# **Chapter 11**

# Plant Reproductive Systems: An Investigative Approach

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## Introduction

This laboratory exercise was developed by the Biology Department at Furman University as one of two plant components for the freshman biology laboratory. One of the important developments shown by plants which make them suited to life on dry land is a change from dependence on water for fertilization to using wind or insects. As we developed this lab, we wanted the students to examine plants in a way that went beyond simply dissecting flowers and examining flower parts. In addition, we wanted this laboratory exercise to continue in the same mode as our other freshman labs by having a strong investigative component paired with the use of scientific techniques.

This freshman level laboratory exercise introduces students to plant diversity by using an investigative approach to study various aspects of plant reproduction. Students study the reproductive structures in ferns and angiosperms and relate these structures to methods of spore, pollen, or seed dispersal.

#### **Materials**

#### Plant Material Needed for the Entire Laboratory

Part A: Reproduction in Ferns

Any fern with visible, mature sori Fern gametophytes: usually found on clay pots in a well-watered greenhouse *Psilotum Equisetum Selaginelia Lycopodium* 

Part B: Comparison of flower structure and pollen in insect vs. wind-pollinated plants. [NOTE: We try to have the students observe at least one composite, one grass, and one "typical" flower.]

#### Fall Season:

Chrysanthemum (insect-pollinated) Abelia (insect-pollinated) Paspalum (wind-pollinated) Ipomoea (unknown insect-pollinated) Chenopodium (unknown wind-pollinated)

Winter Season:

Chrysanthemum (insect-pollinated) Chaenomeles (insect-pollinated) Poa (wind-pollinated) Lonicera (unknown insect-pollinated) Alnus (unknown wind-pollinated)

#### Spring Season:

Chrysanthemum (insect-pollinated) Rhododendron (insect-pollinated) Poa (wind-pollinated) Gladiolus (unknown insect-pollinated) Quercus (unknown wind-pollinated)

#### Materials needed per group of four students for Part A:

Dissecting Microscope Incandescent Lamp White Paper Prepared slides:

> Fern prothallium young sporophyte (Carolina Biological Supply B415) Fern prothallium, antheridia, whole mount (Wards/Turtox B5.813) Fern prothallium, archegonia, whole mount (Wards/Turtox B5814)

#### Materials needed per group of four students for Part B

Dissecting Microscope Compound Microscope Dissecting Instruments such as scales, probes, scissors Prepared slide of mixed pollen, whole mount (Wards/Turtox 91W7001) Mixed pollen whole mount key to pollen Microscope slides Microscope slide covers Pasture pipette Water

#### Materials needed for calibration of the ocular micrometer

Compound Microscope Ocular micrometer Stage micrometer

#### Notes for the Instructor

This laboratory exercise is divided into two parts: PART A: Reproduction in Ferns

PART B: Comparison of Flower Structure and Pollen in Insect vs. Wind-pollinated Plants

We run this laboratory with students divided into teams of two or four. Depending on the time of year, we have different live specimens for the students to use. In part B: Comparison of flower structure and pollen in insect vs. wind-pollinated plants, we always have the students observe a composite, a grass, and a "typical flower". Unknown pollen types will always include an insect-pollinated plant and a wind-pollinated plant. Since the students will have used the ocular micrometer several times in other laboratory sessions before this laboratory, no information is given in the student outline section on how to do the ocular micrometer calibration. However, the portion of the laboratory where students learn to calibrate the ocular micrometer has been included as Appendix B.

# **Student Outline**

#### Introduction

Today, you will investigate a set of adaptations that aided plant development on land: the increasing use of air and animals for gamete and population dispersal. You will look at gamete formation and spore dispersal in ferns (seedless plants), and at flower and pollen structures in angiosperms (seed plants).

#### **Part A: Reproduction in Ferns**

#### **Background: Fern Reproduction**

You will study a common woodland fern of the order Filicales. The Filicales representative is homosporous; meaning only one type of spore (which in this case is air-borne) is produced by meiosis in the sporophyte form of the plant. This spore develops into a single type of gametophyte that produces both male and female gametes to continue the plant's life cycle. You will be observing air-borne spore release from the Filicales's sporophytes.

#### **Observation of spore release from the woodland fern (Order Filicales)**

Observe the underside of the fronds of the Filicales specimen and note the dark, mature sori, each of which contain several sporangia. Select only those that are nearly black, not brown. The dark color is due to the darkened walls of the mature spores within them.

- 1. Obtain a piece of one of the fronds and observe it under your dissecting microscope. Each sporangium in a sorus is composed of a spore sac on top of a thin stalk. The sac has a row of thickened cells extending from the stalk (at the base of the spore sac) to most of the way over the top of the sac. This row of cells, called the annulus, bends back when dried, opening the spore sac and holding many of the spores in its unfolding "lid." After a certain point, the annulus springs back into its original position, hurling the spores away from the sorus.
- 2. Place an incandescent lamp close to the sori under your dissecting microscope in order to accelerate the drying of the sporangia. Observe the sporangia during this drying and note the steps in the release of the spores. Draw representative sori and sporangia.

#### **Observation of fern gametophytes**

The spores released from sporangia land in moist places and germinate into a plant with the haploid chromosome number. This gametophyte generation will produce sperm cells and egg cells,

by mitosis. Observe living fern gametophytes (sometimes called prothallia) if available. Then using low power, examine prepared slides of fern gametophytes, looking for sperm-producing structures (antheridia) and egg-producing structures (archegonia). You will not find antheridia and archegonia on the same prothallus, because they mature at different times. After the egg in an archegonium is fertilized, the sporophyte begins to grow, initially while still attached to the gametophyte. Examine the available slides of this stage. Eventually the gametophyte will wither away, leaving the sporophyte to grow independently.

#### Other vascular non-seed plants

As available, examine live specimens of *Psilotum* (whisk fern), *Equisetum* (horsetail), *Selaginelia* (spikemoss), and *Lycopodium* (clubmoss). While of diverse appearance, these are all plants that are related to ferns, and share the features of vascular systems, dispersal of spores, and free-living gametophyte generations.

#### Part B: Comparison of Flower Structure and Pollen in Insect vs. Wind-pollinated Plants

#### Background

This part of the laboratory deals with the reproductive structures of the angiosperms, also known as flowering plants. There are two main objectives in this exercise. First, you should learn to recognize the main structures in flowers, and appreciate the variations in structural pattern, by handling and dissecting flowers from a variety of different plants. Second, you will observe how floral structure differs in relation to the two most important agents of pollination, wind and insects.

The flowering plants (known technically as Angiospermae, Anthophyta, or Magnoliophyta) represent by far the most abundant, diverse, and successful phylum of plants in existence today, with over 235,000 species known. The characteristic feature of this group is the flower, a set of reproductive structures typically comprising sepals, petals, stamens, and carpels. Sepals are typically green structures that enclose the developing flower bud. Petals lie within the sepals. They are also enveloping structures, but are often large and colorful. Stamens are the male reproductive organs, and produce pollen grains. Carpels are the female reproductive organs, and enclose ovules. After fertilization by a pollen grain, an ovule within its enclosing carpel will develop into a seed within an enclosing fruit. A flower does not necessarily contain all four types of structures; for example, it is quite possible that petals can be missing.

In this laboratory we will concentrate on gross floral anatomy that can be seen with the naked eye or a dissecting microscope. As any professional or amateur botanist can tell you, identification of plants in the field or garden often requires being able to interpret the structures in a flower.

#### **Experimental Protocol: Observation of floral structures**

You are provided with flowers of *Chaenomeles lagenaria* (flowering quince), *Chrysanthemum morifolium* (chrysanthemum), and *Poa annua* (annual bluegrass). The first two of these are insect-pollinated plants, while the grass is wind-pollinated. For each plant, you will also receive a 1-page "Dissection Guide." You should examine and dissect each specimen, using the dissecting microscope where appropriate. Try to identify and understand all the structures indicated on the dissection guide. As you make these observations, try to form some generalizations about the typical structure of wind-pollinated versus insect-pollinated plants.

#### Measurement of pollen diameters

Obtain one of the prepared slides of mixed pollen grains, and the key card that goes with it (which should allow you to recognize the pollen types based on shape and color). Your goal is to measure the diameter of a typical pollen grain from each species. Use 400X magnification on the compound microscope, and determine the conversion factor from ocular units to microns. Each person at your lab table should measure the diameter of 3 or 4 species; the group as a whole should attempt to complete the listing below in Table 11.1. If you cannot find one or two of the species, you may skip them.

In addition to the prepared slides, you should also measure the diameter of *Chaenomeles* and *Poa* pollen. To do so, place a drop of water on a clean slide. Remove a stamen or two from a flower, and crush the anther in the water drop using a dissecting needle. Cover the drop with a cover slip and observe the slide on the microscope. *Chrysanthemum* pollen is on the prepared slide, so you do not need to complete this step for it. Finally, calculate the average diameter for each pollination mechanism.

Pollen Diameters in Microns					
Inse	ct-pollinated	Wind-pollinated			
Chrysanthemum	Nicotiana	Ambrosia			
Dahlia	Rosa	Atriplex			
Hibiscus	Saintpaulia	Fraxinus			
Lilium (either kind)	Salix	Poa*			
Liriodendron	Scrophularia	WIND-POLLINATED AVERAGE			
Malus	Taraxacum				
Chaenomeles*	I				
INSECT-POLLINATED A	AVERAGE				

Table 11.1. FUIGH Diameters in Microin	Table	11.1.	Pollen	Diameters	in	Micron
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\*from fresh specimens, not prepared slide

#### Examination of "Unknowns"

You will also be given specimens of *Lonicera fragrantissima* (bush honeysuckle) and *Alnus serrulata* (alder). A dissection guide accompanies each specimen, but you will not be told the mechanism of pollination. Examine these specimens, noting dimensions of floral structures as you did above. Measure the pollen grain diameter. Attempt to make conclusions about pollination mechanisms, and answer the questions provided.

#### Answer the following questions

- 1. Based on your dissections of *Chaenomeles, Chrysanthemum*, and *Poa*, what generalizations can you make about the overall structure of the flower (especially the petals) in insect-pollinated versus wind-pollinated plants? What hypothesis can you propose that would explain why this pattern exists?
- 2. Based on your observations of pollen from both the fresh specimens and the prepared slide, what generalizations can you make about the pollen of insect-pollinated versus wind-pollinated plants? What hypothesis can you propose that would explain why this pattern exists?
- 3. You were not told what pollination mechanism is used by *Lonicera* or *Alnus*. State what mechanism you think is characteristic of each species, and justify/defend your answers.

#### Acknowledgements

A committee (W. D. Blaker, A. J. Pollard, and L. K. Thompson) did the original write-up for this laboratory from the Biology Department of Furman University. A. Joseph Pollard, Professor of Biology, Furman University, Greenville, SC, did the artwork for figures 1, 6, 7, 8 and 9, and parts of figures 2, 4, and 11.

## **Appendix A Flower Diagrams**

Since the laboratory manual for the Biology Department of Furman University is printed once per year, the Dissection Guides handed out to the students vary, depending on the time of year. The following Dissection Guides have been arranged according to season (fall, winter, spring) and known or unknown pollination mechanisms.

#### Fall Season: Abelia (insect-pollinated)

*Abelia* flowers differ from the "typical" flower diagrammed in your textbook in only a few details. The petals are partially fused into a funnel-shaped structure called a corolla tube. The mouth of the funnel does not have perfect radial symmetry (star shape) but is bilaterally symmetrical. Finally, these flowers have what is called an inferior ovary. The ovary of a flower is the portion that contains the developing seeds; later, the ovary will ripen into the fruit of the plant. An inferior ovary, as in *Abelia*, is located below the point where the petals and sepals are attached. A superior ovary is located within the flower, surrounded by the petals.

Find the indicated structures on your *Abelia* flower, cutting or tearing into the corolla tube as necessary. Measure pollen diameter by dusting an anther in a drop of water on a microscope slide, then covering with a cover slip.



# **Figure 11.1.** Dissection guide for *Abelia*, a fall season, insect-pollinated plant. **Fall Season:** *Paspalum* (wind-pollinated)

*Paspalum* (Bahia grass) is a member of the grass family, Poaceae. This very large family (about 12,000 species) is perhaps the most important in the world, because many important human foods, including wheat, rice, and corn, are made from the seeds of grasses.

All members of the grass family are wind-pollinated. Since they do not attract pollinating animals, their flowers are small and inconspicuous. Perhaps you did not even realize that grasses are "flowering plants" but they do indeed have small, reduced flowers. There are no petals. Sepals are reduced to vestigial structures. The main parts of the flower are the sexual structures: the stamens and carpel. On the other hand, there are often small leaf-like structures called bracts, which sandwich the flower. A flower with its enclosing bracts makes up a unit called a spikelet. To see the flowers of a grass, you will need to use a stereoscopic microscope, and tools such as dissecting needles and forceps to peel away the surrounding bracts.

Examine the forked Bahia grass inflorescence (Figure 11.2A). Select a few spikelets that have brownish-red stamens and stigmas protruding from them. Pluck them off and examine them on the dissecting microscope. The pollen-producing anthers hang out of the spikelet on slender thread-like filaments (anther + filament = stamen). The pollen-receiving stigma is brush-like, to trap pollen from the air.

Using your dissecting tools, peel back the surrounding bracts from the spikelet (Figure 11.2B), to reveal the flower within (Figure 11.2C). It may require several tries to see an intact flower. To measure pollen diameter, place some anthers in a drop of water on a slide, and crush the anthers with your needle. Then cover the drop with a cover slip and look for pollen using the compound microscope.

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Figure 11.2. Dissection guide for Paspalum (Bahia grass), a fall season, wind-pollinated plant.

#### Fall Season: Ipomoea (Insect Unknown), Chenopodium (Wind Unknown)

You have been provided with flowers of two plants: *Ipomoea* (morning glory) and *Chenopodium* (goosefoot). One of these species is wind-pollinated and the other is insect-pollinated. A diagram of the basic structures of each is shown below, to help you get started. Using the information you learned from examinations of *Abelia, Chrysanthemum,* and *Paspalum,* examine these two new species and interpret the structures you see. Prepare pollen slides and measure pollen diameters.

Ipomoea



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**igure 11.3.** Dissection guide for *Ipomoea* (morning glory) and *Chenopodium* (goosefoot), fall season plants. These two plants are used as unknowns, *Ipomoea* is an insect-pollinated plant and *Chenopodium* is a wind-pollinated plant.

#### Winter Season: Alnus serrulata (Wind-pollinated Unknown)

*Alnus serrulata* (alder) is a small tree found along streams in our region. The flowers differ in many respects from "common" flowers. Each flower is either all male or all female. The male flowers are found in dangling inflorescences called catkins. The female flowers are in upright

catkins nearby. Because both male and female flowers occur on the same plant, this species is described as monoecious. If there were separate male trees and female trees, as in both willow and persimmon, the species would be described as dioecious.

The diagram (Figure 11.4) below shows you how to see the flowers. Use a dissecting microscope. In both male and female catkins, the flowers are partially hidden behind small scales called bracts. The male flowers are fairly small; each flower consists of simply four sepals and four stamens. The female flowers are even smaller. Each female flower is basically a carpel, consisting of an ovary and two styles; both sepals and petals are absent in the female flowers.

Collecting pollen is easy, as the tabletop around the plants is probably covered with it. Place some pollen in a drop of water on a slide, apply a cover slip, and measure the diameter at 400X. You are not told the pollination mechanism. Decide the mechanism based on the characters you have observed.





#### Winter Season: Chaenomeles (insect-pollinated)

*Chaenomeles* (flowering quince) has flowers very much like the "typical" flower seen in diagrams in many textbooks. It differs in only a few details. Instead of a single style and stigma, the style branches into five parts, each of which is topped by a stigma. Like the picture in the book, these flowers have what is called an inferior ovary. The ovary of a flower is the portion that contains the developing seeds; later, the ovary will ripen into the fruit of the plant. An inferior ovary, as in *Chaenomeles*, is located below the point where the petals and sepals are attached. A superior ovary would be located within the flower, surrounded by the petals. For purposes of simplicity, Figure 11.5 shows 8 ovules (unfertilized seeds) inside the ovary of the carpel. When you dissect *Chaenomeles*, you will see many ovules in the ovary.

Take one flower from the plant. Examine it first, then use a razor blade or scalpel to cut it in half down the vertical axis of the flower, as seen in the drawing below. Look for the parts that are indicated.



To measure pollen diameter, dust a few anthers in a drop of water on a slide. Apply a cover slip and examine the slide under the compound microscope at 400X total magnification.

Figure 11.5. Dissection diagram for *Chaenomeles* (flowering quince) a winter season, insect-pollinated plant.

#### Fall, Winter, and Spring Season: *Chrysanthemum* (insect-pollinated)

*Chrysanthemum* is a member of the sunflower family, Asteraceae. With over 20,000 species, the sunflower family is one of the largest and most successful families of flowering plants in the world. Characteristically, the flowers themselves are very small, but they are grouped together into a compound blossom called a head, that functions in many ways as if it were a single flower. But it is important to remember that anatomically a head is not one flower, but a grouping of dozens or hundreds of flowers.

Look at the overall structure of a *Chrysanthemum* head (Figure 11.6A); then use your fingers to pull it or a razor blade to cut it into quarters. Ignore for a moment the outer row of "petals", and concentrate on the central "eye" of the head, which is technically called the disk. The disk is made up of many small flowers. Pull out a single, open, disk flower (Figure 11.6B). The disk flower has 5 petals, partially fused into a corolla tube. It has an inferior ovary. The sepals are modified into small scales in other species of this family, the sepals may be silky hairs, like the "parachutes" of a dandelion. Looking at the mouth of the petals, you should be able to see the

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stigma, where pollen grains would land and begin to grow down the style toward the ovary. Inside the corolla tube, you would find the stamens; however, you need not look for them, given the small size of these flowers.

Now turn your attention to the outer "petals" of the blossom that we ignored before. Examine one of these. You will find that at its base there is an inferior ovary, identical to the one you saw in the disk flower. What you hold in your hand is not merely one petal, but an entire flower, called a ray flower (Figure 11.6C). It differs from the disk flower only in that its petals are fused into a long strap, instead of a symmetrical trumpet-shaped corolla tube.

Figure 11.6. Dissection guide for *Chrysanthemum*, a fall, winter, and spring season, insect-pollinated plant.



#### Spring Season: Gladiolus (Insect-pollinated Unknown)

*Gladiolus* flowers should be easy to understand based on this drawing. You will find that the sepals and petals are not clearly differentiated, all are showy and colorful. The ovary is inferior in this case.

Find the indicated structures on your *Gladiolus* flower, pulling aside the petals as necessary. Measure the pollen diameter by dusting an anther in a drop of water on a slide, then covering the drop with a cover slip.

The pollination mechanism is not stated here. You are expected to infer it from the observations you make, and state your conclusions.



Figure 11.7. Dissection guide for *Gladiolus*, a spring season, insect-pollinated plant.

#### Winter Season: Lonicera fragrantissima (Insect-pollinated Unknown)

*Lonicera fragrantissima* (bush honeysuckle) flowers differ from the "typical" flower diagrammed in your book in only a few details. The petals are partially fused into a funnel-shaped structure called a corolla tube. The mouth of the funnel does not have perfect radial symmetry (star shape) but is bilaterally symmetrical. Also, these flowers have an inferior ovary, which you saw previously in *Chaenomeles*.

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Find the indicated structures on your *Lonicera* flower, cutting or tearing into the corolla tube as necessary. You will probably see nectar, a sweet liquid, inside the corolla tube. You also may notice the pleasant fragrance of these flowers (reflected in their Latin name). Measure the pollen diameter by dusting an anther in a drop of water on a microscope slide, then covering the drop with a cover slip.

The pollination mechanism is not stated here. You are expected to infer it from the observations you make, and state your conclusions.







#### Winter and Spring Season: Poa annua (wind-pollinated)

*Poa annua* (annual bluegrass) is a member of the grass family, Poaceae. This very large family (about 12,000 species) is perhaps the most important in the world, because many important human foods, including wheat, rice, and corn, are made from the seeds of grasses.

All members of the grass family are wind-pollinated. Since they do not attract pollinating animals, their flowers are small and inconspicuous. Perhaps you did not even realize that grasses are "flowering plants" but they do indeed have small, reduced flowers. There are no petals. Sepals are reduced to vestigial structures. The main parts of the flower are the sexual structures: the stamens and carpel. On the other hand, there are often small leaf-like structures called bracts, which sandwich the flower. The bracts of grasses have specialized names such as glume, lemma, and palea; however, we will not worry about the exact definition of these terms in this lab. A cluster of one or more flowers with their enclosing bracts makes up a unit called a spikelet. To see the tiny flowers of a grass, you will need to use a stereoscopic microscope, and tools such as dissecting needles and forceps to peel away the surrounding bracts.

Examine the branched bluegrass inflorescence (Figure 11.9A). Select a spikelet with whitish stamens or stigmas protruding. Pluck off the spikelet and examine it using the dissecting microscope. The pollen-producing anthers hang out of the spikelet on slender thread-like filaments

(anther + filament = stamen). The pollen receiving stigmas are brush-like, to trap pollen from the air. In this species, there are usually 3 or 4 flowers per spikelet.

Using your dissecting tools, peel back the surrounding bracts, to reveal a flower within (Figures 11.9B - 11.9D). It requires patience and manual dexterity, and you may end up breaking the flower and needing to reconstruct it. To measure pollen diameter, place some anthers in a drop of water on a slide, and crush the anthers with your needle. Then cover with a cover slip and look for pollen using the compound microscope.





#### Spring Season: Rhododendron (insect-pollinated)

*Rhododendron* (azalea\*) has flowers very much like the "typical" flower in your textbook. It differs in only a few details. The petals of *Rhododendron* are fused into a funnel-like corolla tube. Also, this plant differs from the picture in your book in the position of the ovary. The ovary of a flower is the portion that contains the developing seeds; later, the ovary will ripen into the fruit of the plant. When the ovary is located below the point where the sepals and petals are attached, the arrangement is known as an "inferior ovary." In *Rhododendron*, you will find that the ovary is within the flower, surrounded by the petals, and is thus called a superior ovary. Also, for purposes of simplicity, the picture in your book shows just one ovule (unfertilized seed) inside the ovary. When you dissect *Rhododendron*, you will see many ovules in the ovary.

Take one flower from the plant. Examine it carefully. Tear open the funnel-like corolla tube formed by the petals, and find the stamens and carpel, and identify their component parts as shown on the diagram below. Locate the ovary, at the base of the carpel. Use a razor blade or scalpel to cut vertically through the ovary. Examine the cut surface under the dissecting microscope to see the ovules inside.

To measure the pollen diameter, dust a few anthers in a drop of water on a slide. Then apply a cover slip and examine under the compound microscope at 400X total magnification.

[\*The botanical genus *Rhododendron* includes flowering shrubs commonly known as azaleas, as well as those commonly known as rhododendrons.]





#### Spring Season: Quercus stellata (Wind-pollinated Unknown)

*Quercus stellata* (post oak) is a tree of dry forests in our region. The flowers differ in many respects from the ones you have seen previously. Each flower is either all male or all female. The male flowers are found in dangling inflorescences called catkins. The female flowers are solitary on the twigs nearby. You will probably not get a chance to see the female flowers, but a drawing is provided below. Because both male and female flowers occur on the same plant, this species is described as being monoecious. If there were separate male trees and female trees, as in both willow and persimmon, the species would be described as dioecious.

The diagram below (Figure 11.11) shows you the structure of the very simple male flowers. Use a dissecting microscope to observe them on the specimen.

Take a few anthers and crush them in a drop of water on a slide, or simply collect pollen from the table top if the plant is starting to shed pollen. Put a cover slip on the slide, and measure the pollen diameter at 400X. You are not told the pollination mechanism, but are asked to decide for yourself based on the characters you have observed.



Figure 11.11. Dissection guide for *Quercus stellata* (post oak), a spring season, wind-pollinated plant used as an unknown.

# Appendix B Calibrating the Ocular Micrometer

1. Inserted into the ocular (eyepiece) of the microscope you are using is a glass disc on which a small ruler with arbitrary units has been etched (see Figure 11.12). This is the ocular micrometer. As it stands, these marks do not correspond to any particular measurement like mm or microns. They are just reference marks. (A micron, abbreviated; µm, is one thousandth of a mm and is a common unit of measurement for microscopic distances). However, after you calibrate the ocular micrometer, you can use it to measure actual distances on specimen slides precisely. To calibrate the ocular micrometer, i.e., to assign "real" units to the ruler, you must compare it to a stage micrometer. A stage micrometer is a slide on which an accurately measured ruler with known units of distance has been etched.



Figure 11.12. Diagram of microscope showing the ocular and stage micrometers.

2. Arrange the stage micrometer slide for viewing under low power, and move the slide so that the scale of the ocular micrometer is seen superimposed on the scale of the stage micrometer. The actual distance between the smallest adjacent lines of your stage micrometer is 0.01 mm (or 10 microns). Its entire length is 1.00 mm. Align the left ends of the stage micrometer ruler and the ocular micrometer ruler (see Figure 11.13).



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**igure 11.13**. Diagram showing the comparison between the ocular micrometer scale and the stage micrometer scale.

3. Using the 4X objective, 30 units on the ocular micrometer correspond to what length on the stage micrometer ruler? To what actual distance (using the 4X objective) does one unit on the ocular micrometer correspond? For example, if 30 ocular micrometer units span a distance of 0.6 mm on the stage micrometer (as shown in Figure 11.13), then each ocular micrometer unit equals 0.6 mm/30, or 0.02 mm. Thus, in this example each ocular micrometer unit (oc. mic. unit) would correspond to 0.02 mm or 20 μm, and the calibration factor would be 0.02 mm/oc.

mic. unit or 20  $\mu$ m/oc. mic. unit. Record your ocular micrometer calibration factor in the worksheet below in both millimeters and microns.

4. Repeat this type of calibration for the 10X and the 40X objectives, and record your factors in the table provided below.

Ocular Micrometer Calibration Factors:					
Objective	One Ocular Micrometer unit equals:				
Objective	mm	μm			
4X					
10X					
40X					