

# Traits and Phenotype

## What is a trait?

Biologists use the terms “trait” and “phenotype” interchangeably. They both describe the expressed characteristics of an organism. The color of a leaf, the height of a plant, the smell of a flower – these are all traits. This is not the same as an organism’s genotype, although traits are clearly influenced by genes. The traits of an organism are influenced by the environment as well. Think of a scar on a leaf due to insect herbivory. Or how the amount of sunlight affects the way a plant grows.

The following activity explores this theme.



### Writing or Discussion Activity:

#### Nature or Nurture?

How much do you think you are determined by your genes? How much by your environment?

How much do you think plants are determined by their genes? How much are they determined by their environments?

Does timing matter? For instance, are genes or the environment more important at certain times in life? In the very beginning? When they reproduce? When they die?

What do you think? Use examples to support your opinion.

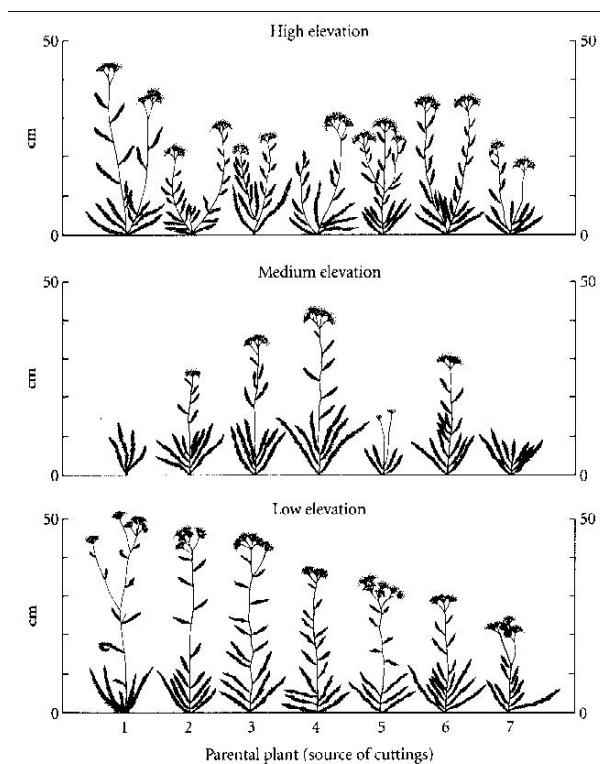
## Variation of Traits

“Variation of traits” refers to the differences in traits among individuals in a *population*. A population is a group of organisms of the same species living in the same place at the same time. *How* we define “same place” is open to interpretation. An ecologist thinks of a population as plants of a certain species in a natural area. A lab scientist thinks of the plants in her growth chamber. Another may be interested in the plants just in one pot. However we define it, some of the plants in our population will be taller, some shorter. Some will have bigger leaves, some smaller.

The variation we find in traits in a population may have a *genetic basis*. One plant may have genes that give it a propensity to grow taller than others. Variation may have an *environmental basis*. One plant may grow taller because it receives more sunlight and has access to more water or nutrients.

How can we tell what influences we are dealing with? Geneticists try to control the variance in the genes, in environment, or in both.

If we’re investigating environmental variance, we want to control the genetic variance – ideally we’d have clones with the exact same genotype. In fact, the famous scientists Jens Clausen, David Keck, and William Heisey took clones of the same plant and grew them at different altitudes in California, and demonstrated the importance that environment has on producing different phenotypes.



From *An Introduction to Genetic Analysis* by Suzuki et al., 1996 by W.H. Freeman and Company, as appears in *The Triple Helix* by R. Lewontin, 2000, by Harvard University Press.

One of Clausen, Keck and Heisey’s experiments is represented in the diagram to the left. Clones of the plant, *Achillea*, were grown at different elevations.

These scientists started with seven different plants, some that were naturally found at high elevations, some at middle ranges, and some at low elevations. Cuttings (clones) were made from each of the seven plants and grown at the different elevations. On the x-axis, the plants are numbered 1-7. Three clones of each line are represented at each of the three elevations.

This is an example of scientists controlling the genetic variation (using clones), and varying the environment. Additionally, since seven clones were used, they also had seven sources of genetic variation. The same genotype grown in different environments produced different phenotypes.

## Model Organism, Ecotypes and RILs

*Arabidopsis thaliana*, sometimes known as mouse-ear cress, is considered a model organism because a large community of scientists has investigated this small plant and a great deal is known about it. This makes it easier to investigate more complex questions about the plant, and much of what is known about *Arabidopsis* applies to other living organisms.



*Arabidopsis* is an ideal model organism because it has a short life cycle (approximately 6 weeks), and can be easily grown in laboratories. *Arabidopsis thaliana* has one of the smallest genomes known in plants: only 157 million base pairs among 5 pairs of chromosomes! In the year 2000, it became the first plant genome to be sequenced. With the structure of the genome identified, the functions of the genes are now being investigated and mapped.

*Arabidopsis thaliana* grows wild in Eurasia and parts of northern Africa. Scientists noticed that the plant traits varied among different environments, such as flowering earlier or later in the year, growing taller or shorter, and possessing greater or fewer leaf hairs (trichomes). Plants of the same species that have distinct traits in a local region are called *ecotypes*. **The two parent lines in this experiment, Columbia and Landsberg, are two ecotypes of *Arabidopsis thaliana*.**

This experiment also uses numerous **recombinant inbred lines (RILs)** developed from crossing the two parent lines, Columbia and Landsberg. Since *Arabidopsis thaliana* is naturally a self-pollinating flower, it is relatively easy to control the genetic inheritance of each generation. Once the original cross is made between the parents (Columbia X Landsberg), the resultant seeds are planted, cultivated, and allowed to **self-pollinate**. The resultant F1 seeds are planted, cultivated, and again allowed to **self-pollinate**. **This process is repeated at least 8 times to develop RILs.** Why? Because each time the plant self-pollinates, it becomes more homozygous and less heterozygous. This technique of controlling the genotype makes it easier to study genes since much of the genetic variation has been eliminated. The **RIL numbers are carefully tracked** with their phenotypic traits, and using molecular techniques, with their genes. These types of techniques allow scientists to map traits onto specific genes or – more often – onto multiple genes interacting together.



Seeds for each RIL will come in a small vial with a number on it (left). These will be planted into soil pellets and labeled (right).

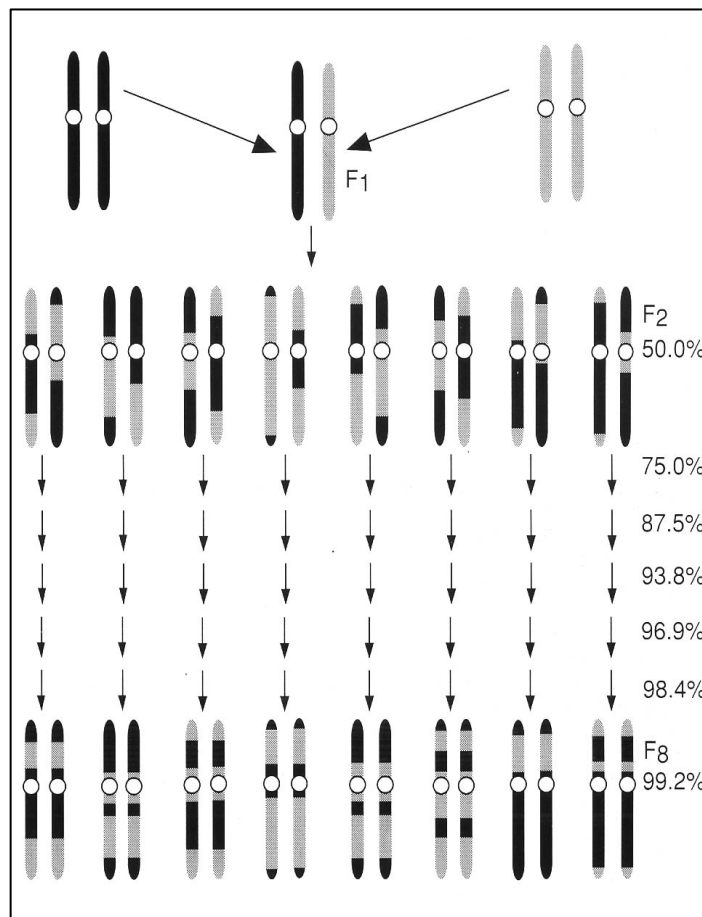
## Inheritance and RILs in Arabidopsis

Using clones is one way of creating different individuals that have little or no genetic variance between them. When comparing the same clone, all the variance then is due to environmental variance.

Another way to limit genetic variance between individuals is to make inbred lines. Geneticists use this method frequently. These inbred lines are called Recombinant Inbred Lines (RILs). Do not be confused by the term “recombinant” in the RILs. They do not contain any foreign recombinant DNA. They are only recombinant in a traditional genetic sense.

In creating RILs, two parents are first crossed. After the original cross, the F1 offspring are then self-fertilized for 8 generations. Selfing each generation reduces the genetic variance.

When a heterozygous individual is self-fertilized, half of the offspring become homozygous. In each subsequent generation, those lines that are homozygous remain homozygous. Those that are heterozygous have a 50-50 chance of becoming homozygous. After eight generations, there is 0.8 percent of the initial heterozygosity (see figure below).



This diagram shows an increase in homozygosity for each generation during the production of RILs. Each of the inbred lines, the RILs, are genetically different from each other. Hence, a large collection of inbred lines show almost as much variation as the initial F1 population, but each line is nearly homozygous.

Within one line, however, all the individuals are essentially identical. So, virtually all the variance we see in phenotypes within a RIL is due to the environment.

The seeds sent to you include the two parent lines, Columbia and Landsberg, and numerous RILs. The difference in the creation of the RILs in these lines than in the description above is that Columbia and Landsberg **are not homozygous**. They start with some amount of heterogeneity. So the RILs we are looking at are more similar to an F2 generation than the F1.

# Model Organism, Ecotypes, RILs

## Study Questions



### Model Organisms

1. What is a model organism?
2. How are model organisms used?
3. What model organisms besides *Arabidopsis thaliana* are there?
4. What are the benefits to using model organisms?
5. What are the limitations to using model organisms?

### Ecotypes

1. Why do you think ecotypes of *Arabidopsis thaliana* arise?
2. What traits might be more adaptive in colder or warmer climates?
3. What traits might be more adaptive in drier or wetter climates?
4. In what ways do you think ecotypes might be different from other ecotypes of the same species?
5. Do you think some of these traits might be related to each other, for instance, certain traits always appear together, or never appear together (for example, small leaves and purple color)?
6. If some traits tend to be inherited together (for example, small leaves and purple color), why do you think that might happen?
7. If you think some traits might be linked, how could you investigate that question?

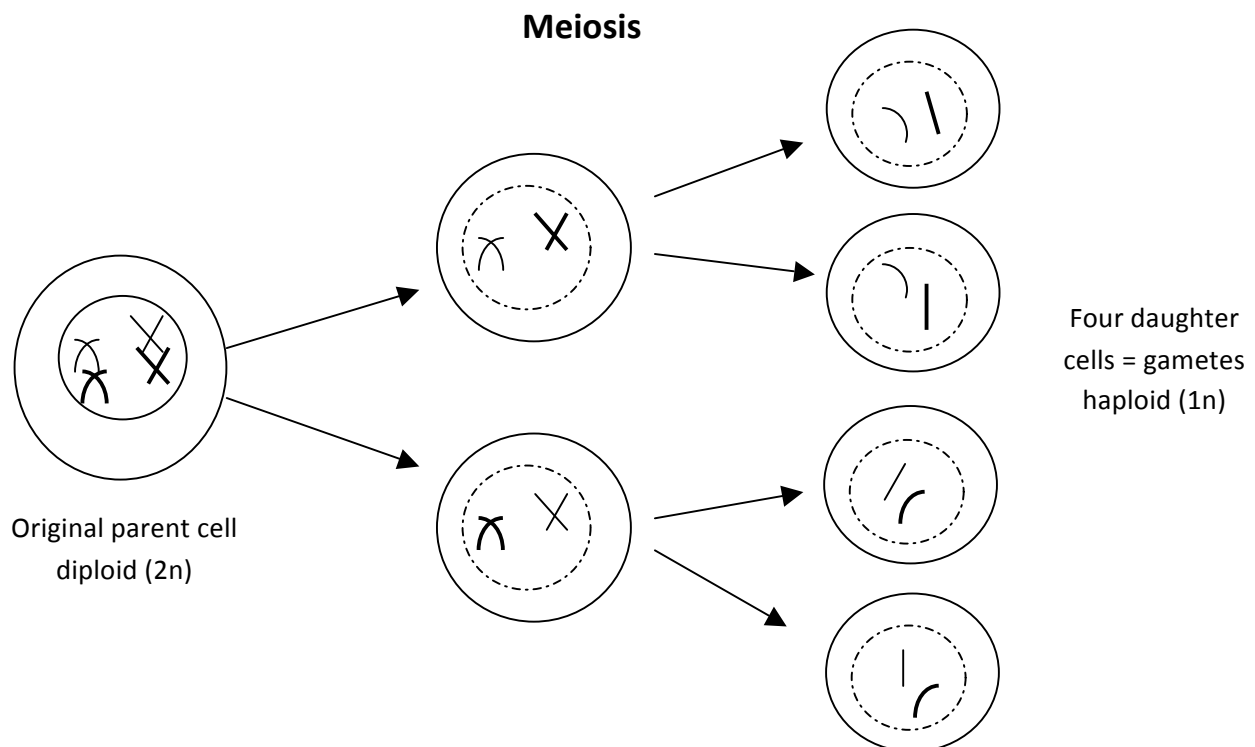
### Recombinant Inbred Lines (RILs)

1. *Arabidopsis thaliana* primarily self-pollinates. What does that mean?
2. How are RILs developed?
3. How does self-pollination help in the development of RILs? How might it be more difficult to develop RILs with a plant that is typically outcross-pollinated?
4. What are the advantages to RILs?
5. What are some characteristics of RILs?

## Genetic Linkage, Recombination and RILs

An organism creates gametes (egg and sperm) through the process of *meiosis*. Recall that meiosis is cell division in which the number of chromosomes in each daughter cell is half the number as in the original cell. Then, when a sperm cell fertilizes an egg cell, the full number of chromosomes is restored. Two chromosomes that have the same genes but that have been inherited from different parents (and therefore may have different alleles) are considered homologous chromosomes, or homologs.

During meiosis, homologous chromosomes sort themselves independently, and the daughter cells can receive unique combinations of chromosomes, some inherited from the mother, and some inherited from the father. Alleles that are on the same chromosome tend to be inherited together, and these traits are considered **linked**. The simple representation below of meiosis illustrates independent sorting of chromosomes. Homologous chromosomes are represented by being the same shape. The darker color represents a homolog inherited from one parent, and the lighter color represents a homolog inherited from the other parent.



You may have studied sex-linked genes, such as male pattern baldness or female elbow angle. These are traits that are inherited with a certain chromosome – they are linked to that chromosome, in this case a sex chromosome. The different traits that Mendel used are completely independently segregated because they are on different chromosomes and the chromosomes are independently segregated during meiosis.

Geneticists can infer which genes are with which chromosomes by having markers, other than sex, for different chromosomes. If two traits of an organism have specific phenotypes that nearly always occur together (and are not determined by the environment, only genes), then the genes for those phenotypes are linked. For instance, if in a certain species of plant, a tall spindly phenotype tends to occur with yellow flowers, and a short stout phenotype tends to occur with pink flowers, we might infer that the gene(s) for growth form (tall spindly vs. short stout) is linked with the gene(s) for flower color (yellow vs. pink). In this hypothetical case, the tall spindly trait and the yellow flower trait tend to be inherited together; and the short stout trait and the pink flower trait tend to be inherited together – but not in different combinations. The fact that phenotypes for different traits tend to occur together is the clue that the genes may be linked.

If genes are *not* linked, the phenotypes for each trait tend to occur randomly with respect to each other – sometimes you get a tall spindly plant with yellow flowers, sometimes a tall spindly plant with pink flowers. If these genes are not linked, the probability that tall spindly plants have yellow flowers vs. pink flowers would be 50% -- just like the independently segregated traits of Mendel. However, if through experiments, we notice the probability that tall spindly plants have yellow flowers *over half the time*, we could infer that these genes are linked – that is, inherited together because they are located together on the same chromosome.

Geneticists have not only studied genes that are linked because they are on the same chromosome and sort independently during meiosis, but they have also been able to figure out which genes are closer to each other *on the same chromosome* and which are farther apart by observing how frequently they are inherited together. This is done by considering recombination.

You may know that during meiosis, crossing-over events occur in which each of the two homologous chromosomes (one from each parent) intermingle and exchange some genetic material. Each chromosome after a crossing-over event has experienced a little **recombination**. Put simply, segments of the chromosomes have been swapped. The diagrams on the next page illustrate crossing-over events in which some genetic material is exchanged among homologous chromosomes.

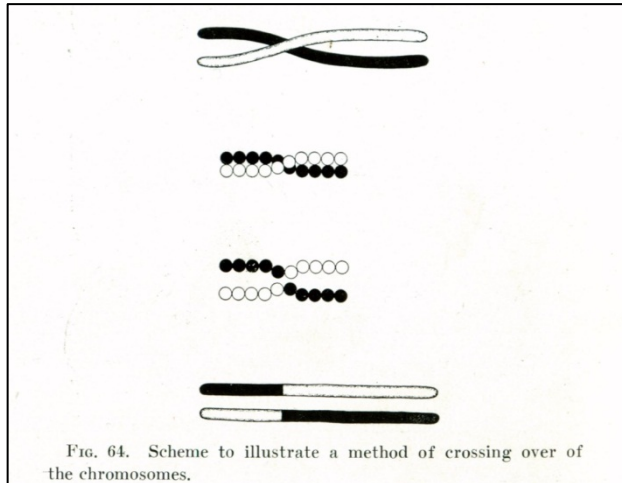


FIG. 64. Scheme to illustrate a method of crossing over of the chromosomes.

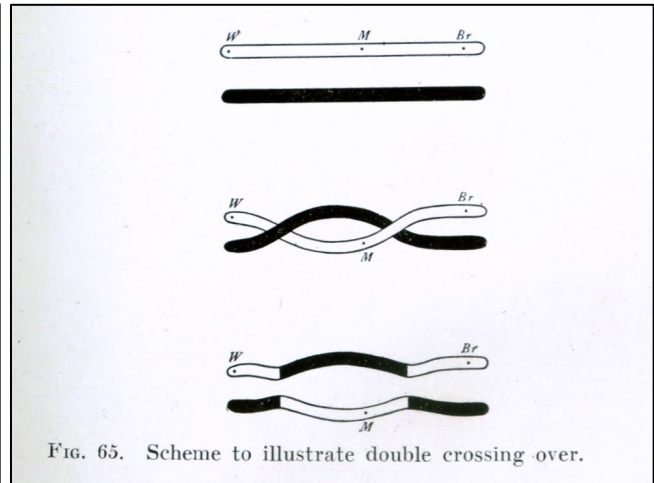


FIG. 65. Scheme to illustrate double crossing over.

**Crossing-over events.** Illustrations by Thomas Hunt Morgan in *A Critique of the Theory of Evolution* (1916), pp. 132-133.

Crossing-over events can separate alleles that were previously on the same chromosome. This is especially true when the alleles are not particularly close together on a chromosome. The chance that two alleles on one chromosome will be separated by a crossing-over event increases with greater distance between them. If they are very close together, crossing-over events will likely not separate them – the alleles will either stay on the original chromosome together, or move to the homologous chromosome together. In this case, the genes are considered to be closely linked, that is, they are located very closely together and tend to be inherited as a unit. The closer two genes are on a chromosome, the more often they will be inherited together.

The higher the probability that different phenotypes are correlated with each other, the closer the associated genes are likely to be on a chromosome. Through experiments, scientists have been able to infer which genes are likely closer and which are further apart. This includes inferring which genes are located on completely different chromosomes, which are on the same chromosome, and how close together they may be on the same chromosome. By studying many traits of an organism and noting the different probabilities that traits are inherited together, geneticists have been able to create linkage maps which show genes in linear arrangement relative to each other based on how closely linked they are. Now that molecular techniques allow scientists to sequence the nucleic acids on DNA and RNA strands, the mapping of genomes has become very sophisticated.

**Recombinant Inbred Lines (RILs)** are specially developed inbred lines of organisms in which genes on each pair of homologs are 99% homozygous (have the same alleles), thereby minimizing genetic recombination between parents. The goal in developing RILs is to create



virtually homozygous genomes in individual organisms. This is possible in plants by creating lines that self-fertilize, so each new generation is formed by having only one parent. With each successive generation, genetic variation is reduced through self-crossing so that homologous chromosomes eventually become nearly homozygous. Think of it this way – with each self-cross, half of the heterozygous genes become homozygous (see Punnett square below). Over many generations of increasing homozygosity, heterozygosity has a smaller and smaller chance of remaining.

### Self-cross of Heterozygous Gene

Aa x Aa

	<b>A</b>	<b>a</b>
<b>A</b>	AA	Aa
<b>a</b>	Aa	aa

Refer back to the diagrams of crossing-over events on the previous page and consider the situation with RILs that have virtually homozygous genomes. If homologous chromosomes have the same allele at each locus, then crossing over events would have no effect. Swapping one allele for the same allele would theoretically have no effect in a homozygous genome. Recombination would cease to happen during crossing-over events. Hence, in RILs, genetic recombination is said to be “fixed.”

When an organism such as a plant has a genome that is virtually homozygous (same alleles) at every gene, we reduce such complications as dominant-recessive interactions, incomplete dominance, co-dominance, and a host of other interactions involving different alleles. Using RILs, it is easier for geneticists to explore traits and their relationships to genes (including mapping genes onto chromosomes) than it would be to use plants that have a lot of genetic variation within each genome. That is why RILs are being used in this module – to minimize the genetic variation in each plant’s genome. In addition, we can assume that all plants from the same RIL have identical genomes. Therefore, we can use multiple plants from the same RIL to create *replicates* in our experiment. Why is using replicates in an experiment an important and valuable practice?

## Experimental Design

### Seed Stock

You should have *Arabidopsis thaliana* seeds from the two parent lines, Columbia (COL) and Landsberg (LER), as well as approximately 48 RILs, which will be labeled with numbers. The genome of each RIL is considered 99% homozygous, meaning the genetic variation has been reduced through controlled inbreeding.



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

### Controlled Variables: Growing Conditions

In order to investigate the genotypic variation among the different genetic lines, the environmental variables should be controlled. All the different seeds, which represent different genotypes, should be grown in the same conditions so that differences among phenotypes can be assumed to be due to genotype and not environment.

**Soil:** We recommend using Jiffy 7 Peat Pellets (see materials). These pellets expand upon soaking, and are wrapped in netting for a self-contained pot.

**Water:** After initial soaking of pellets, plants should be bottom-watered, that is, water placed in the bottom of the tray that the pellets of soil absorb (see instructions). All plants should be watered at the same time, and with the same amount of water.

**Nutrition:** Osmocote pellets (and Miracle Gro solution if growing past three weeks) will be added. Be sure all pots receive the same amount.

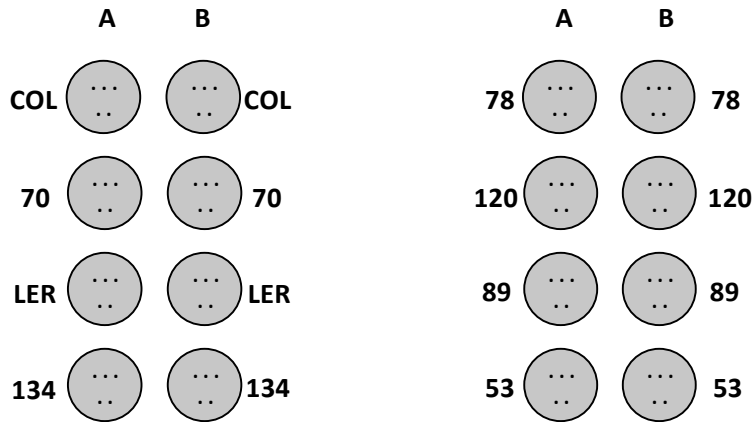
**Light:** A constant source of fluorescent light should be supplied 24 hours a day. This can be accomplished through hanging banks of lights or through constructed light boxes (see instructions). Take care that each pot receives the same amount of light, and that some aren't in shadow or on the edge with less light.

**Temperature:** Room temperature is adequate for growing *Arabidopsis thaliana*. Even though temperature conditions may vary throughout the day in the classroom, pots should be situated so they all experience the same temperature fluctuations (and some aren't in warmer spots than others).

**Independent variables:** The variables we are interested in testing are the different lines of *Arabidopsis thaliana*: Columbia (COL), Landsberg (LER), and the ~48 numbered RILs.

**Dependent variables:** Dependent variables are the phenotypic traits that we observe. These can include the traits with protocols in this module (trichome number, flowering time, plant architecture), and/or can include other traits students are interested in: number of leaves, size of leaves, number of flowers, number of pods, etc.

**Replicates:** Each seed line (COL, LER, or RIL number) will be planted in two pots (or netted pellets). Five seeds will be planted in each pot, so that there are at least 2 pots for each seed line. For instance:



Each circle represents one pellet with five seeds. Each seed line is represented by the letters or numbers at the sides. You will have more lines than are represented here.

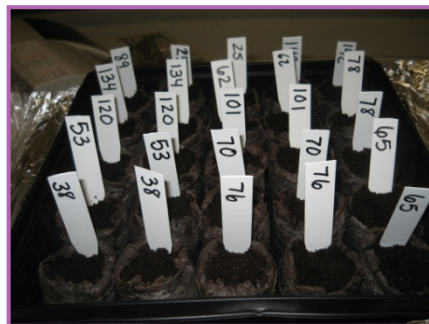
**Pooled Class Data:** Students will be grouped into small teams to work together and communicate with scientist mentors online. The data from student teams can be pooled with other teams within a class to analyze the traits of all RILs in comparison with the parent lines, COL and LER. Pooling data allows for greater confidence in comparing traits among all the lines.

*Top left:* seeds sown and labeled in pellets in one tray.

*Top right:* two trays.

*Bottom left:* seeds germinating.

*Bottom right:* plastic sheets segregating plants so seeds don't fall into neighbor pellets.



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

# Lab 1: Sowing, Cold-treating and Germinating Seeds

## Overview

*Arabidopsis* seeds are tiny! Be prepared to be careful! The seeds can be blown away by a little puff of air that might come with fast movements, laughing, sneezing, coughing, etc. Focus will be important.

You should receive tubes or envelopes with seeds of about 48 RILs, plus the two parent lines, Columbia (COL) and Landsberg (LER). Each line must be kept separate from the others. You will plant 5 seeds of each RIL in two peat pellets (or pots). In other words, for each separate line, you will have 2 replicates pellets (or pots) with 5 seeds each. (See top of page 11)

Once the seeds are in the soil pellets, they will be subjected to cold treatment which stimulates germination, and then placed under light banks or in light boxes.

## Materials:

- *Arabidopsis thaliana* seeds from parent lines Columbia (COL) and Landsberg (LER) and 48 RILs. PlantingScience will provide these.
- Light System
  - Fluorescent light banks, **OR**
  - Home-made screw-in fluorescent bulb in 2 constructed light boxes
- 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.)
  - Access to refrigeration for cold treatment
  - Roll of aluminum foil
  - 100 plastic transparent sheets (one per pellet) for keeping the *Arabidopsis* plants separate when seeds fall
  - 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
  - Flat kitchen knives or spatulas (or wooden popsicle sticks, wooden coffee stirrers)
- Lab equipment and tools
  - Wet lab or table space that can get dirty
  - Beaker 500 ml or larger for watering, pipettes for measuring 2 ml fertilizer
  - Access to refrigeration for cold treatment



## Planting and Germinating Procedure

1. Follow the directions on the Jiffy pellets package, soaking the pellets for the designated time.
2. Pull back the netting on each pellet to open up the space on top.



Package of Jiffy Pellets



Soaked Jiffy pellets



Pull netting back

3. Bury 5 Osmocote granules in a hole  $\frac{1}{2}$ " deep in each soil pellet (a table knife or popsicle stick works for this) and cover.

4. Flatten the soil at the top of the pellet so all the seeds don't fall in one place.



Bury Osmocote pellets



Flatten top of soil

5. Label the pellets according to the number of the RIL or parent line that will be planted in them. You can use pieces of tape on the end of a piece of a wooden applicator stick stuck into the pellet to create a little flag, or use plastic labels. Since each RIL or parent line will have 2 replicates, label one "(A)" and the other "(B)."
6. Take a small square of paper (approx. 3" x 3") and fold it in half. Open it up again and, holding it carefully with the valley pointing down, gently tap the seeds from each vial into the folded paper. Then, gently tap the seeds into the appropriate pellets, counting the seeds as they fall. Put up to 10 seeds in each pellet. Do not bury the seeds – just let them fall on top of the soil. You will have 2 soil pellets for each RIL and parent line – be sure they are labeled.
7. Cover with the tray and pellets with the plastic lid, and wrap each flat in aluminum foil.
8. Cold Treatment: Place in refrigerator for 48 hours (these seeds need cold or a "winter" to synchronize germination).
9. After 48 hours, remove trays from refrigerator, and remove aluminum foil, but keep the plastic lid on. Place in Light Boxes, one tray per crate.

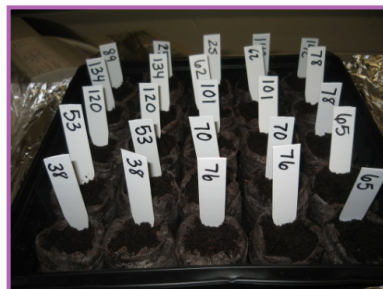
10. Immediately water soil pellets (without fertilizer). **Always bottom-water by pouring water in the tray and letting it soak up into the soil.** Put just enough for a very small amount of water to remain in the tray bottom. Put plastic lid back on.
11. Place in light box or under light banks. Keep the lights on 24 hours a day.
12. Monitor plants every day, but you likely will not need to water the soil again during the first week. Just be sure it stays moist, but not soaking wet.
13. After the first week, plants will have germinated. Move the plastic lid ajar so it is mostly on but lets a little fresh air in. Keep the lid like this for another week.
14. Water as needed. How much is that? As Dr. Griffing says, Arabidopsis are not swamp plants, so they don't like standing water. Always water from below, and water just enough so that any standing water in the tray evaporates every day. You will tend to water about every 4 days. Be sure to water prior to weekends.
15. After plants germinate (about 7 days), water once with fertilizer. To make the fertilizer solution, combine 500 ml distilled water with 2 ml Miracle Gro, and use this full amount per flat (per 25 pellets or pots).
16. Water only *without* fertilizer for another 3 weeks. At approximately 30 days after sowing, fertilize again with water as above (step #14).
17. Fertilize again every other week (step #14).
18. When the plants begin to flower, wrap the plastic transparency around each pellet to keep the plants separated from each other (so seeds from one line don't fall into another line). (See Week 5)

**Top left:** seeds sown and labeled in pellets in one tray.

**Top right:** two trays.

**Bottom left:** seeds germinating.

**Bottom right:** plastic sheets segregating plants so seeds don't fall into neighbor pellets.



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

## Lab 2: Observing Traits – Variation in a Population



1. What is the relationship between an individual and a population?
2. How do you define a population?
3. How do you define the population of *Arabidopsis thaliana* that you will use for your class experiment?
4. List at least four traits that you observe in the population of Arabidopsis.
5. Choose one trait for which your team will observe on several plants today:
6. How will you record your observation of that trait? (by count, measurement, presence vs. absence, color, etc.)
7. Make a data collection table and record observational data for this trait on several plants. Be sure to record the label of each plant along with your observation.
8. Make a graph that depicts your observations among several plants.
9. Does the trait you observed vary among the individual plants? If so, you are observing variation of a trait within a population.
10. What do attribute the variation (or lack of variation) to?
11. How might you find out if any variation you observe is due to genetics, the environment, or the age of the plant?

## Lab 3: Developing the Research Question

This module is designed to investigate traits that vary in phenotypic expression. The different lines of seeds you receive are offspring of two distinct parent varieties of *Arabidopsis thaliana*, Columbia and Landsberg. Each parent expresses the following traits in unique ways. These traits can be used as markers for tracing patterns of inheritance in the recombinant inbred lines.

Phenotypic traits can be thought of in two main groups: *continuous* traits and *discrete* traits:

- *Continuous traits* are the result of multiple genes interacting, which is why they are sometimes called *polygenic* traits. Trait expression for these types of traits vary along a graded continuum. For example, human height is a continuous trait, with possible heights varying along a continuum. These kinds of traits can also often be quantified along this continuum, which is why they are sometimes termed *quantitative* traits. In this module, we will refer to them as *continuous traits*.
- *Discrete traits* are the result of a single gene, and they typically manifest as either one state or another (for example, a purple or white flower in Mendel's experiments). Alleles that are dominant or recessive can be studied by analyzing the proportions of trait expression, such as by using a Punnett square. Predictions can be made when using parent crosses that are homozygous for certain traits, such as traits being expressed in the F2 generation in 1:3 ratios for simple monohybrid crosses, or 9:3:3:1 ratios for simple dihybrid crosses. Discrete traits do not always conform to dominant or recessive principles, and may be co-dominant or have incomplete dominance, so an F2 generation may not conform to the ratios mentioned. But generally we can think of discrete traits as manifesting in an either/or situation, such as yellow *or* green leaves, or yellow *or* green *or* purple leaves. While a continuous trait has a multitude of expressions along a *gradient*, a discrete trait tends to either be present *or* not.

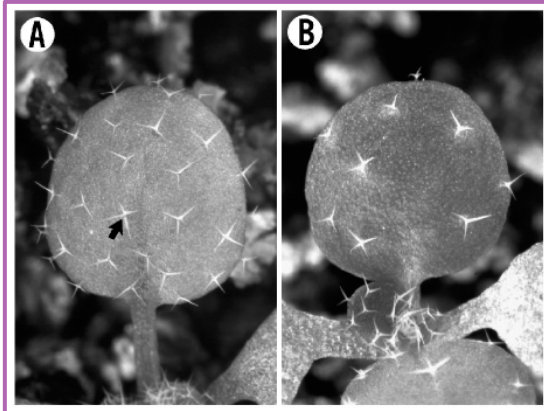
Geneticists are very interested in knowing if certain traits are continuous, or if they are discrete. A discrete trait can be mapped onto chromosomes with some precision, since the gene that influences it is located at one place on a chromosome. By definition, a gene is the segment of DNA in a particular location that influences a trait (or multiple traits). Remember, one gene can have multiple alleles, which might be dominant or recessive (or co-dominant, etc.). A gene, though, is the particular location on the chromosome on which the different alleles may occur.

Continuous traits are the result of multiple genes, and mapping all the genes that influence these traits is more challenging. Geneticists use a number of strategies to help map these genes. For example, one technique is to use RILs, and monitor them to determine what traits each RIL expresses. In addition, geneticists look to see if certain traits may be linked, suggesting the genes that influence these traits are located near each other on a chromosome.





**Trichome (leaf hair) number:** One of the traits is **trichome number**. This trait can best be counted 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](http://www.arabidopsis.org/images/geo_distribution.png)).

**Flowering Time:** Another trait is **flowering time**. The time of flowering is considered in terms of “days after sowing” or DAS. The day the seeds are planted is considered Day 0. Flowering may begin about 21 DAS.



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 seen in some *Arabidopsis thaliana* plants, in contrast to the **wildtype growth form** in others. This trait can be seen about 5 weeks after sowing the seeds.



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.

University of Arizona Biotech Project  
[http://biotech.biology.arizona.edu/Scientific\\_Method/method\\_plants.html](http://biotech.biology.arizona.edu/Scientific_Method/method_plants.html)

## Thoughts to help develop research questions:

What traits will you monitor? Any traits other than the ones listed above?

If you choose to monitor a trait, what kind of data will you need to collect?

How will you record data?

How many samples will you need to collect, and how will you get them?

Will you collect separate data as separate teams, or will teams collect data that will then all be pooled together to be analyzed?

If the whole class is collecting the same data to be pooled together, how can you be sure everyone is collecting data in the same way?

What patterns would you expect to see in your data if a trait is a continuous trait?

What patterns would you expect to see in your data if a trait is a discrete trait?

What patterns would you expect to see in your data if a trait is linked to another trait?

## Monitoring traits:

Decide what, if any, traits the whole class will monitor, and the **protocols** by which they will be monitored. Some traits include those things that can be measured, or quantified. Not all traits need to be measured – there are qualitative traits too, such as color, texture, etc., which can be included.

Don't forget some traits include time, such as: how many days after sowing do the plants appear above the soil, or develop their first true leaf, or develop their first flowers? Traits such as these that depend on a developmental stage may only be observable during that time. For instance, a seed only exists until it germinates. A flower only flowers for a limited time.

## Observations, Data and Pooling Data



We often think of data as numbers; however there are other kinds of data as well. For instance, categorical data includes such things as shapes, colors, names of species, etc. Categorical data can also include groups of numbers that are bounded or “binned” (0-10, 11-20, 21-30, etc.). Images can also comprise data.

How might you measure something like color? Scales are often developed to identify what level of “greenness” exists, for instance. Sometimes paint chips from a paint store can be helpful in developing such a scale.

How will you **record** data? Computer databases are a great way to compile data, but may we suggest you first record your data on paper in your lab notebook? (Have you ever lost electronic files? Made computer formatting errors that shifted columns around?) You can compile and analyze data either on paper or by using a database program such as Excel.

How will data from the whole class be **pooled** together from all the teams? While individual teams should keep track of their own data, data from all teams should be pooled together in one place. Will that be in a computer database? Or on large poster paper hanging in the room? Or in some other way?

If data are pooled, are they all collected in the same manner? A **protocol** is a clear method that different people use to collect the same data. What would happen if different people used different ways of measuring, or different categories? Could the data be compared?

A close-up photograph of a spreadsheet with a blue background. The image shows several rows of data with positive numerical values. The values are: +2.688, +5.000, +1.500, +1.125, and +1.062. The numbers are slightly blurred, suggesting a shallow depth of field.

## Lab 5: Counting Trichomes

What is a trichome?

The picture to the right shows the basal rosette arrangement of leaves on *Arabidopsis thaliana*. Do you see the little hairs scattered over the leaves? These are trichomes.



Public Library of Science: *Small RNA Pathways in Plants*. PLoS Biol 2/5/2004; e107.  
<http://dx.doi.org/10.1371/journal.pbio.0020107>

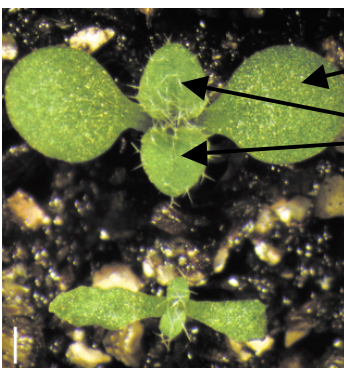
Why might a plant have trichomes? Do you think they might be advantageous to a plant?

Creating a structure like a trichome requires energy for the plant. Do you think a plant could have too many trichomes? Too few? Do you think trichomes might be more advantageous in some environments over others? Which ones?

Do you think there are different numbers of trichomes on the larger vs. the smaller leaves, on older vs. younger leaves? If you want to compare trichome number between plants, how will you do this if even on one plant there are differences between leaves?

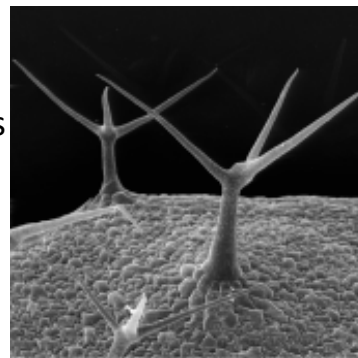
*Arabidopsis* scientists have *standardized* the practice, so they can all compare similar data. They choose the first leaf on each plant and count trichomes on that leaf.

So, which is the first leaf? In the photo below and left, you see four “leaves”. The *very first* leaf-like structures that emerge from the seed are called **cotyledons**, and are **not** considered true leaves. Cotyledons enfold the embryonic plant between them inside the seed. When the plant germinates, the cotyledons open like two hands and emerge first. Then the **first true leaves** emerge from between the cotyledons. You will be counting trichomes on the first true leaves. Click here to see how cotyledons and the first true leaves emerge: [http://www.prep.biochem.vt.edu/expinfo/expinfo\\_growth.html](http://www.prep.biochem.vt.edu/expinfo/expinfo_growth.html)



Cotyledon

First true leaves  
(choose one)



Each trichome has three prongs on it. Be sure you count each as *only one* trichome and *not 3!*

## Trichome Counting Procedure

1. Each student team should have carefully labeled RILs they are responsible for counting.
2. Practice looking for trichomes on leaves.
3. Each plant in each RIL will be counted. Each RIL should be in its own soil pellet or pot, and the plants may be a bit tangled. Identify each separate plant.
4. Identify the cotyledons and the first true leaf. Handle the plants as little as possible, as handling can damage them. You can use a pencil or dissecting needle to separate the soil between plants a little if necessary.
5. Using a dissecting microscope or hand lens, count the trichomes on the first true leaf of each plant and carefully record the counts.
6. Is it enough for one person to count only once? How can you repeat the counts so you are confident you are relatively accurate?
7. Make notes and drawings in your lab book of observations, questions, problems, etc.
8. How will you make sense of your data? What can you tell about your data already? Are the numbers of trichomes between plants similar or really different? How do the numbers compare between RILs?
9. How can you summarize your data to make sense of it?
10. Scientists usually plot their data in graphs to look at trends and patterns. What kind of graph will you use?
11. Compare your results to those of other teams in the class. Do you notice any patterns or trends?



## Directions for Recording Data for Trichome Counts

You should have 2 pots or pellets with seeds from the same RIL or parent. These are replicates, and they should be labeled #1 and #2.

In each pot, approx. 5 seeds were planted. You may or may not have 5 plants now. Each plant needs to have data entered for it. Record each individual on a separate line. If you don't have 5 plants, just fill in what you do have, and leave other lines blank.

It is good practice to have your data collection checked by someone else. If you are able, have 2 team members count trichomes on the same leaf. Record each count in these 2 columns.

Were your counts similar? If so, then just average them and enter that value here. However, if they were very different, then one person likely made an error. See if you can figure it out. If one person made an error, enter the other person's count here. Maybe you need a third count. However you resolve the differences, enter the agreed value here.

Enter RIL number or parent code (COL or LER) here

RIL #	Pot #	Ind Plant	Trichome # (First Counter)	Trichome # (Second Counter)	Trichome # (Agreed Value)
	1	A			
		B			
		C			
		D			
		E			
	2	A			
		B			
		C			
		D			
		E			
			Average =		

Calculate the average value of trichome number per leaf for this RIL or parent line. This average number will be entered into the whole class data sheet so you can compare average trichome number for the different RILs and parent lines.

## Trichome Data Collection Sheet

Team Name \_\_\_\_\_ Date \_\_\_\_\_

RIL #	Pot #	Ind Plant	Trichome # (First Counter)	Trichome # (Second Counter)	Trichome # (Agreed Value)
	1	A			
		B			
		C			
		D			
		E			
	2	A			
		B			
		C			
		D			
		E			
			Average =		

RIL #	Pot #	Ind Plant	Trichome # (First Counter)	Trichome # (Second Counter)	Trichome # (Agreed Value)
	1	A			
		B			
		C			
		D			
		E			
	2	A			
		B			
		C			
		D			
		E			
			Average =		



## Analyzing Data and Looking for Patterns

Why do we collect data anyway? The data from our observations constitute *evidence*. OK, evidence for what? For any claims we make or conclusions we draw from our experiments. So how do we draw conclusions?

Scientists often look for patterns and make comparisons. Is one group of plants taller than another? Is one group more purple than another? If two groups are different, is it enough of a difference to be “significant,” or are they similar enough it’s hard to tell if they’re truly different?

If we conclude two groups are different, how might we determine if the difference is genetically based, environmentally based, or a

combination of both? We often make predictions based on our prior understanding (for instance, we would expect to see a 3:1 distribution of a typical dominant-recessive discrete trait in an F<sub>2</sub> generation of homozygous parents). We can test our actual results against our prediction. If we see the pattern we predict, we can conclude that our evidence supports our prediction.

When we use measurements to collect data from a group, we can analyze them by finding the mean, range, standard deviation, etc.

Categorical data can be analyzed in proportions such as a green to yellow proportion of 3:1. Another example might be percent of total seeds that actually germinated (such as a 72% germination rate).

Graphing data is a way to look for patterns. Sometimes scientists graph the same data in different ways before they see the patterns. Bar graphs may be helpful for certain types of data. Line graphs or scatter plots for others. Pie charts are a great way to graph percentages and proportions.

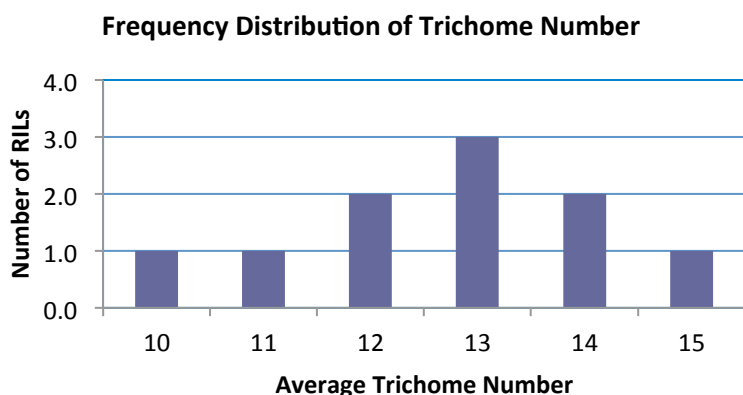


## Lab 6: Analyzing Trichome Data

### Overview

What kind of patterns do you notice in your data? If trichome number were a discrete trait, you would notice plants within a population had a discrete number of trichomes, for example, 13 (or a couple discrete numbers, such as 4 and 13). If trichome number were a continuous trait, you would notice the number of trichomes across a population of plants exhibits a continuum.

When you have data that vary, but are roughly within a common range, you can describe that data set by figuring an *average* (or *mean*), *mode*, and *range*. Mode is the most frequently represented value. Range is the lowest value subtracted from the highest value. Range gives you a sense of how spread-out the values are. *Variance* is another calculation that describes the spread.

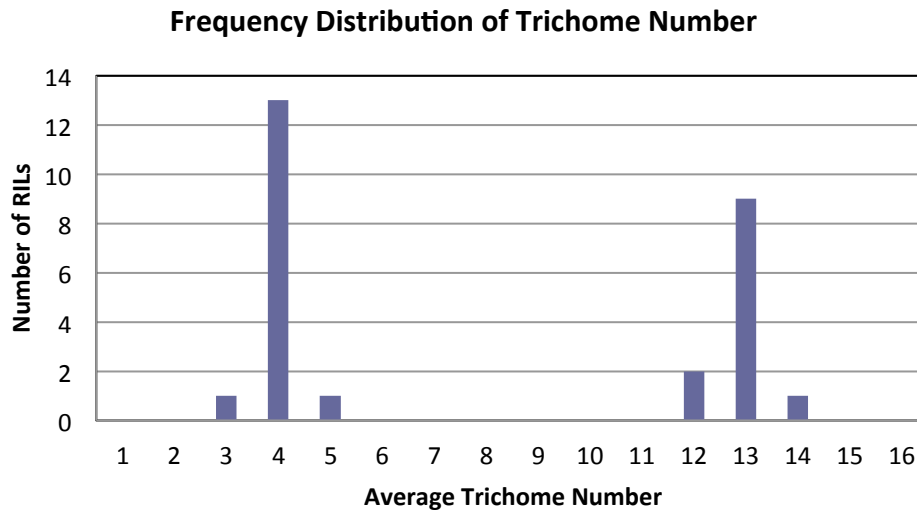


Frequency distributions are a way of plotting a data set that has repeated values. Look at the graph at the left. Can you tell what is represented here?

Frequency distributions plot the number of samples against a value or range of values. In the graph above using hypothetical data, the numbers of RILs are plotted against the average number of trichomes. Reading the graph from left to right, we can see that one RIL had an average of 10 trichomes, one RIL had an average of 11 trichomes, two RILs had an average of 12 trichomes, three RILs had an average of 13 trichomes, and so on. In this sample set, the most common average value (*mode*) is 13 trichomes. Frequency distributions make some patterns visible. In the graph above, you can see a tendency for the average trichome number to center around 13, with values higher and lower decreasing in frequency the farther away they are from 13. In statistics, this type of bell-curve frequency distribution is called a “normal distribution.”

When we analyze average values of a trait among the RILs, we would expect to see some form of a normal distribution of values if a trait is a continuous (quantitative) trait. In addition, the range of the distribution would likely be roughly between the average value of each parent line, in our case, Columbia (COL) and Landsberg (LER).

What would a frequency distribution look like if a trait were a discrete trait? *Continuous* traits have a frequency distribution reflecting a *continuous* range of values among RILs. However, *discrete* traits would show a frequency distribution reflecting *discrete* ranges of values among RILs. For instance, consider the graph below representing hypothetical (not actual) data.



Do you notice how in this frequency distribution, the data cluster around two values that most frequently represented, 4 and 13? Those two values would be considered modes, and so this distribution is statistically considered a “bimodal distribution.” In addition, because there are virtually no intermediate values, we would say the average number of trichome is essentially one of two *discrete* values. This is not a continuous distribution, but rather a *discrete* distribution. The average number of trichomes could be expected to be *either 4 or 13*. We do not see a wide, continuous distribution of values, but rather discrete values, with very little (but notably some) variance around these discrete values.

### How Can You Tell If a Trait is Continuous or Discrete?

In order to conclude whether a trait is a continuous trait or a discrete trait, you will create frequency distributions from the data you and your classmates collect. You will look at these graphs and analyze them for patterns. If you see well distributed data along a range of values, such as a normal curve will show, that indicates the trait is a continuous trait. If your frequency distribution graph shows tight clusters of data, then you would conclude the trait is a discrete trait.

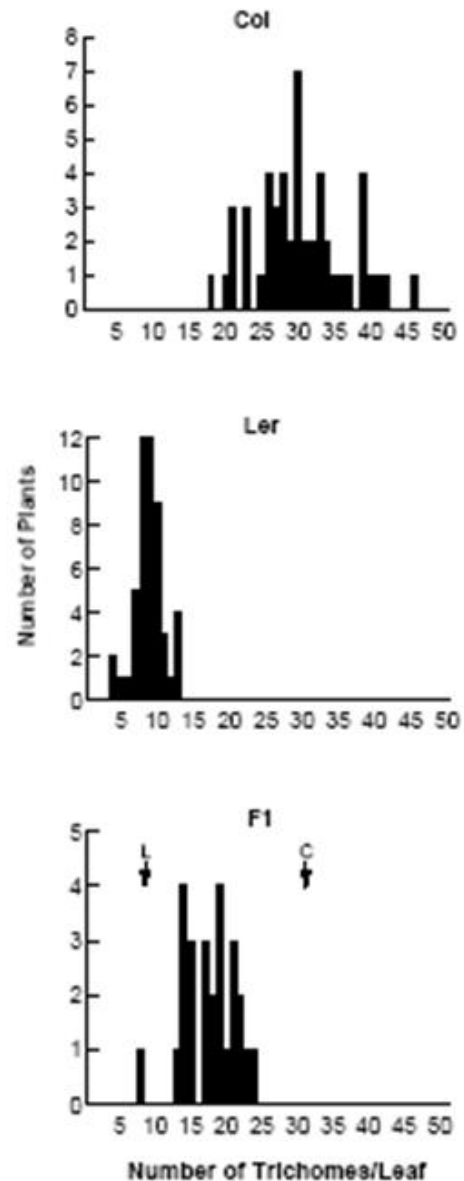
Knowing that the RILs are offspring of the parent lines, Columbia and Landsberg, it would also be interesting to compare the trichome number in the RILs with those of the parent lines. Larkin et al. (1996, Development 192: 997-1005) collected data on trichome number in both parent lines and the F1 offspring, and their data are reproduced at the right.

These are frequency distributions, with the number of trichomes per leaf on the x-axis, and the number of plants on the y-axis. The top graph represents trichome number in a sample of Columbia plants, and the middle graph represents trichome number in a sample of Landsberg plants. In both of these graphs you get a sense of the range, mode and average of trichome number in each parent line.

The bottom graph shows a frequency distribution of the number of trichomes per leaf in F1 offspring produced by crossing the Columbia and Landsberg lines. Notice that the mean values of both parent lines from the top two graphs are represented by crosses.

You can see how in this experiment the F1 offspring show an intermediate average number of trichomes as compared to the parent lines.

You will be calculating and representing your data in a similar way (see next page).



## Data Analysis Steps

1. In your class, you should have average trichome number figured for all the RILs and the parent lines. Set the parent line (COL and LER) data aside for the moment and do not use them in the initial analysis.
2. Pool together all your data from all teams and class members (except for data in the parent lines) into one data set.
3. Sort the data from lowest to highest values (see sample data at right).
4. Next, we will make a frequency distribution. However, rather than having a mark on the x-axis for every value, we are going to group the data into “bins” to make a “binned histogram”. So instead of having single marks for values of 1, 2, 3, 4, 5, 6, 7, and so on, we will create categories with values of 1-5, 6-10, 11-15, 16-20, and so on. Then you will count how many RILs have values (average number of trichomes) that fall into each category. This way, we can make a frequency distribution that shows patterns more easily.

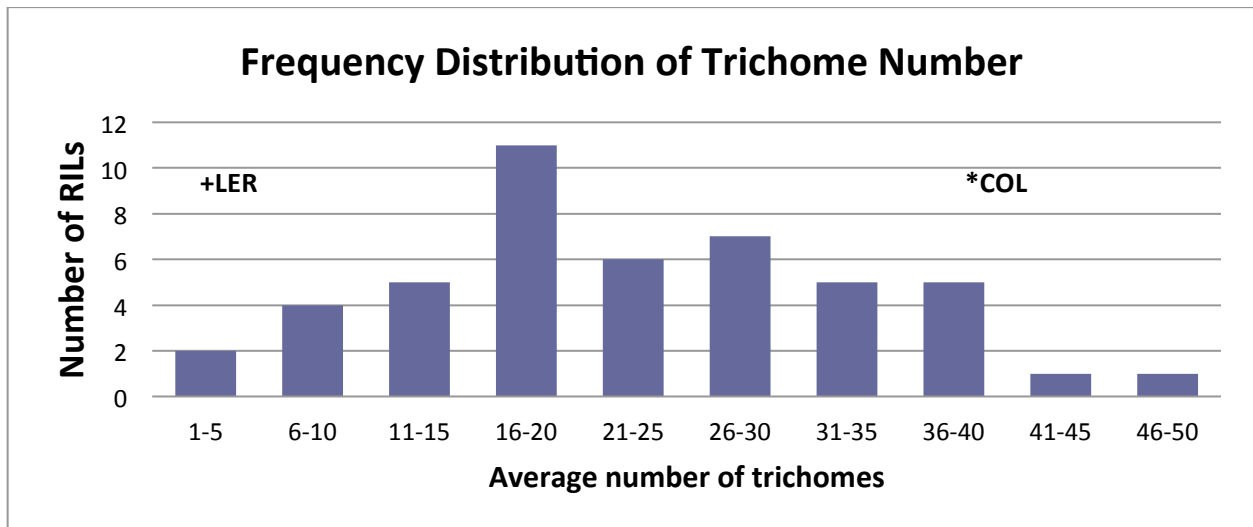
Create a table with your binned categories, and the number of RILs that have those average values. That table might look something like this below:

RIL	Average Trichome Number
98	5
102	5
82	6
53	8
32	9
52	9
61	12
59	13
36	14
104	14
34	15
50	16
57	16
64	17
108	17
123	17
41	18
33	19

Bin	Number of RILs
1-5	2
6-10	4
11-15	5
16-20	11
21-25	6
26-30	7
31-35	5
36-40	5
41-45	1
46-50	1

Note that if you have a very wide range of values, you might want your bins to be bigger, for instance, 1-10, 11-20, etc. On the other hand, if you have a smaller range of values, you might want your bins to be smaller, for instance, 1-3, 4-6, 5-9, etc.

- Next, you will graph your data, with the binned categories on the x-axis, and the number of RILs on the y-axis. See the example below.
- Now look at your average number of trichomes for each parent line, Columbia (COL) and Landsberg (LER). Insert indications of these values above the bars. (See below)



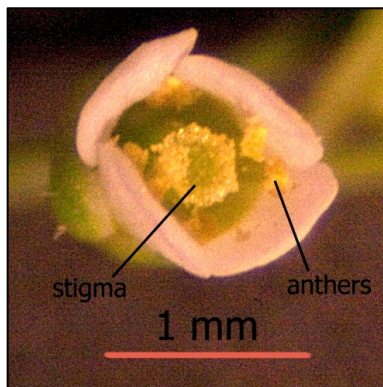
- Based on your frequency distribution, would you say the trait is a continuous trait or a discrete trait? (Be sure to look at your own data and graphs, and not the one above.)
- Based on where the values for the parent lines are in the distribution, would you say the values in the RILs are distributed as a continuum between the parent values, or do they represent some other pattern?

## Lab 7: Recording Flowering Time

For this PlantingScience experiment, standard flowering time will be recorded in number of days after sowing (DAS). Days After Sowing (DAS) can be figured by counting from the date of sowing (Day 0) to the date of first flowering for each individual plant.\*

The day that you see the white petals of the first flower appear between the outer green sepals of the bud is to be considered the day of flowering. The day of sowing is considered Day 0. The first day after sowing is considered Day 1, and so on. The top photo below indicates what to look for.

If flowering occurred on a weekend or holiday when you were not present to monitor the plants, you can estimate which day flowering occurred. A video (posted on the PlantingScience website) made from lapse-time photos with hours since sowing labeled on the images can help you estimate how long flowers appear (roughly 2 days) before petals wither and the silique begins to form and elongate.



\* Arabidopsis scientists use a variety of ways to record flowering time, including counting the number of rosette leaves at time of flowering. Your scientist mentors may suggest some of these alternate ways, and please feel free to incorporate these methods in your data collection.

## Flowering Time Data Sheet

Team Name \_\_\_\_\_ Student Name \_\_\_\_\_

RIL #	Pot #	Ind Plant	Date of Sowing	Date of First Flowering	Flowering in Days After Sowing (DAS)	
	1	A				
		B				
		C				
		D				
		E				
	2	A				
		B				
		C				
		D				
		E				
				Average =		

RIL #	Pot #	Ind Plant	Date of Sowing	Date of First Flowering	Flowering in Days After Sowing (DAS)	
	1	A				
		B				
		C				
		D				
		E				
	2	A				
		B				
		C				
		D				
		E				
				Average =		

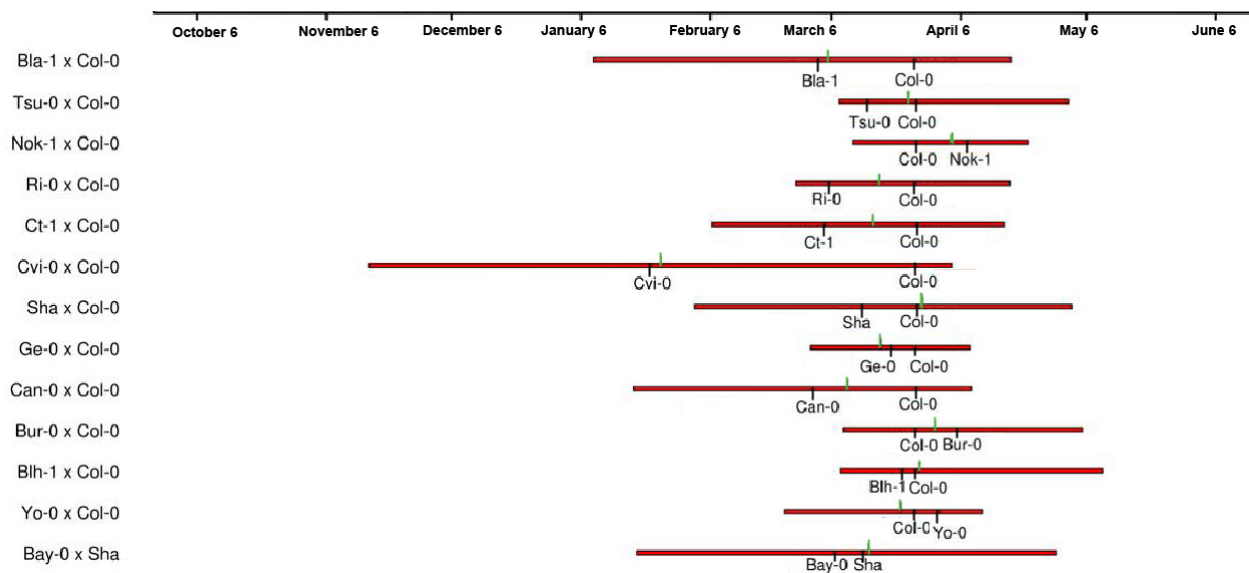
# Variation in Flowering Time as a Continuous Trait

## Background

Flowering time, the time between germination and the appearance of the first flower is critical to plants. It is essential for plants to form flowers at a time when pollination and seed production can proceed under favorable conditions. If the plant flowers too early or too late, the environmental conditions may favor disease-producing organisms or conditions of light and temperature that are not good for pollination and seed production. To get it right, plants monitor the day-length (photoperiod) and ambient temperature. Day-length and temperature vary widely with geographic location. Therefore, different accessions (Columbia and Landsberg in PlantingScience) have widely varying flowering times, reflecting their different original habitats.

Where, genetically, does this variation come from? This is one of the questions that will be answered by the data from the PlantingScience observations. It is clearly multigenic, with genes that are involved in temperature sensing and light sensing. Natural allelic variation will occur in the genes involved in temperature sensing. Hypothetically this will occur in the genes *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) which are regulators of temperature-sensing. Also, genes involved in light reception, like *CRY2*, a gene for a photoreceptor, will show variation.

The fact that flowering time is multigenic is shown by the range of flowering times when examining the RILs from different parental crosses, as shown below<sup>1</sup>. The red line shows the variation. The green line on the red line shows the median of the distribution. The black lines on the red line show the average flowering time for each parental accession. As you can see, we do not have the *Ler* x *Col-0* variation. This is what we will be determining in PlantingScience.





Genetic variation is a game of balance. We expect variation in genes that are involved in determining the latest possible flowering time. For example, if plants can grow for an extended time without flowering, they will produce more leaves and root area, thereby ensuring the best nutrition for the developing embryos of seeds once flowering occurs. On the other hand, plants need to avoid stressful conditions that may damage seed production, such as drought or elevated temperatures in late summer. So what is the balance that has been selected by natural selection? We can find this out by knowing which genes are involved in the variation of flowering time. We can begin by learning how variation in flowering time is linked to, or associated with other genetic markers, such as the Erecta gene.

<sup>1</sup> Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M., Bergelson, J., Cuguen, J., and Roux, F. Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. PLoS Genet 6(5): e1000940. doi:10.1371/journal.pgen.1000940

## Lab 8: Bolting Plants and the Erecta Phenotype

1. Study the basic plant structure of *Arabidopsis thaliana*.\*
2. Notice that *Arabidopsis* plants have a basal rosette of leaves, and a stem that grows tall on which the small cauline leaves (leaves on the stem) and flowers form. The stage of development when this stem forms and grows is considered “bolting.”
3. The erecta phenotype cannot be distinguished until the plants bolt and flowers begin to form. This phenotype is characterized by an overall plant architecture of being squat and sturdy with less spacing between the cauline leaves, the flowers and mature seed capsules than the wildtype.



University of Arizona Biotech Project  
[http://biotech.biology.arizona.edu/Scientific\\_Method/method\\_plants.html](http://biotech.biology.arizona.edu/Scientific_Method/method_plants.html)

4. Assume the erecta phenotype is either a dominant or recessive discrete gene, and the RILs are the F2 generation where the F1 is heterozygous ( $Ee$ ) for this trait. After crossing the two F1 heterozygous parents ( $Ee$ ), the offspring are the F2 generation. Using a Punnett Square, predict what ratio of F2 offspring (RILs in this case) you expect to see if these assumptions hold true. Predict expected ratios if the erecta phenotype were dominant and if it were recessive.
5. Record which RILs you categorize as having the erecta phenotype and which have the wildtype phenotype.
6. Pool the whole class data.
7. What proportion of the overall RILs had the erecta phenotype?
8. How does your actual data compare with your predictions?
9. Do your data support the prediction that erecta is either a dominant or recessive discrete trait? What is your evidence?
10. Are there other possible explanations to account for the erecta phenotype? How might you test these?
11. **Before your flowers produce seeds, make a tube of the transparency sheets, taping it at the top and bottom, and inserting it directly into the soil pellets. This will keep the seeds separated in each RIL.**



\* A good reference for *Arabidopsis* anatomy is the PREP Online Lab Notebook at [http://www.prep.biochem.vt.edu/expinfo/expinfo\\_anatomy.html](http://www.prep.biochem.vt.edu/expinfo/expinfo_anatomy.html)

## Study Questions for Erecta Phenotype in Arabidopsis

1. What advantage or disadvantage would a short, erect stature have for a plant?
2. Is a category considered a kind of “data”?
3. How do you know if a plant has this erecta trait? How do you “score” this trait?
4. How will you compare your prediction to your actual data?
5. How similar do your actual data need to be to your predictions to be considered conforming to them? How dissimilar to be considered different from your predictions?
6. How will you represent your data on the erecta phenotype?  
(Hint: tables, graphs, and diagrams are ways to represent data visually)

## Lab 9: Analyzing Data to Investigate Linked Traits

Traits are considered linked if they are always inherited together. For instance, in fruit flies, eye color and wing length are linked.

What causes traits to be linked? Generally, the genes that determine linked traits are located close together on the same chromosome so that they tend to be inherited together. Understanding what traits are linked together is important in order for scientists to map gene functions onto genomes. In addition, using DNA and/or RNA molecular markers as traits, geneticists can map trait functions onto specific genes.

In this investigation, we will look at whether a continuous trait and a discrete trait are linked. For our purposes here, we will look at whether any continuous trait is linked to the erecta trait.

### You will need:

- Data from a continuous trait.
- Data from the erecta vs. floppy trait (a discrete trait).

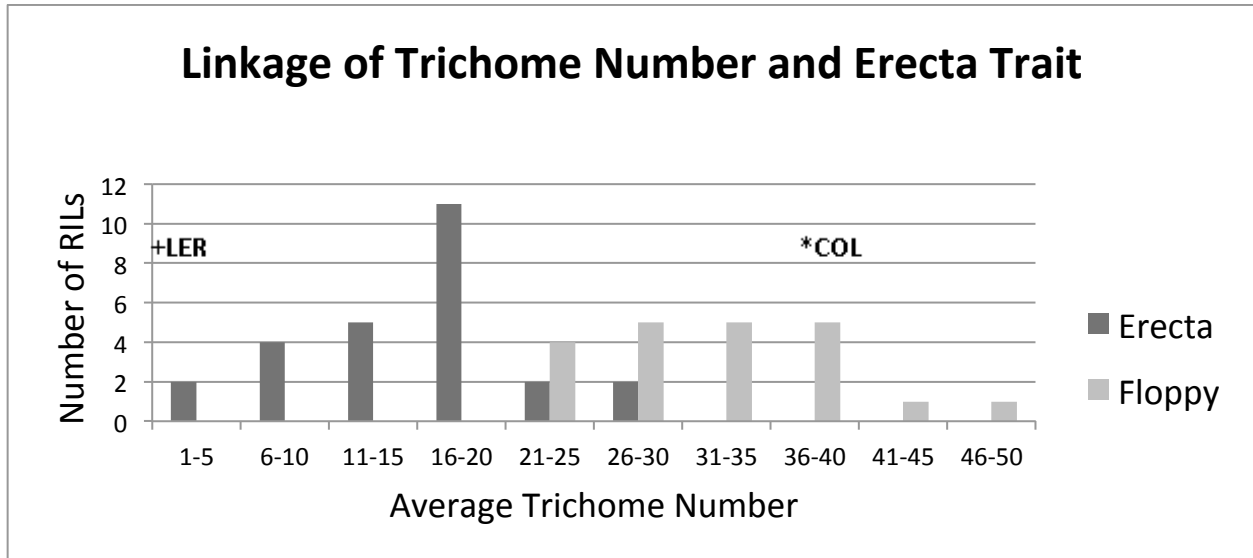
### Steps for Analysis:

1. Create a table of pooled data from your class with both the continuous data and the erecta vs. floppy trait together according to RIL. See the sample to the right.
2. Sort the data by continuous trait value, from lowest to highest.
3. Create binned categories for your continuous trait data, and distinguish how many erecta and how many floppy RILs are in each bin. See sample below.

RIL	Average Trichome Number	Erecta vs. Floppy
78	19	Erecta
105	19	Erecta
45	20	Erecta
46	20	Erecta
65	21	Erecta
44	22	Erecta
125	23	Floppy
22	24	Floppy
48	24	Floppy
55	25	Erecta
39	26	Erecta
147	26	Erecta
85	27	Floppy
17	28	Floppy

Bin	Number of Erecta RILs	Number of Floppy RILs
1-5	2	0
6-10	4	0
11-15	5	0
16-20	11	0
21-25	2	4
26-30	2	5
31-35	0	5
36-40	0	5
41-45	0	1
46-50	0	1

- Based on your binned table, create a frequency distribution like the one below. You will have two types of bars shown representing the two forms of the discrete trait. One type of bar will represent the number of RILs in the binned category that have the erecta phenotype, and the other type of bar will represent the number of RILs in the binned category that have the floppy phenotype.
- Superimpose the values of the parent lines above the bars (COL and LER).



#### Forming a conclusion about linkage:

If your graph looks similar to the one above, with most of the erecta phenotype distributed on one side of the distribution of the continuous trait, and the floppy phenotype on the other side, the discrete trait and the continuous trait would appear to be linked. In other words, a higher number of trichomes is generally inherited together with the floppy phenotype, and a lower number of trichomes is generally inherited together with the erecta phenotype.

If, however, you have both phenotypes of the discrete trait (e.g., both erecta and floppy) distributed throughout most of the binned categories of the continuous traits, then you do not have evidence for linked traits. In other words, a greater (or lesser) value of trichome number does **not** tend to be inherited together with a particular phenotype of the discrete trait.

## Lab 10: Collecting Seeds

These RILs and parent lines can continue to be maintained for continuous generations, as these plants self-pollinate. If you collect the seeds, you can conduct the experiments in the following term. The seeds stay good for several years, so you can actually store them and use them years later.

### Materials

- 50 vials to place seed from each RIL and parent line (COL and LER)
- Sharpies to label vials
- Clear white paper

### Readiness to collect seed

To collect seed, the plants will need to produce mature seeds and seed pods (siliques) – these will turn from yellow to brown as they mature.

### Precautions to not mix seeds among RILs

Remember, these seeds are very tiny! Every precaution must be taken not to mix seed between RILs; otherwise the future experiments with the seed will not work. To separate and handle the plants, you will have already made a tube of overhead transparency inserted into each RIL soil pellet. Carefully separate each soil pellet out and remove the transparency.

### Harvesting seed

1. Label each vial with the appropriate RIL numbers and parent codes (COL and LER).
2. Cut a piece of white paper in quarters.
3. Fold each quarter in half.
4. With the folded paper between your thumb and forefinger, gently rub the ripe siliques so that the seeds fall carefully into the fold of the paper.
5. Carefully pour the seed from each separate RIL and parent line in the appropriately labeled vial.
6. Make sure to completely brush away the seed from the tabletop and paper before collecting another RIL.