Developmental Biology Laboratory

POLLEN TUBES & FLOWERING PLANT FERTILIZATION

SUPPLIES:

Flowers--Each student should bring at least one flower to lab. The instructor will also bring a bouquet of flowers. Beware of compound flowers, such as daisies, though a few for comparison is nice. Also beware of lilies purchased at the florist, whose anthers may have been removed.

Fluorescent microscope (outfitted with a digital camera and access to color printer)
Stereo microscopes and light sources for each pair of students
Dumont #5 forceps—two pairs per pair of students
Single edge razor blades and micro-scalpels
Wooden handle pointers, insect pins, other dissection tools
Large format, fine quality sketching paper
A variety of pencils (ranging from 4B to 4H)
Colored pencils, Estompe/blenders, Moldable erasers
Pencil sharpeners
Glass slides, and large coverslips (22x40mm)
Plastic Pasteur pipettes
5ml syringes filled with vacuum grease and tipped with yellow pipette tips
Fluorescent dyes:
- Auramine O at 0.1% in 1.5M HEPES ~pH7 (for Sporopollenin)
- Calcofluor White at 0.01% in HEPES (for Cellulose)
- Aniline Blue at 0.1% in HEPES (for Callose)
- Congo Red at 0.1% in HEPES (for pollen tube walls)

Solidified Pollen Growth Medium in small Petri dishes (protocol below)

In this lab, you will observe and compare reproductive structures in a variety of flowering plant taxa. Observations will be recorded in drawings and in fluorescent micrographs. The class will be divided into two groups – one group will dissect and draw first, then work with the fluorescent scope in the second half of the session; the other group will begin with fluorescent microscopy. Lastly, you will dust pollen onto pollen growth medium, to observe *in vitro* germination and pollen tube elongation 24 hours later.

DISSECTION & DRAWING:

- Select a few flowers from the class bouquet for comparison with those you brought yourself.
- Hastily doodle a single flower of each kind (include a doodle of the whole umbel or compound flower, if necessary).
- Label all visible structures on your doodle.
- Remove sepals and petals to find the interior reproductive structures.
- Now doodle the interior of your flower, labeling all visible structures.
- Write short notes as you begin your dissection--for example, count the # of petals in one whorl, or the total # (many in a rose!). Is your flower radially symmetrical? Are there fused structures?
- Select one male and one female structure for more careful illustration, under the stereo microscope. For example, you may pop out an ovule, or zoom in on the stigma papillae for the female. Or you may draw only the filament or include the anther for the male. You do not need to label your careful drawings, but do note the flower's common name and the name of the structure somewhere on the page.
- Fill the art paper with your drawings, using blenders and graphite pencils first, so that you can erase. Invest AT LEAST 20 minutes on each of your two drawings.

Pay attention to proportion and shapes (no perfect circles, no perfect ovals, etc), and work SLOWLY to follow each contour. Use color pencil gradually, adding light layers of color, blending as you go. Pay

attention to light and shadow – to make your drawing look 3-D, deepen shadows by making the color both darker/more intense and "cooler" (toward blue, purple).

FLUORESCENT MICROSCOPY:

Prepare your samples by dissecting them carefully. You will make two slides, from any 2 on this list:

1. Anther (in sections if it is large) – stain with Auramine O or Calcofluor White
2. Stigma (dust pollen on first if necessary, cut in sections if it is large) – stain with Auramine O or Calcofluor White
3. Dissected ovary of previously pollinated flower – stain with Congo Red or Aniline Blue
4. Dissected style of previously pollinated flower – stain with Congo Red or Aniline Blue

These stains will help you to see pollen on the anthers, and the pollen previously dusted on the stigma, as well as pollen tubes traveling within the style, and possibly approaching ovules in the ovary. Note that Aniline Blue stains the callose plugs scattered along the pollen tube length, so you will see "dashed lines" indicating the pollen tubes.

Mounting your sample:

- Place your sample in the middle of the slide.
- Select a coverslip (be careful you don't have two)
- Squeeze a bead of vacuum grease around the edge of the coverslip (using the syringe tip to keep it fine and leaving at least one opening).
- Flip the coverslip onto the sample, sticky side down.
- Press gently (while watching through the stereo microscope). Don't crush your sample, but do make gentle contact with it.
- Inject the appropriate dye beneath the coverslip, using a plastic Pasteur pipette to flood all around the sample (bubbles are fine, as long as they aren't exactly where you're planning to focus).
- Take your sample to the fluorescent scope, where the instructor will speak to you about Stokes shift, fluorescent filter cubes, and how to capture images.
- Look at other students' samples, as well as your own
- Tape images into your notebook, writing beside the images the common names of the flowers from which you took the samples, the dye you used, and the structures' names.

***IN VITRO* POLLEN GERMINATION & TUBE ELONGATION:**

Dust pollen from different flowers onto solidified pollen growth medium (PGM; see below). Under the stereoscope, gently touch anthers down to the agar surface, without making deep divots; pollen will come off into the surface moisture.

- Put the lid on the dish, and leave it on the benchtop.
- Return ~24 hours later to see that some of the pollen will have germinated. Elongated pollen tubes will be visible by stereomicroscope on the surface of the PGM.
- You may also scoop pollen grains and tubes out of the agar, and place them on glass slides, cover with a coverslip (as described above), and stain with Congo Red, for viewing on the fluorescent scope.
- Capture images or make drawings for your notebook, with written notes about the common name of the flower from which the pollen came, the hours since you dusted the pollen on the PGM, and characteristics of the germination--was it through an aperture in the pollen wall? Is the pollen tube projecting into the agar, or upward into the air? Is the tube branched? Is the tube straight or wavy?

Solidified PGM

(from Dr. Zhengbio Yang's laboratory, University of California, Riverside)

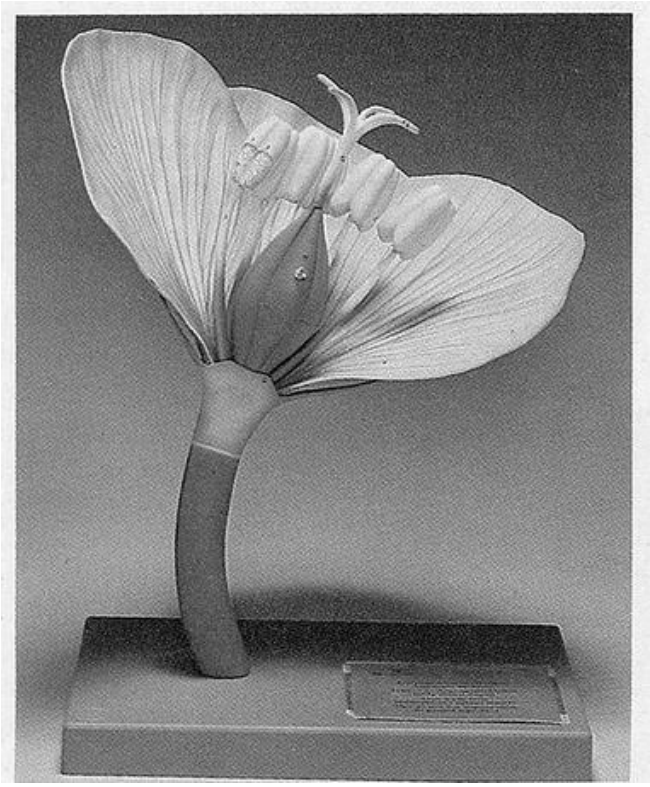
18% Sucrose	1mM $CaCl_2$	1mM $MgSo_4$
0.01% Boric acid	1mM Ca $(NO_3)_2$	0.5% Noble agar (Difco)

Dissolve sucrose first, then add other ingredients. pH to 7.0. Finally add agar, place medium in a hot water bath and let the contents boil until agar completely dissolves (3-4 minutes). There is no need to autoclave the medium. Pour medium onto Petri dishes (35mm diameter mini dishes). Store dishes at 4 ^{0} C for up to 6 weeks.

BACKGROUND

FLOWER STRUCTURE

Flowering plants are called **angiosperms**, from the Greek words *angeion*, meaning "vessel," and *sperma*, meaning "seed." They are distinguished from gymnosperms, whose seeds are *gymnos* (naked) on the scales of their cones. Flowers contain sterile appendages (**sepals and petals**) along with their fertile organs (stamens and carpels). Stamens are also called androecium (house of man) and are composed of a slender stalk with a two-lobed **anther** at its end, containing pollen sacs. The **carpels** or gynoecium (house of woman) enclose one or more **ovules**. Usually the lower part of the carpel contains the **ovary**, with enclosed ovules, while the upper part, the **stigma**, receives the **pollen** (often on finger shaped cells called **papillae**). The stigma is connected to the ovary by the **style**. The ovary becomes the fruit (vessel) containing the seeds, and can be dry like a seedpod or nut, or juicy like a pear or sweet pepper. Flowers can contain either one or the other of the fertile organs, or both at once. Similarly, a given plant may contain one or both sorts of flowers. If both female and male flowers occur on the same plant, it is called monoecious (from the Greek words for "one house"). If the male and female flowers are found on separate plants, the species is dioecious (two houses).



Practice naming flower structures by connecting the following labels to the appropriate parts of the Carolina Bio Supplies flower model shown:

SEPAL

PETAL

FILAMENT

ANTHER

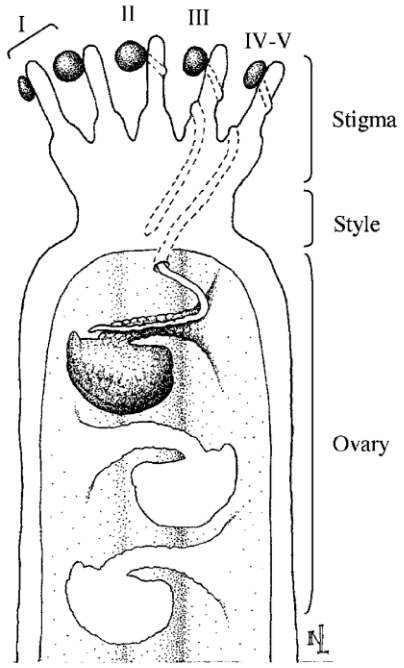
CARPEL

STIGMA

OVARY

STYLE

Where would one find the POLLEN? The OVULES? Is this flower male, female, or both?

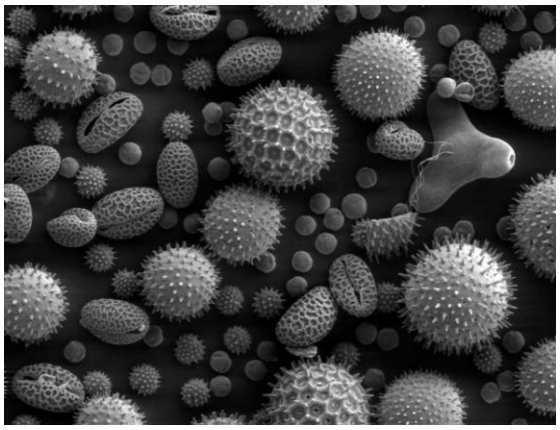


Pollination is only the first step—the delivery of the pollen to the stigma. After pollination, sperm nuclei must escape the pollen grain's walls and be conveyed to the ovules, by the construction of individual **pollen tubes**. The empty pollen grain walls are left behind on the stigma. This transport of sperm nuclei through the style and ovary is analogous to the movement of human sperm through the uterus and fallopian tubes.

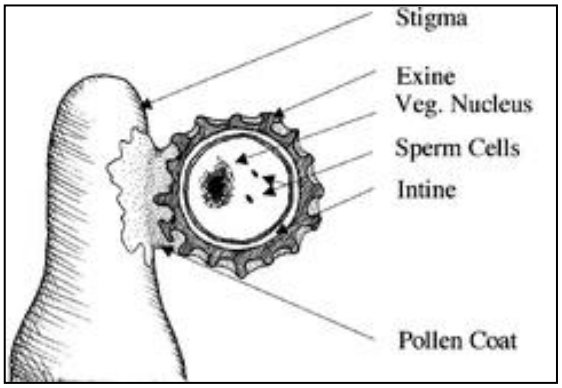
SELF QUIZ
Rewrite the following statement so that it is correct:

"During pollination, pollen grains move down the style and into the ovary, where the ovules are fertilized."

POLLEN GRAINS



Pollen grains vary considerably in size and shape, ranging from less than 20 micrometers to over 100 micrometers in diameter. Beneath a fatty substance called **pollen coat**, lies the outer cell wall called **exine**, whose sculpturing is unique for each species. Pollen also have an inner cell wall, much like any other plant cell called the **intine**. Interior to the intine are the plasma membranes. Exine is made of a remarkably durable substance called **sporopollenin**, and unlike flowers and fruits, pollen grains are very well represented among fossils, providing a rich record of old climates and plant variants.



Pollen development involves a special mitotic division that results in two cells within the same wall. One cell is called the tube or **vegetative cell**, and the other is called the **generative cell**. The generative nucleus divides again, giving rise to two **sperm nuclei** (one sperm nucleus will ultimately unite with the egg to make the zygote, while the other will unite with the polar nuclei and go on to form the endosperm; this process is called **double fertilization**). When pollen is mature, the anthers **dehisce** and shed the grains, so that they can be carried by a vector

(wind, insect, mammal or bird) to the stigma of the same or a different receptive flower. Once on the stigma, the pollen grains rehydrate and germinate a pollen tube (see I – V in above Figure).

POLLEN TUBE ELONGATION

Soon after pollen grains adhere to a flower's stigma, pollen tubes emerge from the grains and invade the pistil tissue *en masse*, winding their way basally toward the individual ovules, passing up those ovules that have already interacted with other pollen tubes. Despite the cells' thick external walls, this process depends upon cell-cell interactions, polarity, adhesion, motility and pathfinding behaviors similar to those studied in metastatic cells or axonal growth cones in animals. Unlike axonal outgrowths, pollen tubes transport all of their cellular contents close to their tips, periodically sealing off the empty older portion of the tube with a **callose plug**. It is this callose that we will stain with the dye Aniline blue.

Pollen grains will germinate and form pollen tubes *in vitro*, and such cultures have proven useful for studying cell characteristics and navigation. Culturing pollen tubes in the presence of different extracts has helped to identify key guidance cues for directed tube extension. Positive signals appear to emanate from the ovules and to be coordinated with negative signals or repellents. Given the large number of *in vitro* studies conducted, surprisingly few *in vivo* images have been captured of pollen tubes moving naturally over and within each region of the carpel. Your images in class today could be the first for that particular species!

A FEW REVIEW ARTICLES ON POLLEN TUBES

Edlund, A.F. Swanson, R. and Preuss, D. (2004) Pollen and Stigma Structure and Function: The Role of Diversity in Pollination. *Plant Cell* 16, S84-92.

Geitmann A. (2010) How to shape a cylinder - Pollen tube as a model system for the generation of complex cellular geometry. *Sexual Plant Reproduction* 23, 63–71.

Wilhelmi L.K. and Preuss, D. (1997) Blazing new trails: Pollen tube guidance in flowering plants. *Plant Physiol.* 113, 307-312.