red determinants for color, and *a* and *b* are white determinants for seed color, we can construct a dihybrid Punnett Square to predict the ratios of offspring.

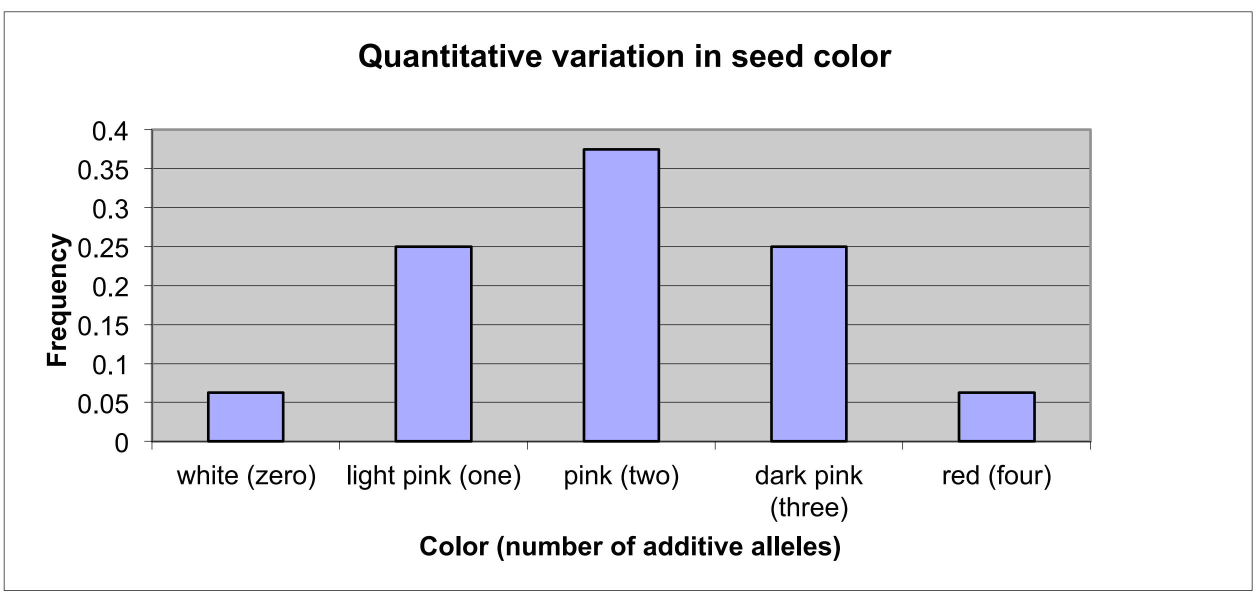
Punnett Square		Sperm			
		ab	aB	Ab	AB
Egg	ab	aabb (white)	aaBb (light pink)	aAbb (light pink)	aAbB (pink)
	aB	aaBb (light pink)	aaBB (pink)	aABb (pink)	aABB (dark pink)
	Ab	Aabb (light pink)	AabB (pink)	AAbb (pink)	AABb (dark pink)
	AB	AaBb (pink)	AaBB (dark pink)	AABb (dark pink)	AABB (red)



In the hypothetical case illustrated at left, the F2 proportions of phenotype have been figured from the Punnett Square above.

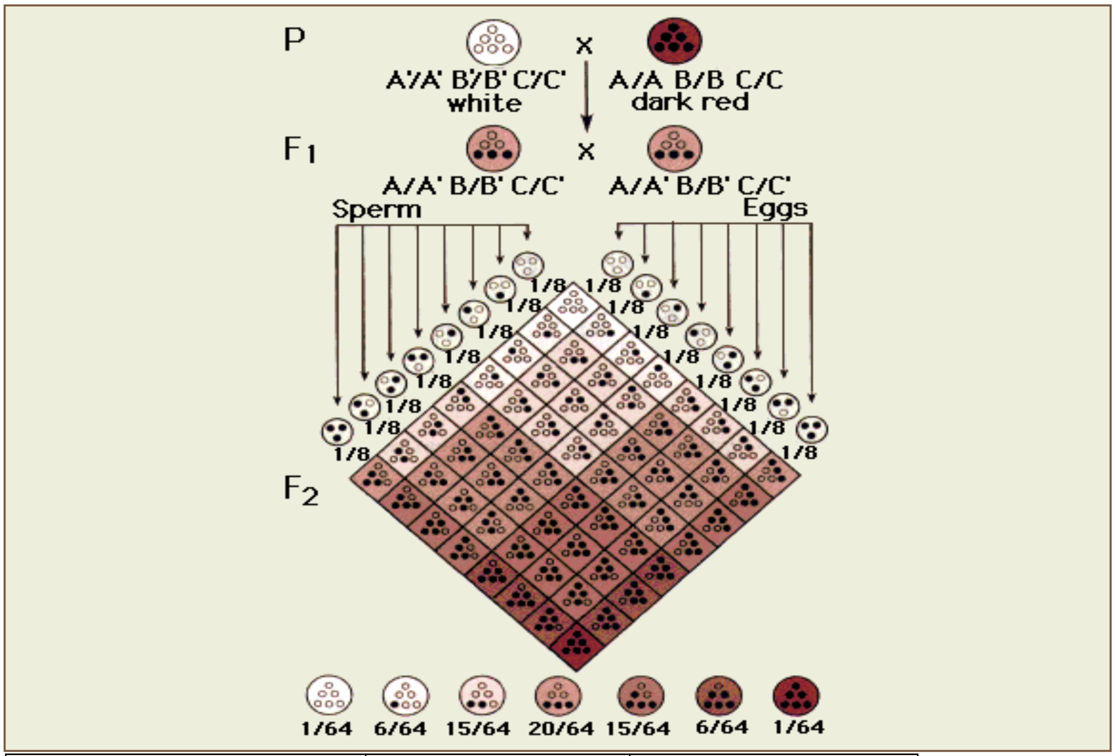
Color (# of additive red alleles)	Number of individuals	Frequency
White (zero)	1	0.0625
Light pink (one)	4	0.25
Pink (two)	6	0.375
Dark pink (three)	4	0.25
Red (four)	1	0.0625
Sum	16	1.0000

To find the number of genes contributing additively to a polygenic character, look at the mean of the distribution. Note that the mean number – the midpoint of the distribution – is two additive alleles. If the two alleles contribute equally to the phenotype, then the mean is the number of genes involved. In this case there are two genes involved and the mean has two additive alleles.



Now let's take the actual case of seed color of hexaploid wheat studied by Herman Nilsson-Ehle in Swedon (1909). Many organisms are diploid, meaning they have 2 sets of homologous chromosomes. A hexaploid has 6 sets of homologous chromosomes. Wheat is a stable hybrid of three different diploid species. This is called an allopolyploid.

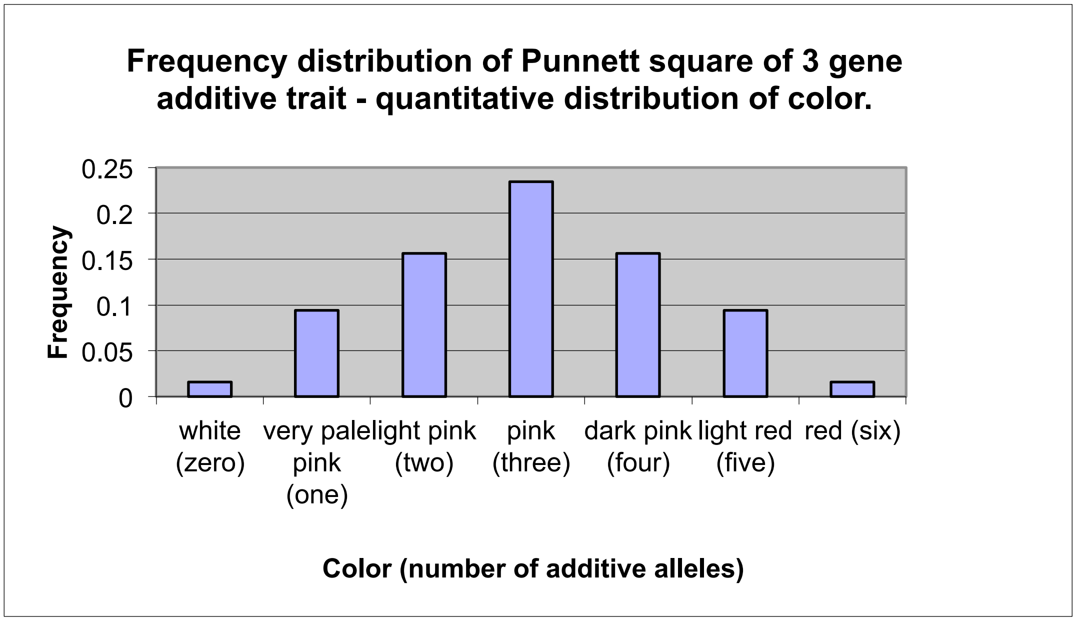
We will call the red alleles A, B, and C, and the white alleles A', B' and C'. These alleles contribute to seed color additively. The figure below shows the Punnett Square of the outcome of an F2 cross.

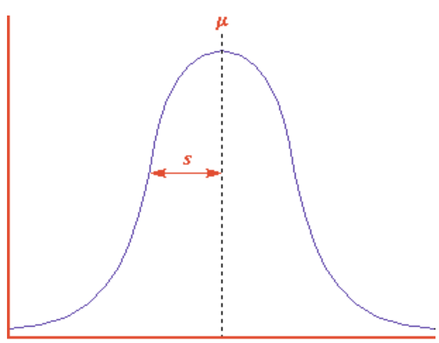


Color (number of additive alleles)	Number of Individuals	Frequency
White (zero)	1	0.015625
Very pale pink (one)	6	0.09375
Light pink (two)	10	0.15625
Pink (three)	15	0.234375
Dark pink (four)	10	0.15625
Light red (five)	6	0.09375
Red (six)	1	0.015625
Sum	64	1.000000

Continued on next page

We can then plot the frequency distribution predicted by the Punnett Square of the 3 gene additive trait (frequencies from the table on previous page). As each allele contributes to the color, and 6 alleles are involved in all, we see the distribution of a simple quantitative trait.





Add in a little environmental variation and human inability to absolutely determine color differences, and the steps become "smoothed out", as many quantitative traits do when their phenotype frequencies are plotted.

The basic form of the continuous, rather than step-wise, curve is called a bell curve, or Gaussian curve (left). It has two important properties – the **mean**, or midpoint, **μ** (pronounced *mu*). The mean can be related to the number of genes controlling the character. The other important property is the width of the curve, ***s***, or phenotypic **variance**. Variance is a measure of the spread or range of values. Generally speaking, if you have two similar sets of data, and one has a wider range of values, it will have a larger variance. The square root of the variance is called the standard deviation. The phenotypic variance is due to both genetics and the environment.

$$Vt = Vg + Ve$$

total variance = genetic variance + environmental variance

To determine the environmental variance, V*e*, make the line inbred so there is little or genetic variance between individuals. All of the variance then is due to environmental variance.

Instructions:
Light box construction for growing plants

Overview

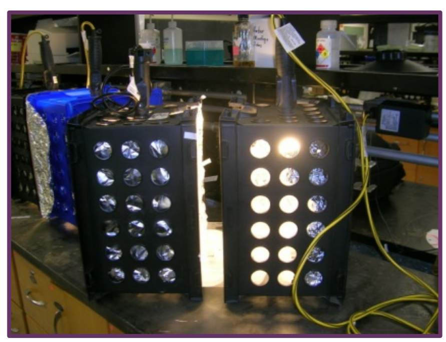
Two plastic filing crates (AKA "milk crates") will be positioned on their sides, long side vertical. The open sides will face each other, creating an enclosed space. A screw-in fluorescent light will be fixed at the top of each upended crate, and they will be lined on the sides with aluminum foil. You will need 2 light boxes, i.e., 4 plastic crates, per class.

Materials for each light box (need two light boxes – 4 plastic crates – per class):
From office or big-box store:

- 4 Light Housings: Home Series Incandescent Trouble Light. 15 ft. 18/2 gauge (cord thickness) – unscrew protective head. $6 ea. X 2 = $24
- 4 Plastic File Crates: Sterilite Officeware Legal.Letter 1693 Black (or other color). $5 ea. X 2 = $20
- 4 Fluorescent Screw-In Light Bulbs: Sylvania (or other brand) 23W 120V 60Hz 0.330 A CF23EL/Mini. Basically the equivalent of a 100W screw-in incandescent light bulb (could also probably use the 18W, 75-Watt equivalents, but these would be less desirable.) $2 ea. X 2 = $8

Construction of Light Box

1. Turn plastic crates on ends, long sides vertical
2. Unscrew protective head of light housings
3. Place light housing on outside of crate, at a hole in the middle of the "top" of each crate as it stands upended
4. Screw the fluorescent light bulb from the *inside* of the crate into the housing. The plastic crate should be in between the housing and the light. Do this for each crate.
5. The inside space will be where the trays of Jiffy plugs will be placed.
6. Plug in the light housings, and you're good to go!



The light housing is on the outside of the crate, and the light bulb on the inside. You're good to go!

Resources and References

About *Arabidopsis thaliana* – life cycle, traits, anatomy, cultivation

- Partnership for Research and Education in Plants (PREP) Online Lab Notebook: http://www.prep.biochem.vt.edu/
    - *Arabidopsis* timeline: http://www.prep.biochem.vt.edu/timeline/
    - Growth and development: http://www.prep.biochem.vt.edu/expinfo/expinfo_growth.html
    - Anatomy: http://www.prep.biochem.vt.edu/expinfo/expinfo_anatomy.html
    - Growing *Arabidopsis*: http://www.prep.biochem.vt.edu/expinfo/expinfo_videos.html
- Video of Arabidopsis growth: http://www.arabidopsis.org/info/arabgrowth.mov
- The Arabidopsis Information Resource (TAIR): http://www.arabidopsis.org/portals/education/aboutarabidopsis.jsp
- TAIR Video of Arabidopsis seed germinating: http://www.arabidopsis.org/portals/education/movies/germination.mov
- Arabidopsis Biological Resource Center: http://abrcoutreach.osu.edu/
    - Handling *Arabidopsis* plants and seeds, Arabidopsis Biological Resource Center, Ohio State University: http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/handling.htm
- Plants in Motion time lapse video of early leaf development: http://plantsinmotion.bio.indiana.edu/plantmotion/starthere.html
- University of Arizona Biotech Project http://biotech.biology.arizona.edu/Scientific_Method/method_plants.html
- Geographic distribution of *Arabidopsis thaliana* ecotypes: http://www.arabidopsis.org/images/geo_distribution.png
- Wyatt, S. and Ballard, H. 2007. Arabidopsis ecotypes: A model for course projects in organismal plant biology and evolution. American Biology Teacher.69: 477-481.

As a model organism:

- National Institute of Health, Model Organisms http://www.nih.gov/science/models/
- National Center for Biotechnology Information, Model Organisms http://www.ncbi.nlm.nih.gov/About/model/index.html
- Howard Hughes Medical Institute, A Brief Guide to Model Organisms http://www.hhmi.org/genesweshare/e300.html
- The Arabidopsis Information Resource (TAIR), Education and Outreach, About Arabidopsis http://www.arabidopsis.org/portals/education/aboutarabidopsis.jsp
- Partnership for Research and Education in Plants (PREP) Online Lab Notebook http://prep.biochem.vt.edu/timeline/
- Video introduction to Arabidopsis as model research plant by PREP http://www.youtube.com/watch?v=foHiKrlY9Qc&list=UUQgD6eojgUpAe4x3vmO4hOw&index=10&feature=plcp

General genetics:

- Gene Inheritance and Transmission: http://www.nature.com/scitable/topic/Gene-Inheritance-and-Transmission-23

Analyzing traits:

- Video tutorial – Measuring Leaf Area with Adobe Photoshop 3: http://youtu.be/E3O-V6WLw0g
- Genetics and Statistical Analysis: http://www.nature.com/scitable/topicpage/Genetics-and-Statistical-Analysis-34592
- Test Crosses: http://www.nature.com/scitable/topicpage/Test-Crosses-585

Traits determined by genes or environment:

- Stanford Encyclopedia of Philosophy, The Genotype/Phenotype Distinction, Richard Lewontin http://plato.stanford.edu/entries/genotype-phenotype/
- Gene, Organism and Environment, Richard Lewontin – YouTube video http://www.youtube.com/watch?v=we4ZzjKxFHM
- Scitable article: http://www.nature.com/scitable/topicpage/Phenotypic-Range-of-Gene-Expression-Environmental-Influence-581

Linked genes:

- Meiosis (short animated video): http://youtu.be/D1_-mQS_FZ0
- Griffiths AJF, Miller JH, Suzuki DT, et al. An Introduction to Genetic Analysis. 7th edition. New York: W. H. Freeman; 2000. Sex chromosomes and sex-linked inheritance: http://www.ncbi.nlm.nih.gov/books/NBK22079/
- Griffiths AJF, Miller JH, Suzuki DT, et al. An Introduction to Genetic Analysis. 7th edition. New York: W. H. Freeman; 2000. The discovery of linkage: http://www.ncbi.nlm.nih.gov/books/NBK22076/
- Griffiths AJF, Miller JH, Suzuki DT, et al. An Introduction to Genetic Analysis. 7th edition. New York: W. H. Freeman; 2000. Linkage maps. http://www.ncbi.nlm.nih.gov/books/NBK21827/
- Scitable article on genetic linkage: http://www.nature.com/scitable/topicpage/thomas-hunt-morgan-genetic-recombination-and-gene-496

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