WISCONSIN FAST PLANTS

Flowering and Pollination: Pollination Biology

Concepts

Pollination is the process of mating in plants; it is the precursor to double fertilization. In flowers, pollen is delivered to the stigma through a wide range of mechanisms that insure an appropriate balance in the genetic makeup of the species. In brassicas, pollen is distributed by bees and other insects. The flower is the device by which the plant recruits the bee. Bees and brassicas have evolved an interdependent relationship.

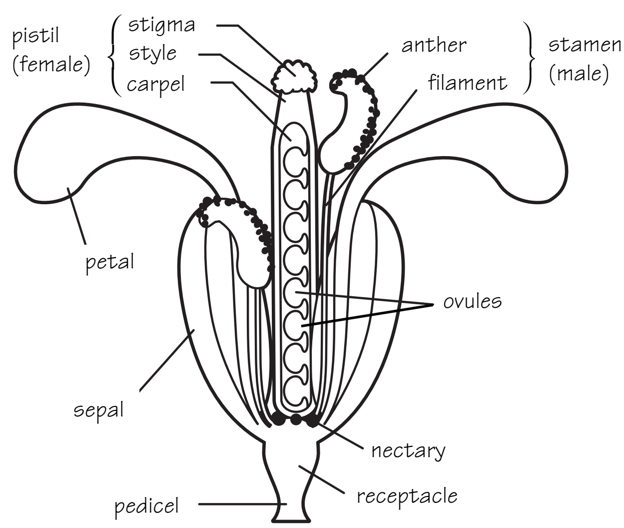
Background

What is a flower? In human eyes it is something to enjoy, with color and fragrance. For many plants, flowers are vital organs of reproduction containing both male and female gametes. For bees and other nectar-feeding animals, flowers are a source of food.

Symbiosis is the close association of two or more dissimilar organisms. Such associations can be beneficial to both organisms (*mutualistic*) or detrimental to one *(parasitic)*. Symbiotic relationships among species occur frequently in nature. When the two or more species in symbiosis evolve in response to each other, they are said to *coevolve*. Under close examination each symbiotic relationship stands out as an example of miraculous complexity which has emerged. The *coevolution* of bees and brassicas, each dependent upon the other for survival, is such a relationship.

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.

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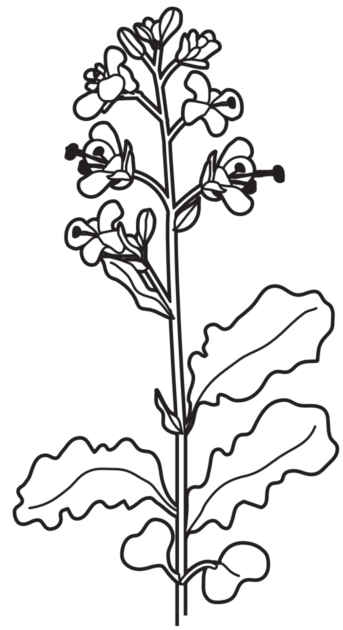
Investigations of a Gametic Kind

The Developmental Clock



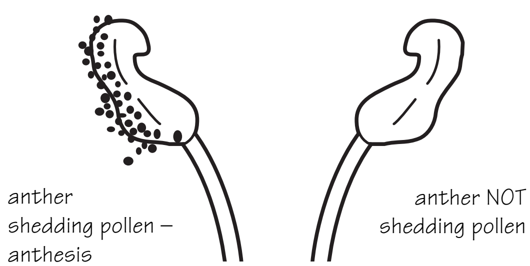
At about Day 7 in the Fast Plants life cycle, you may have looked down on your plants from the top view and noticed a tightly packed whorl of buds, some of which were larger than others. Each successively smaller bud represented a time interval in the developmental clock of Fast Plants, later marked by the appearance of new flower buds on the shoot.

As your Fast Plants begin to flower, normally the lowest flower on the shoot opens first, followed by the next highest and so forth. By recording the time when the first flower opens and counting the number of successive flowers that open in the intervening 24 hours, you can calculate the average time between successive developmental events that initiate a flower on the shoot apical meristem.

- Can you calculate the number of hours between successive flowers on your Fast Plants?
- How many flowers would you predict will open between the time your first flower opens and when you pollinate your plants?

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.

Gametogenesis

The production of sperm and eggs involves a fundamental sequence of events that characterizes most higher plant and animal life. In plants, meiosis precedes gamete formation and establishes the initial events of sexual reproduction by exchanging and sorting the genes on the chromosomes into the vehicles of genetic transmission and reception that we call gametes: the eggs and sperm.

In Fast Plants the developmental process known as *microsporogenesis* occurs in the developing anthers when the first flower bud of the apical whorl is about one millimeter in diameter and leads to the production of pollen. Within the anthers specialized tissues undergo meiosis to form the *microspores* that eventually become pollen grains. Pollen is the immature stage of the *microgametophyte*, which does not become fully mature until it germinates on a stigma and forms a pollen tube containing two sperm cells and a tube nucleus.

At about the same time as the anthers are developing, tissues within the developing pistil of the immature flower bud give rise to a series of ovules. Within each ovule meiotic divisions in the process of *megasporogenesis* lead to the production of *megaspores*. Through the process of *megagameto-genesis* the megaspore develops into a mature *megagametophyte* or embryo sac (Raven, Evert and Eichorn, 1992).

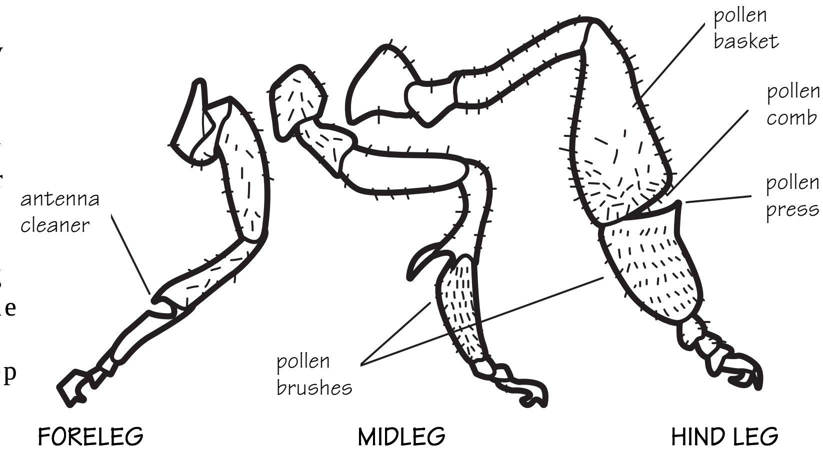
The Flower and The Bee: Pollination

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.

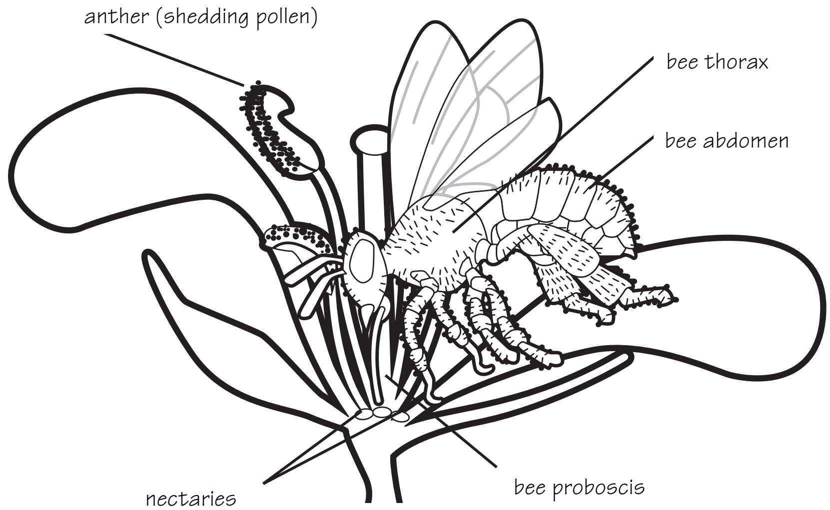
Each foreleg is equipped with an *antenna cleaner*, a deep notch with a row of small spines, which is used to brush pollen from the antennae. Using the large flat *pollen brushes* on the midlegs, the bee removes the pollen from its head, thorax and forelegs. The pollen is raked off the brushes by the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



Are We Compatible?

For Fast Plants and many though not all brassicas, the act of pollination does not insure fertilization and seed formation. Some brassicas contain recognition compounds called glycoproteins which are unique to each plant. These compounds enable the plant to recognize "self," resulting in the abortion of the plant's own pollen. This genetically controlled prevention of fertilization with "self" pollen is called *self-incompatibility*. Only "non-self" pollen is able to germinate and effect fertilization.

In order for pollen germination and fertilization to occur, pollen must travel from one brassica plant to the stigma of a different brassica plant in the process of cross-pollination. Bees take care of this problem naturally as they move from plant to plant in search of nectar and pollen. AstroPlants are therefore *cross-compatible* and *self-incompatible*.

ACTIVITY

Making Beesticks, Pollinating and Observing Pollen

Introduction

As their plants come into flower, students need to be prepared to pollinate. The act of pollination is the prelude to the beginning of a new generation that starts with double fertilization. In preparation for pollination of their plants, students will need to understand the developmental biology leading to the production of male and female gametes (sperm and egg) and the concepts associated with the coevolutionary relationships between flowers and their pollinators.

Question: How is effective pollination carried out?

Sample Hypothesis: The transfer of adequate numbers of viable, compatible pollen from the anthers of one plant to the stigmas of another plant will result in effective pollination.

Design

- With a beestick as the vector, pollen is collected from the anthers of various plants and transferred to the stigmas of other plants.
- Students will record data on their Floral Clock Student Data Sheet.
- It is important for each class to have at least **two extra sets** of four film can wick pots (16 plants) to serve as **unpollinated control plants**. The control plants should be planted along with and maintained in the same manner as the experimental plants.

Time Frame

A period of 16 days from the sowing of seed is required for the growth of the AstroPlants and the completion of the activity. Making beesticks will require about 15 minutes and should be done one to two days prior to pollination. Observation of the bee and a lesson on the relationship of bees and the Fast Plants flower in pollination could take one 50 minute class period. The pollination will require one 50 minute class period.

Learning Objectives

In participating in this activity students will:

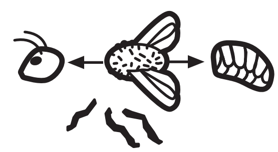
- understand flowering as the sexually mature stage of plant development;
- understand where and how ovules and pollen originate (male and female gamete formation);
- explore the parts of the flower and the role that each part plays in reproduction;
- observe the reproductive tissues of plants, including pollen and stigma, under magnification;
- understand the interdependent coevolutionary relationship of bees and brassicas; and
- begin the process of reproduction in their Fast Plants by performing a pollination using a beestick, setting the stage for future developmental events.

Materials

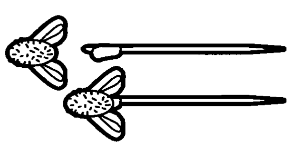
- 8 flowering Fast Plants (Day 14 to 16) 2 plants in each film can wick pot
- forceps
- two dissection strips (see WFPID *Dissection Strips*)
- 2 cm wide clear adhesive tape
- dried bees
- toothpicks
- glue (e.g., Duco® Cement)
- hand lens or microscope

Procedure: Making Beesticks

1. One to two days prior to pollination, students should make beesticks. While making beesticks the teacher may wish to have students observe the anatomy of a bee, focusing on the legs and proboscis, to reinforce an understanding of the design (role and function) of the bee in relation to the flower.



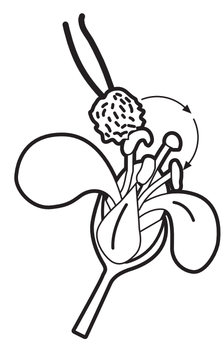
2. Carefully remove the legs, head and abdomen of the dried bee, leaving the fuzzy thorax. Pollination can be performed with "whole bee" beesticks as well.

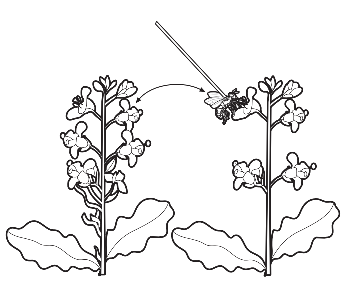


3. Place a drop of fast-drying glue on the tip of a toothpick. Carefully push the toothpick into the top of the thorax of the bee. Remove the wings. Let the beesticks dry overnight.

Procedure: Setting the Floral Clock

1. At a time between **Day 14** and **Day 16** when five or more flowers are open on each plant in the film can, cross-pollinate all open flowers on each plant with a beestick 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.





Moving to other plants and repeating the rolling motion over the anthers and the stigma of each pistil, students should make sure to deposit pollen collected on the beestick on to the stigma of each flower. Students from one group may wish to "fly" their bees to flowers of other groups. Buzz!

2. Take the **top open flower** of the first plant and carefully remove it with a forceps. Place it on the sticky tape of a dissection strip.

3. While observing with a hand lens or microscope, carefully remove the flower parts, noting their relative positions on the receptacle, **making sure not to damage the pistil**. Refer to the illustration on page 43 for help in identifying the floral parts. You and your students may want to test the nectaries for the presence of glucose (see WFPID *The Hunt for Glucose - A Flower's Treasure*).

4. Slip the ruler on a second dissection strip (without tape on it) under the first strip. Each student should measure the length of the pistil from the receptacle to the top of the stigma and record the pistil length on his/her Floral Clock Student Data Sheet.

5. Remove the top open flower of the second plant, measure the length of the pistil and record the data on the Floral Clock Student Data Sheet in the column under the number of the second plant.

   - With higher powered microscopes students could observe magnified views of pollen trapped on the stigmas of dissected flowers or on a beestick.

   - While pollinating, students might also observe the pollen trapped on the tape of a dissection strip.

   - Alternatively, look at the pollen on the stigmas or on the beestick with a hand lens.

   - A beestick with pollen can be rolled over the sticky tape on the dissection strip. Observation under a magnifier will reveal pollen attached to the bee setae.

magnified pollen grains on bee setae

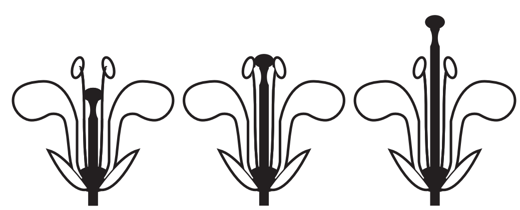
6. Note that flowers on the plants are produced and open in a sequence spiraling up the flower stem. Starting with the next flower down from the one that you removed, **number the remaining flowers as 4, 3, 2 and 1** as shown in the illustration on the next page, with number 1 being the oldest flower.

   - With a sharp pair of fine scissors, carefully snip off all additional remaining flowers below flower number 1 (including side shoots), leaving only the four open flowers that have been numbered.

   - Snip off the developing apical flower shoot and buds above the four remaining open flowers. This is known as *terminalizing* the plant.

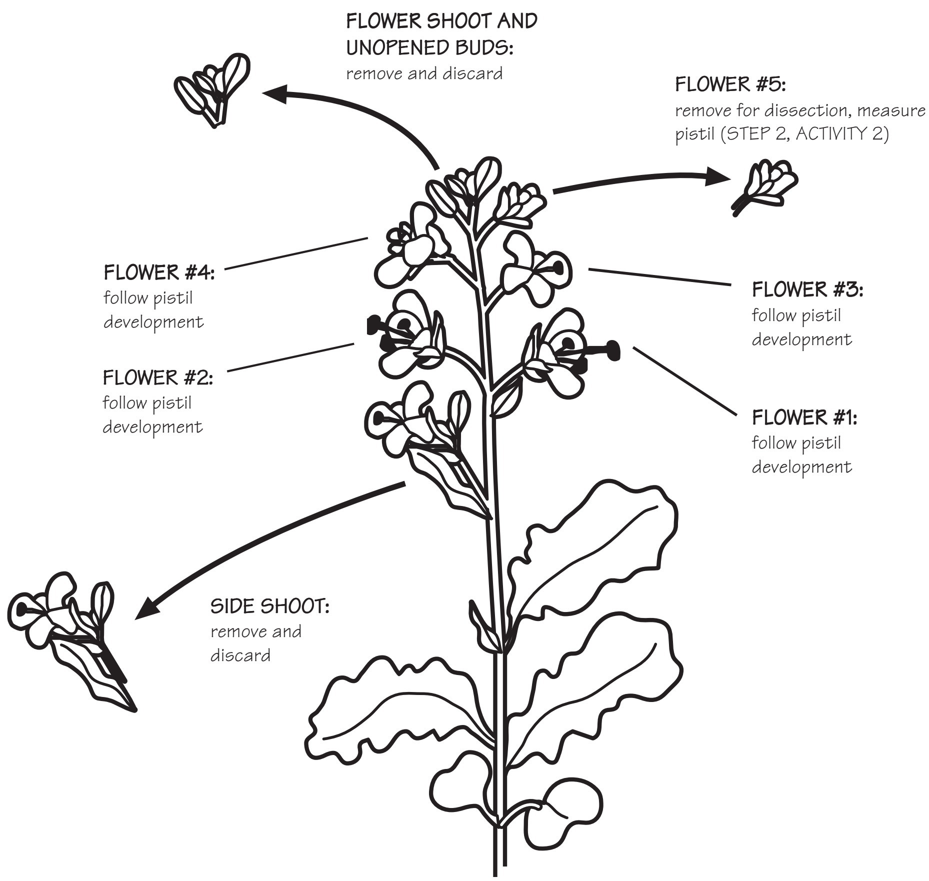
7. Returning to the four remaining flowers, note the position of the stigmas relative to the tall anthers. Is the stigma below (-1), equal to (0) or above (+1) the tall anthers? Record this information for each flower of both plants on the Floral Clock Student Data Sheet.

   - Is there a relationship between flower age and the position of the stigma relative to the level of the four tall anthers?



8. Be sure to measure the length of the uppermost open flower of each plant in the set that was grown for the unpollinated controls. Data for the unpollinated control sets should be recorded on a separate Floral Clock Student Data Sheet.

9. Before leaving the plants be sure that each of the remaining stigmas has been adequately pollinated by the beestick. Can you see any pollen on the stigmas? Check with a hand lens.



Concluding Activities and Questions

The completion of pollination sets the floral clock at "0 dap" (days after pollination). Activities 5 and 6 in the section "Double Fertilization and Post-Fertilization Events" involve observing, recording and analyzing pistil development as an indication of successful pollination. In participating in these activities students will complete their Floral Clock Student Data Sheets. After completing Activity 4, have students consider the following:

- What is the relative importance of each flower part in relation to pollination and sexual reproduction? Are some parts more important than others? Why?

- Does the pistil continue to elongate after the flower is functionally open? Even if the flower is not pollinated?

- How many flowers on average open each day once the first flower has opened?

- What is the average amount of time (in hours) between the development of one flower and its nearest neighbor?

> "People from a planet without flowers would think we must be mad with joy... to have such things."
>
> \- Iris Murdoch

Extensions

For activities on observing and experimenting with pollen germination in vitro under the microscope and on observing compatible and incompatible pollen-stigma interactions and pollen tube growth in the style and ovaries, see WFPID's *Pollen Germination* and *Pollen-Stigma Interactions and Pollen Tube Growth*.

With a separate third set of plants, try self-pollinating and compare the amount of seed produced with the seed produced by the cross-pollinated plants.

Floral Clock Student Data Sheet

Student Name ______________________

Plant Type: (circle one)

pollinated   unpollinated

Group Number ____________

Environment

Irradiance: _____ no. of bulbs _____ distance of plants from bulbs

_____ wattage of bulbs or $\mu E m^{-2} s^{-1}$ measured under bulbs

Average daily temperature of growing environment: _____ ˚C

Nutrient used: ____________

Root medium used: Peatlite ____ Specify other: ____________

Seed stock: ____________

Date	dap	Character/Activity	Plant No. ____ Measurements				Statistics				Plant No. ____ Measurements				Statistics			
	0	pistil length top flower (mm)																
		number pollinated flowers																
		Flower/Pod Number	1	2	3	4	n	r	x	s	1	2	3	4	n	r	x	s
	0	note position of stigma above, at or below anthers (+1, 0, -1)*																
		pistil length (mm)																
		pistil length (mm)																
		pistil length (mm)																
		est. no. developing seeds (#)																
		pistil length (mm)																
		pistil length (mm)																
		harvest & count seeds/pod																
		viability test (+ / -)																

* when noting stigma position: +1 = above anthers, 0 = at level of anthers, -1 = below the level of anthers

dap = days after pollination, n = number of measurements, r = range (maximum minus minimum), *x* = mean (average), s = standard deviation

The dap column is left blank on this data sheet because the timing of activities depends on the rate of development in each students' plants.

Floral Clock Class Data Sheet

Date ________________

Name ___________________________________

Environment

Irradiance: _____ no. of bulbs _____ distance of plants from bulbs

_____ wattage of bulbs or $\mu Em^{-2}s^{-1}$ measured under bulbs

Average daily temperature of growing environment: _____˚C

Nutrient used: _____________

Root medium used: Peatlite _____ Specify other: _____________

Seed t stock: _____________

How to use this Class Data Sheet:
This Class Data Sheet can be used for the teacher or students to compile data from one measured character (e.g., pistil length, 14 dap) taken by students who have completed the Floral Clock Student Data Sheet. Each group of students should compile summary statistics for the group's eight plants. These statistics should then be entered in the chart below. From the Group Data chart, the class data can be derived.

Variable Measured _________________________ Date Measured _____________ dap of Measurement _____________

Group Data

Group	Statistics					Group	Statistics			
	n	r	x	s			n	r	x	s
Group 1						Group 7				
Group 2						Group 8				
Group 3						Group 9				
Group 4						Group 10				
Group 5						Group 11				
Group 6						Group 12				

Class Data

Statistics			
n	r	x	s

dap = days after pollination, n = number of measurements, r = range (maximum minus minimum), *x* = mean (average), s = standard deviation