



PlantingScience Foundations of Genetics: Traits, Variation, and Environment in Rapid Cycling *Brassica rapa*



Teacher's Guide

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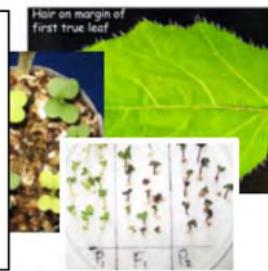
PlantingScience Foundations of Genetics:

Overview and Planning

Growing System Logistics

Plants, Genetics, Environment

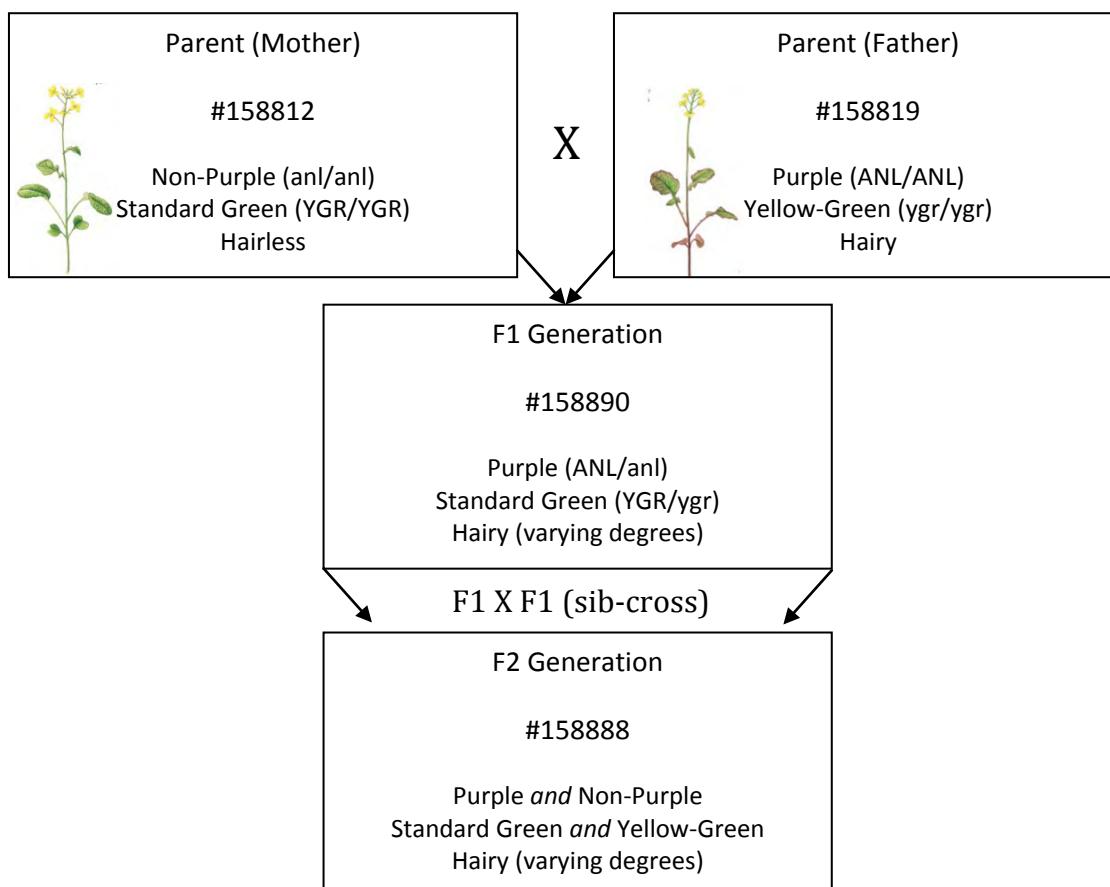
Glossary and Appendices



This section is dedicated to carrying out data collection, analysis, and sense-making throughout the life cycle of *Brassica rapa*. As a reminder, the parent lines, the F1 generation, and the F2 generation (which students are growing) are shown below in diagram with their respective phenotypes indicated.

Remember that the environment plays a large part in an organism's phenotype. The use of two nutrition levels in the experimental design is meant to accentuate this. Please refer to Appendix F for further background information (in the Appendices section).

- **Discrete Trait for Purple / Lack of Purple.** Anthocyanin is a purple pigment, especially visible on the stems, hypocotyls, under cotyledons, and at leaf tips. A single gene, the anthocyaninless gene (*anl*), in Wisconsin Fast Plants® regulates whether or not anthocyanin will be expressed.
- **Discrete Trait for Standard Green / Yellow Green.** The yellow-green gene (*ygr*) in Wisconsin Fast Plants® determines whether the leaves will be standard green or yellow-green in color.
- **Quantitative Trait for Hairiness (Trichomes).** Multiple genes work in concert to regulate the number of small hairs, or trichomes, on leaves and stems. There are varying degrees of hairiness.



Recording, Analyzing and Sharing



Keeping a Lab Notebook – Sharing with Mentors

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

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!

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



+2.088
+5.000
+1.500
+1.125
+1.062

can compile and analyze data either on paper or by using a database 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?



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 F₂ 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 our data is a way to look for patterns. Sometimes scientists graph the same data in different ways before they see the patterns. (See p. 15, “Analyzing Data”.) 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.



Sharing and Presenting

Every scientist shares her or his investigations and results.

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.

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.

Monitoring Traits

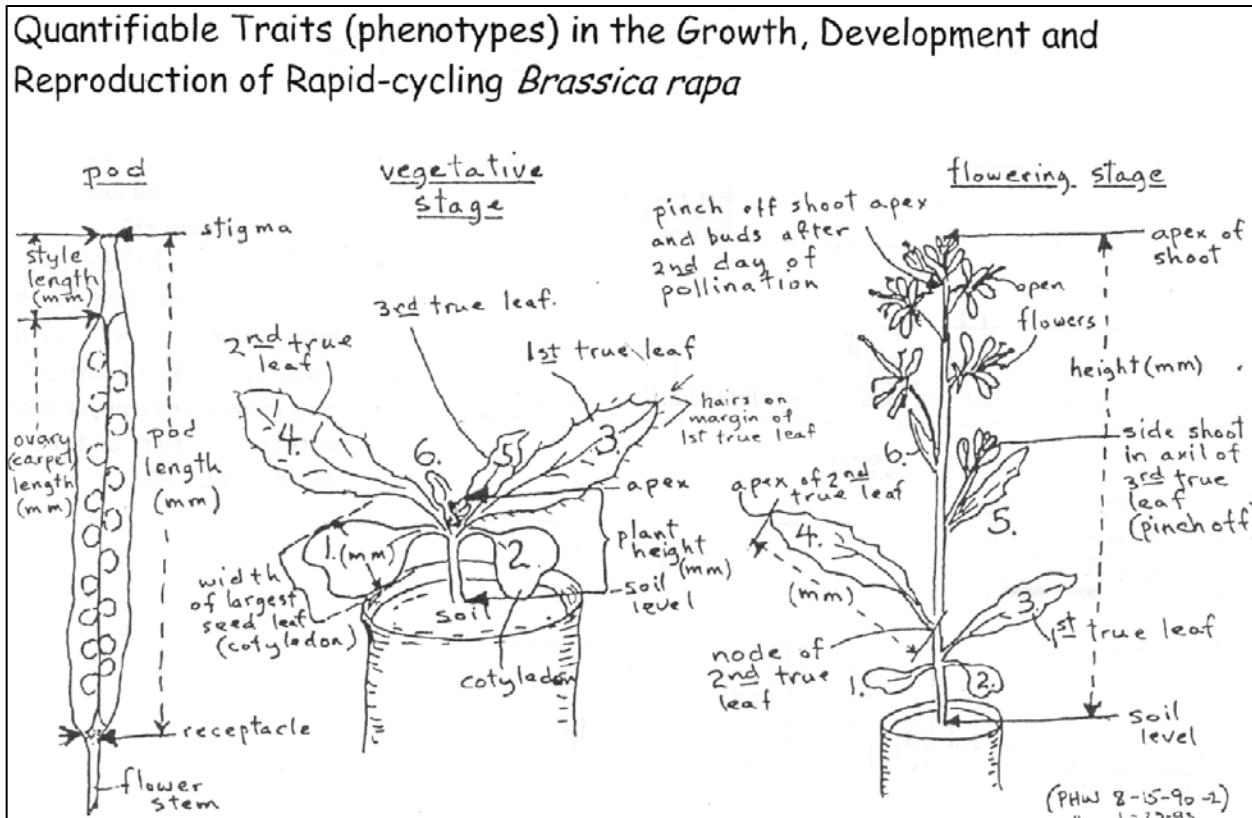
As the plants grow and develop, practice observing traits by tracking the changes of chosen traits of your plants. Below is a diagram suggesting traits that can be measured. See p. 14 for a sample data sheet.

Decide what traits the whole class will monitor, and the **protocol** 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.

For additional information on *Brassica rapa* traits, see:

http://www.fastplants.org/pdf/activities/WFP_growth-development-06web.pdf



Seeds



Observe Seed Traits

What is a seed? Is it alive or dead, or something else? What is a seed made up of? Are all seeds the same? What direction is “up” for a seed? How do we describe traits? How do we compare traits?

Materials:

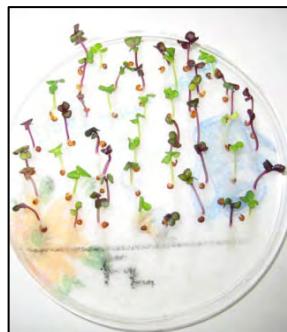
- *Brassica* seeds on transparent tape
- Flat rulers
- Magnifying glasses or dissecting microscopes
- Lab journals



Observe seeds to explore what traits they have, and create a list (such as color, shape, size, markings, etc.). Drawing seeds freehand in lab journals is a useful way to observe and note traits.

Decide what traits on which to collect class data. Have teams gather data, pool as a class, and discuss.

Extension: For activity comparing *Brassica* seeds with pinto bean seeds, with data sheets, see the FastPlants website: <http://www.fastplants.org/pdf/activities/gettingacquaintedseed.pdf>



Germinate Seeds for Observation

Here, you germinate extra seeds in petri dishes with paper toweling set in a container of water. The paper toweling serves as a wick to keep seeds moist. You will likely only have enough seeds for a demonstration dish per class.

Materials:

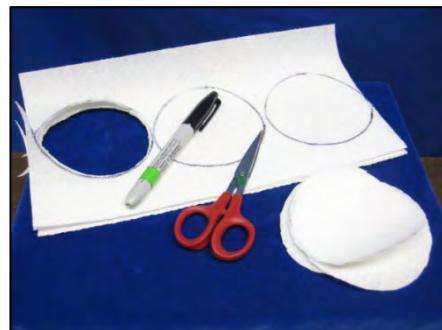
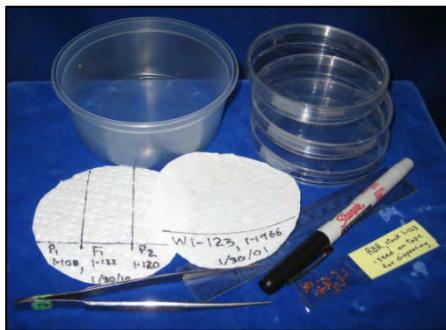
- Extra *Brassica* seeds (seeds not used for student plantings)
- Petri dishes
- Paper toweling
- Waterproof markers
- Large pipettes or eyedropper
- Container to set petri dishes in with water



Procedure:

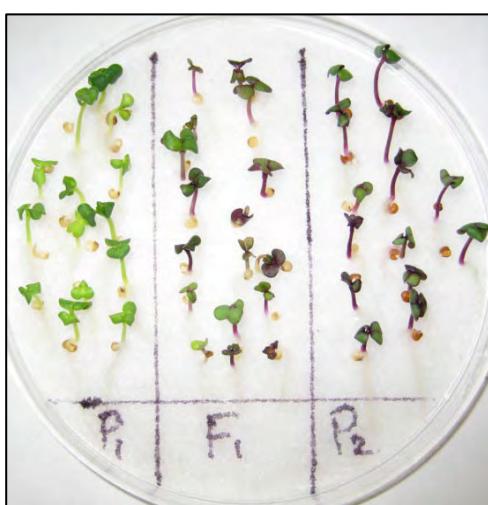
1. Draw and cut circles of paper toweling as shown, using the petri dish lid (larger half) as the proper size.
2. Label the paper towel to the seed line(s), the date, and the time.

3. Set the paper towel into the petri dish lid (larger half), and moisten it with the pipette.
4. Place *Brassica* seeds on the paper towel.
5. Cover with the petri dish bottom (smaller half).
6. Put water in container to about $\frac{1}{4}$ full.
7. Place petri dish(es) at steep angle ($80\text{-}90^\circ$) in the water container so that the bottom 2 cm of the petri dish(es) are below the water surface.



Extensions: For an activity on testing which way of the seed is “up,” see the FastPlants website:
<http://www.fastplants.org/pdf/activities/germlaunchingtheseed.pdf>

For more on measuring and observing seed germination, see
http://www.fastplants.org/pdf/activities/WFP_growth-development-06web.pdf



Left: Seeds germinated in petri dish from both parent lines (P1—Mother, P2—Father), and the F1 generation. Note the color traits. **Right:** Seeds germinated in petri dish from F2 line. Note the variation in color traits in the F2.



Population and Variation

Writing or Discussion Activity: What is a Population?

What population do you belong to? The population of people in your city or town? In your state? In your country? Can you belong to more than one population at a time? Do you belong to sub-populations, such as people of a certain age? Is it necessary to define a population to know who belongs to it or not? Are all the individuals in a population identical, or is there variation?

How do you think a population of plants is defined? How might a population of plants that grows in the wild be defined? Are plants that grow in a lab considered part of a population? Do seeds belong to a population? Are there differences between individual plants in a population?

Why Do Individuals in Populations Differ?

Biologists define a population as a localized group of individuals of the same species that are capable of interbreeding and producing fertile offspring. One of the fundamental attributes of individuals within a population is that they vary in observable characteristics referred to as **phenotypes** that can be observed and measured. Phenotypes are influenced by the set of genes carried by an individual (**genotype**), and the environment that influences the expression of genes. For example, some people are genetically predisposed (genotype) to express high blood cholesterol, but only exhibit heart disease (phenotype) when they eat a high fat diet (environment).

Within each individual's set of genes, there are different forms of genes called **alleles**. It is common to describe a genotype using a set of letters, each variant denoting an allele. For example, if a gene has two alleles, and one allele is dominant over the other, we can denote the dominant form as 'F' and the recessive form as 'f'. Given that organisms are **diploid** and carry two sets of genes (one from each parent), there are three possible allelic combinations for each gene: FF, Ff, ff. And that is the variation for just one gene. When you consider all the genes in an individual and allelic variation at each gene loci, added to chromosomal rearrangements and environmental influences, that's a lot of variation! The more alleles there are for a gene coupled with an ever changing, variable environment, the more potential exists for diversity of phenotypes in a population.

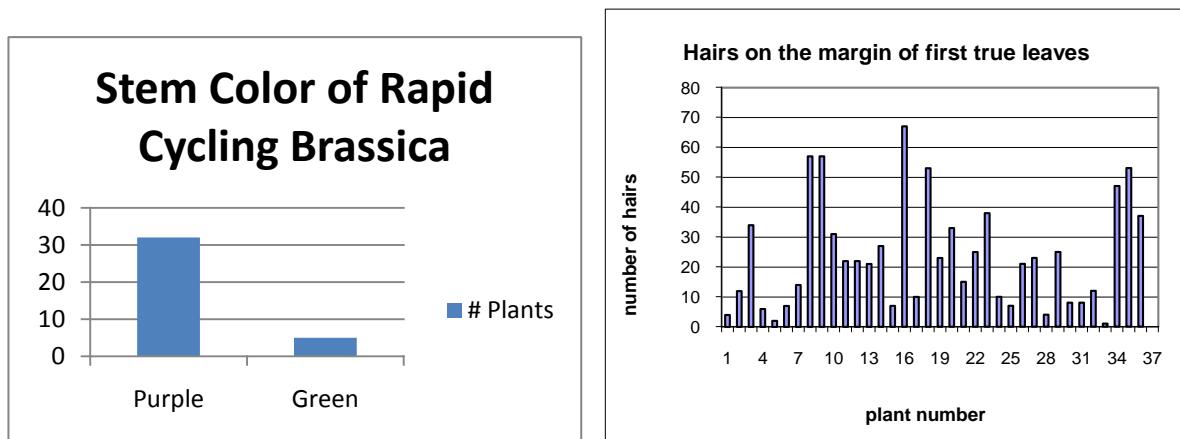
Discrete and Quantitative (Continuous) Traits

What's the Difference?

The observable expression of traits in an organism is its *phenotype*. A plant may have red flowers, heart-shaped leaves, deep roots – these are considered its phenotype – the expressed traits. Some traits are not directly observable, such as immunities or biochemical reactions.

Discrete traits (also known as discontinuous traits) are the result of one gene, and are often definitive traits, such as yellow vs. green color, or smooth vs. wrinkled seed. Even though one gene is the determinant of the trait, there are usually more than one allele, and often dominant and recessive alleles. A discrete trait can often be scored as either one form or the other, or the presence or absence of a trait. Discrete traits conform to Mendelian patterns of segregation, usually practiced with students through Punnett Squares.

Quantitative traits (also known as polygenic or continuous traits) are the result of many genes interacting within the organism, and often vary along a continuum. They are often measured, and an average value (such as leaf length) calculated among a group of individuals.



Above: Graphs of variation patterns common in discrete traits (left graph) and quantitative traits (right graph).

Questions:

Does the environment of a plant affect the expression of traits? Are there some traits that are more affected by the environment than others?

How can you tell if a trait is a discrete trait (determined by one gene), or a quantitative trait (determined by multiple genes)? How might you make a prediction that a trait is a discrete one, and test your prediction?

Discrete Traits in *Brassica rapa*

Analyze Population for Discrete Traits

This module is designed to investigate traits that vary in phenotypic expression. The F2 seeds are specially cultivated descendants of two distinct parent varieties of *Brassica rapa* (see p. 1 in this section). Each parent expresses the following trait in unique ways. We'll use these traits as markers for tracing patterns of inheritance in the offspring you will grow.

Record observations for each plant (they should be numbered). Are they the purple or the non-purple phenotype? Are they the standard green or the yellow-green phenotype? See descriptions below. Have teams pool their data and analyze the proportions of each phenotype within the population of F2 plants. How do their results compare with predictions made by using Punnett squares?

The Punnett square on the following page for the monohybrid cross examines only the Purple vs. No Purple trait. The Punnett square for the dihybrid cross examines both traits below.



Purple vs. No Purple. Anthocyanin is a purple pigment found in many plants, including Wisconsin Fast Plants®. Anthocyanin is best observed when the plants are 4-7 days old. Look on the stems and hypocotyls, under the cotyledons, and at the leaf tips. A single gene, the anthocyaninless gene (*anl*) in Wisconsin Fast Plants® regulates whether or not anthocyanin will be expressed. In the homozygous, recessive form (*anl/anl*), anthocyanin expression is completely suppressed, and the plants appear a bright green color, or the “non-purple stem” phenotype (above right). If the genotype is *anl/ANL* or *ANL/ANL*, then anthocyanin is expressed at varying levels and the plants are the “purple stem” phenotype (above left).



Standard Green vs. Yellow-Green. The yellow-green gene (*ygr*) in Wisconsin Fast Plants® determines whether the leaves will be yellow-green or standard green in color. In the homozygous, recessive form (*ygr/ygr*), the leaves appear a pale, yellow-green color, which is the “yellow-green leaf” phenotype. If the genotype is *ygr/YGR* or *YGR/YGR*, then the leaves appear green, which is the “standard green leaf” phenotype.

Refer to the diagram on page 1 to see the Parent Stock, and the F1 generation. The seed comprising the F2 generation was produced by crossing F1 with F1.

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Monohybrid Cross with Fast Plants: F₂ and Test Cross Phenotypic Recombination Classes Parental Cross: Non-Purple X Purple

F₁ Phenotype: Purple

F₂ Phenotypic Recombination Classes

\backslash F ₁ male gametes	Purple 1/2 wild-type	Non-Purple 1/2 mutant
F ₁ female gametes		
Purple 1/2 wild-type		
Non-Purple 1/2 mutant		

Test Cross Phenotype: Non-Purple

Test Cross Phenotypic Recombination Classes

\backslash F ₁ male gametes	Purple 1/2 wild-type	Non-Purple 1/2 mutant
Test Cross female gametes		
Non-Purple 1/1 mutant		

Genetics Made Easy with Fast Plants



Dihybrid Cross with Fast Plants:

F₂ Phenotypic Recombination Classes

Parental Cross: Non-Purple, Green X Purple, Yellow-green

F₁ Phenotype: Purple, Green

F ₁ male gametes F ₁ female gametes	Purple, Green 1/4 2 wild-type traits	Non-Purple, Green 1/4 1 mutant, Non-Purple 1 wild-type, Green	Purple, Yellow-green 1/4 1 wild-type, Purple 1 mutant, Yellow-green	Non-Purple, Yellow-green 1/4 2 mutant traits
Purple, Green 1/4 2 wild-type traits				
Non-Purple, Green 1/4 1 mutant, Non-Purple 1 wild-type, Green				
Purple, Yellow-green 1/4 1 wild-type, Purple 1 mutant, Yellow-green				
Non-Purple, Yellow-green 1/4 2 mutant traits				

Genetics Made Easy with Fast Plants

Extensions: Selecting and pollinating based on discrete traits, see Appendix B)

Investigating Mendelian Genetics with Wisconsin Fast Plants® on the FastPlants website

<http://www.fastplants.org/pdf/activities/WFPgenetics-06web.pdf>

A simulation program designed for exploring predictions of crosses made with Wisconsin Fast Plants® (you need to set which traits the parents have): <http://www.fastplants.org/genetics/Introductions/index>

Quantitative Traits and Data Analysis

Quantitative (or Continuous) Traits

Most traits are determined by multiple genes (polygenic). In these cases, the traits tend to vary among individuals in a population along a continuum or range, since they are determined by the interaction of many genes (along with environmental influences). These traits can be quantified, either as a measure (e.g., height, length, days), as a count (e.g., number of leaves), or along a scale (e.g., “hairiness” on a scale of 1-10). So, for instance, plants within a population may vary in height within a range at a particular time in their development (see hypothetical data on pages 15-16).

Quantitative traits that can be easily explored in *Brassica rapa* include the following:

- Days for seedlings to emerge
- Height on given days after sowing
- Number of leaves on given days after sowing
- Length of leaves (be sure to create a protocol consistent throughout the class regarding which leaf on the plant will be measured, and how it will be measured)
- Days to flower
- Number of pods

There are clearly many more that can be explored. A sample data collecting sheet is on the following page.

Calculations

From the raw data collected, the following can be calculated for the class population of plants, and comparisons can also be made between the two treatments.

- Average (mean)
- Range (high minus low value)
- Standard deviation (a measure of variance)

For more information on understanding these, please see the FastPlants website: “Variation: Data Organization, Display and Analysis” <http://www.fastplants.org/img/sandbox/uploads/113.file.pdf>

Rapid Cycling *Brassica rapa* Growth Data Sheet

Team Name _____

Date _____ Days After Sowing _____

Environment

- Light (controlled): _____ no. of bulbs _____ distance of plants from bulbs _____ wattage of bulbs
 - Temperature (controlled): Average daily temperature of growing environment _____ °C
 - Nutrient used (varied in 2 treatments): _____

Number of measurements:

Average:

Range:

St. Dev.:

Analyzing Data

Once you collect data of your quantitative traits, and pool it together from all students and teams in your class, how can you organize it so you can see trends, or so you can compare treatments? Graphing data allows you to visualize the trends, and graphing data in different ways can be helpful. Below is a set of hypothetical data graphed with the x-axis representing the plant ID numbers, in ascending order. You can see there is a certain amount of variation in plant height on this day in the population.

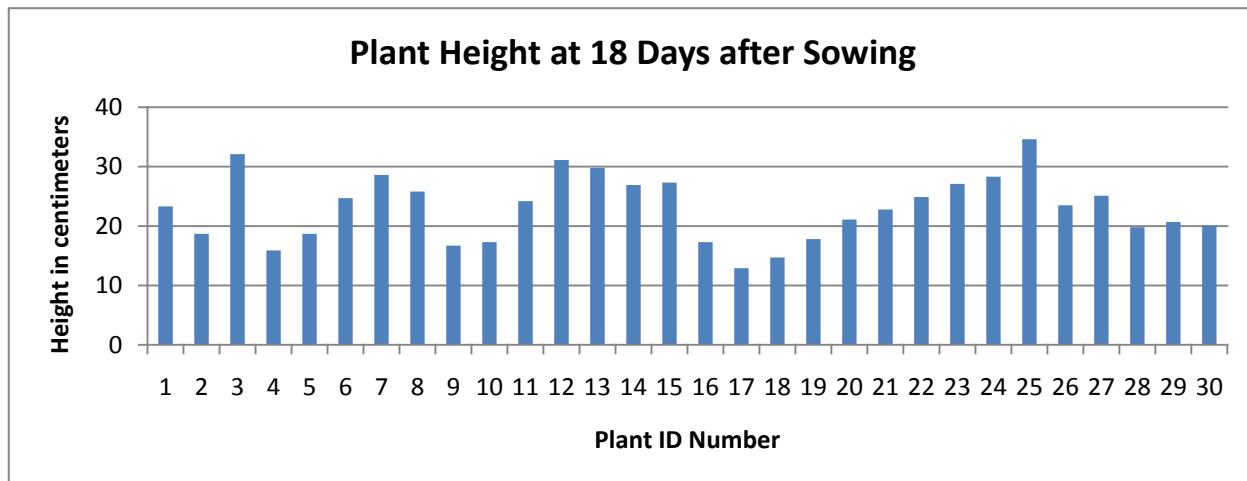


Figure 1. Plant height represented in ascending order of plant ID number.

These same data can be shown organized by height in ascending order, as below. This graph was created in Excel by sorting data by height, and then formatting the horizontal axis to be linked to the Plant ID Number column.

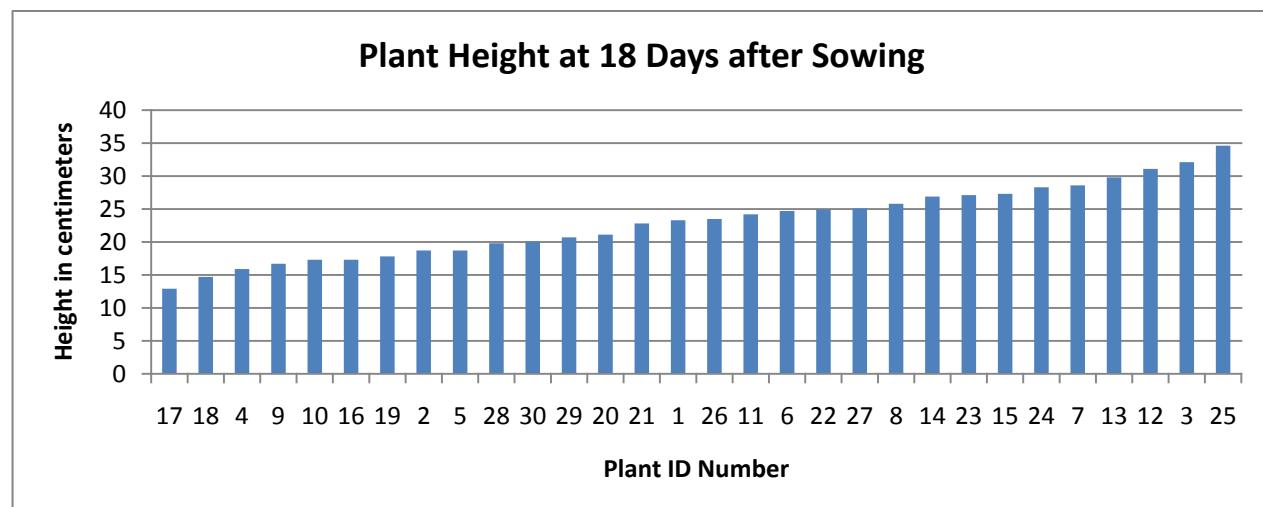


Figure 2. Plant height represented in ascending order of height. Notice that the plant ID numbers are *not* in ascending order.

Graphs showing central tendencies. The previous graphs showed variation in plant height, and the range of that variability. But it's a little difficult to get a sense for an average, or how many plants are at the extreme measurements and how many are in the middle. A good way to organize a data set to look at these central tendencies is to prepare a frequency distribution, sometimes called a binned histogram. This shows you the frequencies, or the numbers of plants, that fall within a certain range of measurements (a "bin"). In the graph below, the data have been organized in 7 bins with 5.0cm ranges. You can see that the majority of plants fall in the 20.1 – 25.0 cm category. Those data follow a nice "bell curve" shape, and the average value falls roughly in that central range.

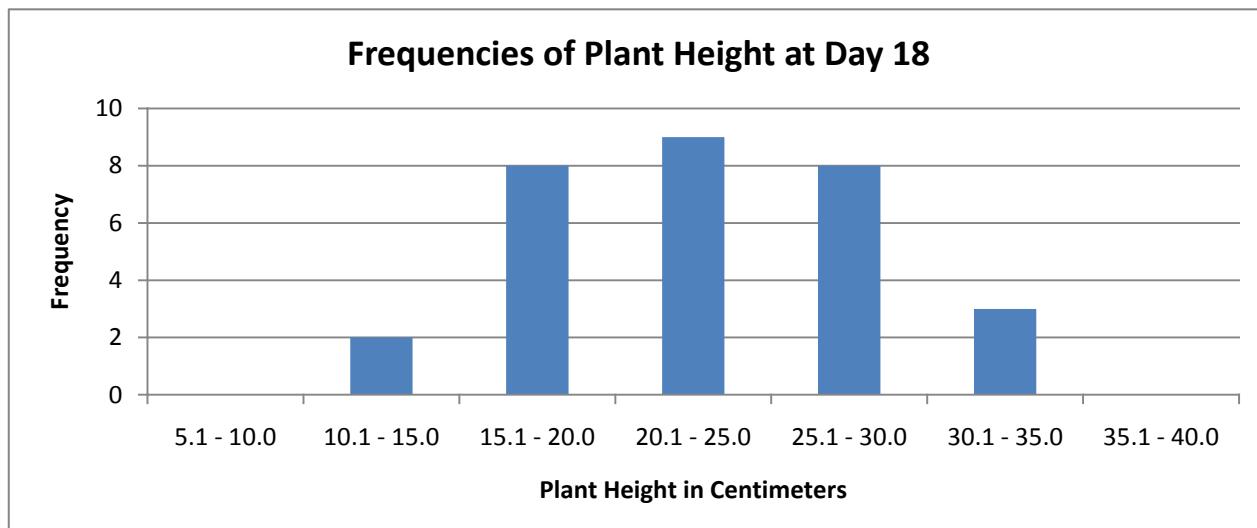


Figure 3. A binned histogram showing frequencies of plants with measurements in 5.0 cm ranges.

You might want to have categories with smaller ranges.

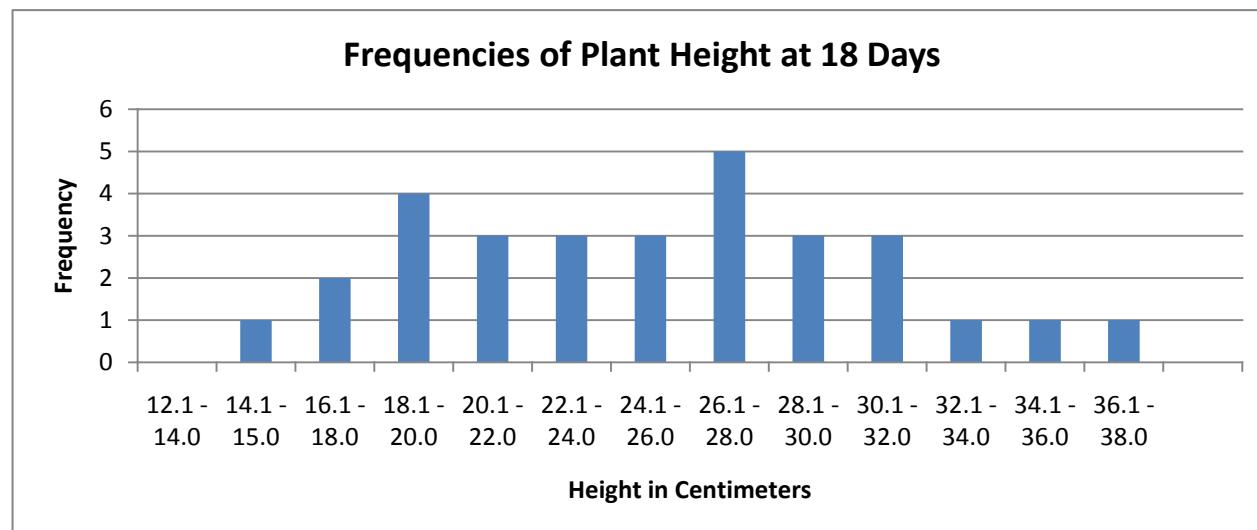


Figure 4. A binned histogram showing frequencies of plants with measurements in 2.0 cm ranges.

For more information on making frequency distributions and binned histograms, see Appendix D and Appendix E.

Quantitative Trait – Trichomes

An observable trait that might escape the notice of students, but which lends itself easily to investigating variation and inheritance is hairiness. Varying numbers of hairs can be seen along the stem, on upper and lower surfaces of leaves, on leaf edges, and even on some buds. These hairs are called **trichomes**.

The number of hairs found on these Wisconsin Fast Plants® are variable, quantifiable, and heritable. The Fast Plants have been specifically developed to study this trait. One of the parents in the lines we are working with has virtually no trichomes (mother). The other parent has a high number of trichomes (father).

The expression of the hairy phenotype appears to be under the control of a number of genes (polygenic). When students collect data on

trichome counts of the F2 population, and graph these data, they are likely to find patterns of central tendency.

If you have also grown parent and F1 seed stock, it will be particularly interesting to compare the data from the F2 generation to these other lines.

Perhaps the best location to count trichomes is on the leaf margin of the first true leaf (the first leaf to emerge after the cotyledons). The following pages provides guidance in data collection.

For more information on the hairiness trait, see Appendix D. Also, for an **extension in selection and pollination** to grow another generation (selected for hairiness), see Appendix C.

Questions for Discussion

- 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?

Activity: Counting Trichomes (continued on next page)

Materials per student team:

- Well labeled plants in their bottles
- Dissecting microscopes (or hand lenses)
- Lab notebooks / class data set
- Graph paper or database

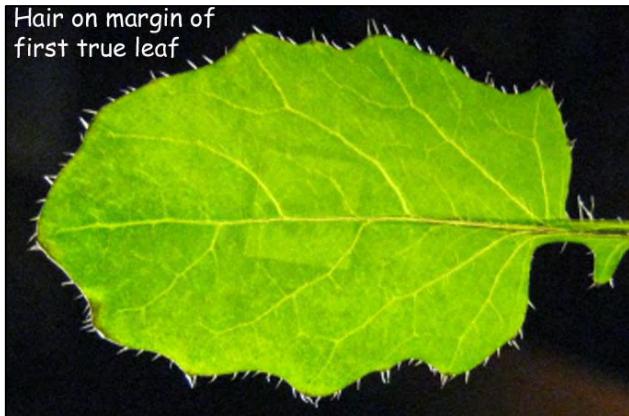
Teaching tip: Scientists need to develop in their own minds a “search image” for something new they are looking at. It takes time to become familiar with something new. You and your students will benefit from allowing time to get to know trichomes and practice counting them.

Counting Trichomes

What is a trichome?

The picture to the right shows a leaf from *Brassica rapa*. Do you see the little hairs scattered on the margins (edges) of the leaves? These are trichomes.

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

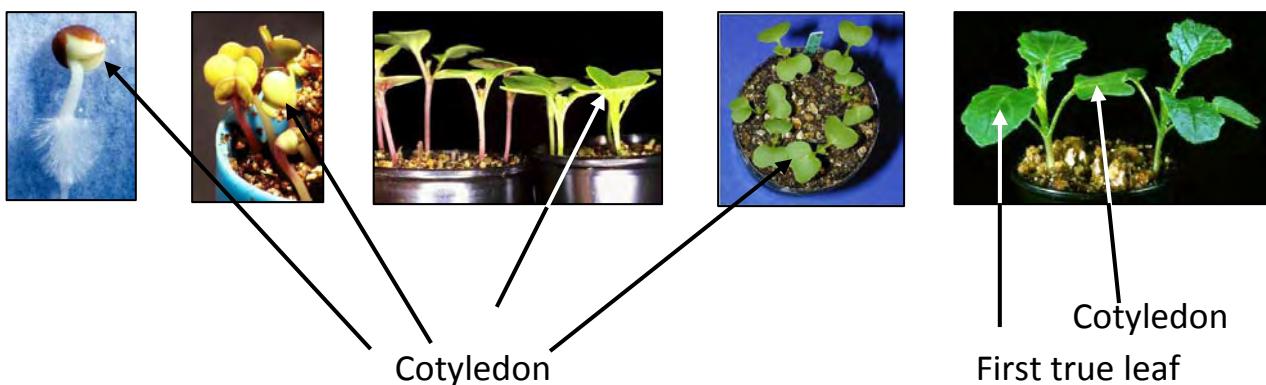


Creating a structure like a trichome requires energy for the plant. Do you think a plant could have too many trichomes? Too few? Do you think trichomes might be more advantageous in some environments over others? Which ones?

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?

Brassica 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 series below, you see a seed germinating. 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. *Brassica* cotyledons have a distinct shape to them that is somewhat heart-like.



Trichome Counting Procedure

1. Each student team should have carefully labeled plants they are responsible for counting.
2. Decide how you will record data – database, lab notebook? Why might it be important to write data by hand even if you will record it electronically? Have you ever had computer glitches?
3. Practice looking for trichomes on leaves.
4. Each plant in each bottle will be counted. Identify each separate plant.
5. 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. If the plants are already well developed, you can cut the first true leaf off the plant to count.
6. Using a dissecting microscope or hand lens, count the trichomes on the first true leaf of each plant and carefully record the counts.
7. Is it enough for one person to count only once? How can you repeat the counts so you are confident you are relatively accurate?
8. Make notes and drawings in your lab book of observations, questions, problems, etc.
9. 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 bottles?
10. How can you summarize your data to make sense of it?
11. Scientists usually plot their data in graphs to look at trends and patterns.
What kind of graph will you use?
12. Compare your results to those of other teams in the class. Do you notice any patterns or trends?



Comparing Traits of Plants in Different Environments

So how much *does* the environment influence a plant? To explore that question, we can compare the plants grown in the two different nutrition environments.

In our case, we have many F₂ seeds of *Brassica rapa* that are crosses between two known grandparents. So although each plant may have somewhat different genes from the others, they are generally similar.

The environmental variable we adjusted is the type of nutrition they receive. One subpopulation of plants received an adequate amount of fertilizer pellets when the seeds were planted (4 pellets per plant). The other subpopulation received only 1 pellet per plant, so it had a low amount of fertilization. The difference between the low and the high fertilization is 1:4. You hopefully labeled the bottles that received a high and low number of fertilizer pellets carefully. That way we can compare data from the two subpopulations.

You can compare the mean (average), and the spread (range, variation) between each

subpopulation (2 different nutrition levels). Are they similar or different? If you call them similar, how confident are you? If you call them different, how confident are you? What is your evidence?

One of the best ways to compare the trends is to convert your data into a frequency distribution, sometimes called a frequency histogram or binned histogram.

When you look at a histogram, you can see several things. For instance, two groups may have the same mean (average), but they could have a much larger range! Alternatively, they could have about the same range, but have very different average values.

Compare your data of your quantitative traits between the two nutrient populations.

Which traits do you think are inherited? Do they appear to be influenced by the nutrition environment? How confident are you in this conclusion? What evidence do you have to support it?

Question for Discussion or Writing

Do you agree or disagree with the idea that **phenotype** is an expression of an organism's **genotype** interacting with the **environment**? In simple form, that can be expressed by the equation:

$$P = G \times E$$

(Phenotype = Genotype interacting with Environment)

Use evidence to support your opinion.

Additional resources on this theme can be found on the Fast Plants website:
<http://www.fastplants.org/activities.variation.variation.php>

Flowers and Pollination



The appearance of flowers in your *Brassica rapa* plants provides an opportunity to review flower anatomy and plant reproduction. These plants require outcross pollination to produce seeds, and students can play the role of artificial bee.

Questions for Writing or Discussion

- What happens if a flower doesn't get pollinated?
- Does a plant need an insect for it to be pollinated? Is this different among different species of plants?
- Would some insects be better at pollinating *Brassica rapa* than others?
- In a natural population, what kinds of traits might get selected for?
- In an artificial population, what kinds of traits might get artificially selected for (e.g., agricultural or research)?
- Why do some plants produce more seed than others?

Pollination Activity

Students will need to make “bee sticks” to act as pollinators. Making bee sticks requires about 15 minutes and should be done one or two days prior to pollination. Observation of the bee and a lesson on the relationship of bees and the Fast Plants could take one 50 minute class period. The actual pollination will take about 15 minutes, unless pollination is being conducted with selection, in which case more time should be allocated.

Materials to make bee sticks:

- A few dozen dried bees. You can check with a local apiary, or you can order them through Carolina Biological Supply.
- Toothpicks
- Fast drying glue



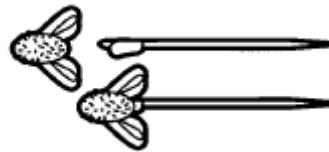


Making a bee stick

1. Carefully remove the legs, head and abdomen of the dried bee, leaving the fuzzy thorax. Pollination can be performed with “whole bee” bee sticks as well.



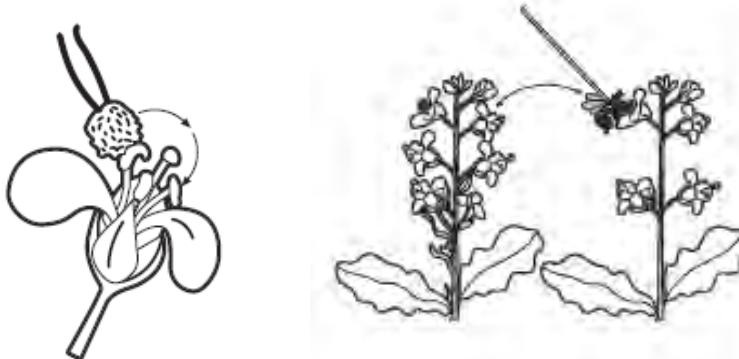
2. Place a drop of fast-drying glue on the tip of a toothpick. Carefully push the toothpick into the top of the thorax of the bee. Remove the wings. Let the bee sticks dry overnight.



Pollinating

Around Day 14 – 16 after sowing, when 5 or more flowers are open on each plant, cross-pollinate all open flowers on each plant with a bee stick by gently rolling the bee thorax back and forth over the anthers of flowers of several plants until yellow pollen can be observed on the hairs.

Move to other plants and repeat the rolling motion over the anthers and the stigma of each pistil, making sure to deposit pollen collected on the bee stick onto the stigma of each flower. Students from one group may wish to circulate your bee sticks to flowers of other groups. Repeat the process for 2-3 days among newly opened flowers.



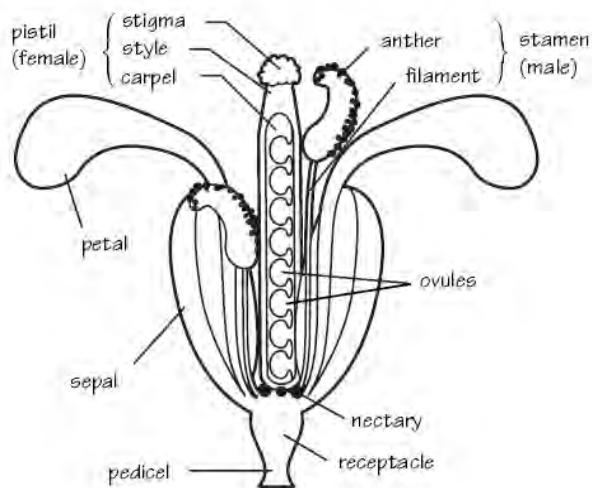
Extensions: Rather than random pollination, as described above, you can select certain plants to cross with each other based on traits, either discrete traits (see Appendix B, p. 56), or quantitative traits (see Appendix C, p. 59).

The Flower

Most flowers have the same basic parts, though they are often arranged in different ways. The five main parts of a flower are the *sepals*, *petals*, *stamens*, *pistil* and *nectaries*. The sepals are the green leaflike structures at the base of the petals that protect the developing flower. The petals are the colored leaflike structures within the sepals.

The stamen has two parts, the *anther* and the *filament*. The anther contains the pollen grains, which contain the male gametes.

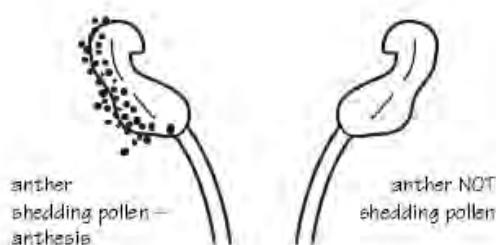
The pistil usually has three parts, the *stigma* (which receives the pollen), the *style* (the neck below the stigma) and the *carpel* (or ovary). Brassica (Fast Plants) flowers have two fused carpels, separated by a thin membrane. The carpels house the *ovules*, which contain the female gametes.



Sugar-rich nectar is secreted by the specialized nectary tissues strategically located in the flower to ensure that nectar-gathering animals will receive pollen from anthers and transmit it to stigmas.

When is a Flower Open?

The answer to this question is not always as straightforward as it might appear. As you observe the progression of flowers opening, you will notice that as the buds swell the sepals are pushed apart by the enlarging anthers and emerging yellow petals. Eventually the petals (which are slightly rolled) fold outward about halfway up their length, flattening and spreading to reveal their bright yellow color. At this time you might conclude that the flowers were open.



From the perspective of mating, a flower is open when it is capable of providing and receiving pollen. Thus, not until the anthers on the filaments of the stamens open up (*dehisce*) and release their pollen is a flower functionally open.

The shedding of pollen is known as *anthesis*. When you observe a succession of flowers at the shoot apex of your Fast Plants you will observe whether anthesis has occurred by noting the release of the powdery yellow pollen from the anthers. A hand lens can be helpful in detecting anthesis.

Sometimes a flower is inhibited from male function by excessive heat or genetic male sterility. As mentioned in WFPID *Understanding the Environment*, anthers may fail to dehisce at very high relative humidities. This problem can usually be corrected in a few minutes by circulating air over the plant with a fan.

The Flower and The Bee: Pollination

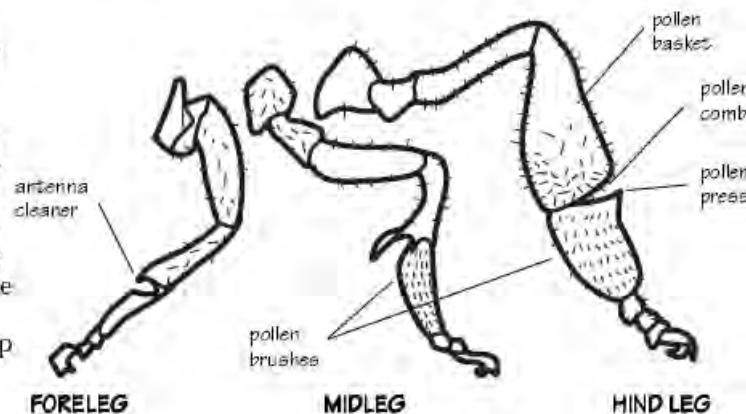
Brassica pollen is heavy and sticky – unable to be easily wind-borne. For brassicas, bees are marvelously coevolved pollen transferring devices (vectors).

Bees depend on the flower for their survival. Sugars in the nectar provide carbohydrates to power flight and life activities. Pollen is the primary source of proteins, fats, vitamins and minerals to build muscular, glandular and skeletal tissues. A colony of bees will collect as much as 44 to 110 pounds of pollen in a season.

A worker bee foraging for pollen will hover momentarily over the flower and use its highly adapted legs for pollen collection (see illustration). The bee's three pairs of legs are evolved to comb pollen from the bee hairs and pack it into the *pollen basket* for transport to the hive.

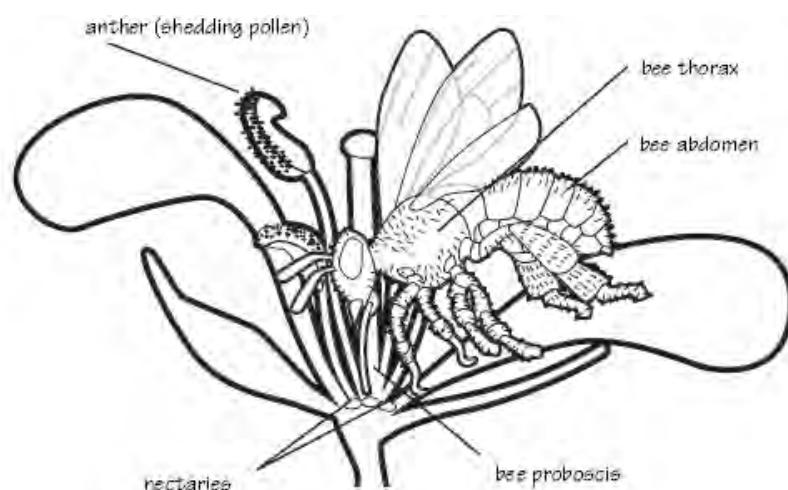
Each foreleg is equipped with an *antenna cleaner*, a deep notch with a row of small spines, which is used to brush pollen from the antennae. Using the large flat *pollen brushes* on the midlegs, the bee removes the pollen from its head, thorax and forelegs. The pollen is raked off the brushes by the *pollen combs* and packed into the baskets by the *pollen press*.

Bees are members of the insect family Apidae, which are unique in that their bodies are covered with feather-like hairs (setae). The bright yellow flower petals act as both beacon and landing pad for the bees, attracting them to the flower and guiding them to the nectaries. The bee drives its head deep into the flower to reach the sweet nectar secreted by the nectaries and brushes against the anthers and stigma. Quantities of pollen are entrapped in its body hairs.



As the bees work from plant to plant, pollen on the bee setae is carried from flower to flower. The transfer of pollen from the anther to the stigma is known as *pollination*. When pollen is transferred from one plant to another, the process is called *cross-pollination*.

As the bee forages, cross-pollination occurs and genetic information is widely transferred. Some pollen grains are deposited on the sticky surface of each stigma and each compatible pollen grain sends a tube through the style to the ovule to complete fertilization. Within three days of fertilization, petals drop and the pistil begins to elongate to form a pod as the seeds develop inside.



Plant Reproduction

Brassica rapa flower



Sepals	Petals	Stamens	Anthers	Pollen
Pistil	Style	Stigma	Ovary	

1. The photo above is a *Brassica* flower. Using your living plants as a reference, label the anatomical parts listed above on the photo.
2. What does pollination mean?
3. What does fertilization mean?
4. Are pollination and fertilization the same thing?

5. Where on the plant is pollen produced?
6. Where does pollen need to be delivered in order to pollinate a flower?
7. Can a flower pollinate itself?
8. What is self-pollination?
9. How might a plant prevent self-pollination from occurring?
10. What is outcross-pollination?
11. If a plant relies on outcross pollination, how does pollen get moved from one plant to the next?
12. What are the genetic consequences of being outcross- or self-pollinated?
13. Some plant species are only self-pollinated. Some are only outcross pollinated. What might be the advantages and disadvantages of each pollination strategy?

Seed Production as a Variable Trait

Seed production is largely hidden to the eye, except that students will see the seed pods grow out from the flower. Seeds need to mature and dry, and watering of plants should cease.

Exploring Seed Production

1. View the video of the life cycle of *Brassica* again at <http://www.fastplants.org/intro.lifecycle.php#day2>
2. What part of the flower is swelling to form the seed pods?
3. Are all the plants producing the same number of seeds?
4. What factors do you think affect how many seeds a plant produces?
5. Once the seed pods are mature and the seed pods are growing yellow, count the number of pods on each plant. Record these data.
6. Then, visually estimate the number of ovules developing into seed in the attached pods by holding the pods to a light to see the seeds inside.
7. Once the pods begin to turn brown, they can be harvested. It is important to keep the seeds from each individual “mother” plant separate from the others. For best results, open the pods over a piece of scotch tape. You could clump the seeds in groups of 5 on the tape to make counting easier.
8. The longer the seeds can be kept dormant, the better the germination rates will be for the next generation.



Top: Seed pods maturing on the plants as flower petals wither around them.

Far left: Estimate the number of developing seeds by holding the pod up to the light.

Near left: Immature developing seed inside the pod.