

Expt. How do flowering plants do it without flagella? The journey to find an egg. What causes pollen grain germination and tube growth?

File: F12-07_pollen

Modified from E. Moctezuma & others for BSCI 442 (H. Sze, Univ Maryland)

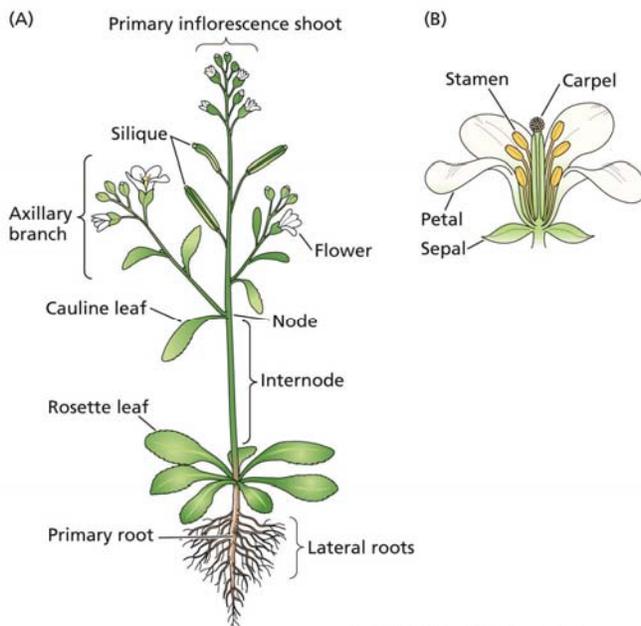
Objectives:

- Observe. What are the parts of a flower? What does each part look like and what is its job? Where are male and female organs? What are pollen grains? What do they do?
- Test: what is needed for pollen to germinate and to grow a tube growth?
- How can one observe and measure germination and tube growth? Analyze results?
- Learn to present a short oral report to the class and participate in a group discussion.

Introduction

Ancestors of land plants lived in marine or aquatic environment. These ancestors reproduced sexually by motile sperms that could swim to the egg. As plants colonized land, the reproductive structures evolved as fertilization could no longer depend on water. The largest and most successful plant group on land is the flowering plants, or **angiosperms**. From giant eucalyptus trees to minuscule duckweeds, from desert cacti to water lilies, these familiar plants dominate the world. They play a significant role in providing food and shelter for animals and other organisms. Angiosperms derive their name from the Greek words *angion* (vessel) and *sperma* (seed). The flower contains the vessel that houses the seed. It is also the organ for sexual reproduction.

How do land plants reproduce? Early land plant, like a moss, developed structures to protect gametes from drying. The egg is formed inside a protective vase-like structure with a long neck and a small opening at the top. Sperms formed in the male organ could swim down the neck to find the egg. Other land plants evolved so the egg was formed and completely protected from the environment by layers of cells that sealed the egg from the outside. How can sperms reach such eggs? How can sperms from one plant fertilize another when they are physically apart. Answer: sperms are delivered to another plant inside a pollen grain, a structure that protects the sperms from drying out as they are carried over long distances. To break the seal that entombs the egg, each pollen grain grows a tube that can navigate through the female tissues and then target an ovule containing an egg.



To understand this process, look at a flower. A typical flower is composed of modified leaves arranged in four **concentric circles** on the end of an enlarged portion of the stem. **Sepals** form the outer (and lowest) whorl of floral parts. Petals make up the second whorl which are often colored to attract insects or birds that serve as pollinators. Plants that are wind-pollinated or that are self-pollinated do not have colorful petals, such as the model plant *Arabidopsis*.

Fig. 1. Arabidopsis plant and flower (Taiz Fig 16.1)

The third whorl consists of the male portion of the flower, the **stamens**. On top of the stamen, the **anther** is a four-chambered structure where **pollen** is produced. The male gametes are contained within pollen grains. The innermost whorl is the female part of the flower that contains one or more **pistils** (or **carpels**). The **stigma** is the receptive tip where the pollen lands; the **style**, is an elongated tubular structure through which the pollen tube grows after pollen germination; and the **ovary** is at the base. Arabidopsis had multiple ovules within two fused carpels. Only flowering plants produce flowers and seeds.

Alternation of Generations in Flowering Plants

Flowering plants reproduce sexually and undergo alternation of generations. That is, the life cycle of involves a diploid ($2n$) sporophyte stage and haploid (n) gametophyte stage. After meiosis, the male gametophyte generation (e.g. pollen) is very reduced and is enclosed within and dependent on the parent sporophyte. On the female side, meiosis and cell division produces a gametophyte with 8 cells. Thus flowering plants are **heterosporous** (produce two kinds of spores). The two types of spores are called **microspores** (small- male) and **megaspores** (large-female).

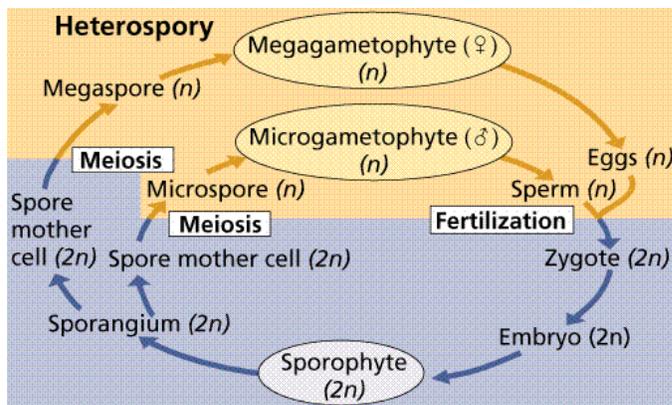


Fig. 2 Life cycle of plants. Plants undergo alternation of generations: the $2n$ spore-producing plant (sporophyte) alternates with the haploid gamete-producing plant (gametophyte). Pollen, the male gametophyte, has 3 cells. The female gametophyte has 8 cells. Animal life cycles have meiosis followed immediately by the formation of gametes (sperm or egg). (Image from Purves et al., *Life: The Science of Biology*, 4th Edition, by Sinauer Associates and WH Freeman)

Pollen Development

The diploid male sex organ of the flower, the **anther**, encloses four compartments containing microspore mother cells. Each one of these cells undergoes meiosis to produce haploid **microspores** ($1n$). The haploid microspores grow and mature into haploid male gametophytes, known as **pollen grains**, which are released and travel to the female. The pollen houses the sperm gametes.

Embryo Sac Development

The female **ovary** contains the diploid female sex organs of the flower called the **ovules**. Specific cells in the ovules undergo meiosis to produce haploid **megaspores**. One of these megaspores grows and develops into the female gametophyte or **embryo sac** within which develops an **egg cell**.

Flowering Plant Pollination

Pollination, the transfer of pollen from the male anther to the female stigma, must occur before seed plants can reproduce sexually. Pollen grains have a limited life span. Depending on the species, pollen may be viable for only a few hours up to several weeks after it is released from the anther. Many plants rely on wind to aid in pollination. However, most flowering plants use animal agents to transfer pollen.

A primary function of flowers is to attract pollinators with colorful petals, scent, nectar, and pollen. In exchange for moving their pollen around, flowers have evolved many rewards to attract pollinators. The most common attraction is food, either **nectar** (a sugar solution) or pollen, which is high in protein. In many plants, nectar is produced in **nectaries** most commonly found in the flowers.

Pollen tube growth and Fertilization

After landing on the right stigma, the pollen grain germinates. The sperm in the pollen grain must be delivered to the egg, located in the ovary. The pollen grain grows a tube from the stigma, through the style, and down to the ovary. When the tube finds and reaches the ovule, the tip of the tube bursts. The two sperms are released into the ovule. One sperm fertilizes the egg in the embryo sac to produce a **diploid zygote (2n)**, which grows into an embryo enclosed in a seed. The other sperm fuses with the two polar nuclei to form the **endosperm** that provides food for the developing embryo. This event is called **double fertilization** and is unique to flowering plants. Thus, **seeds** are the mature ovules that contain the embryos and the ovary develops into the **fruit**, a structure for seed dispersal.

When the seed germinates in the soil, the young embryo uses the energy stored in the **cotyledons** to start growth. One of the reasons that flowering plants are so successful today is that their seeds and fruits are adapted for dispersal in many different ways.

Materials

Lilies are in bloom	– tiger, yellow, red all v. good
---------------------	----------------------------------

Stock Solutions: made and sterilized by autoclaving.

Medium content	MW	Stock conc	final
sucrose	342	10%	10%
Boric Acid(H ₃ BO ₃)	61.83	1 %	0.01%
Calcium Nitrate.4H ₂ O	236.15	300 mM	3 mM
KCl		300 mM	3 mM
MgSO ₄		100 mM	1 mM

Part A. Observe and know the parts of flowers

1. Obtain a flower per group. Examine its form with the dissecting microscope.
2. Identify and count the number of sepals, petals, stamens, and pistils. Use a dissecting microscope to help you see stamens and pistils.
3. Now, use tweezers to remove the sepals and petals. Look at the stamen and pistil using the dissecting microscope.
4. Draw and label the **stamen, anther, filament, pistil, stigma, style, and ovary**.

PART B. Test Pollen Germination

Pollen grains are able to germinate and to extend a tube in the absence of the female organ, if a suitable nutrient media is provided. Here you will test which nutrients are required and discuss why.

Pollen germination solution

Medium content	MW	amount	stock
sucrose	342	2 g in 50 ml	
Boric Acid(H ₃ BO ₃)	61.83	0.1g in 10 ml	1%
Calcium Nitrate.4H ₂ O	236.15	Make 20 ml	0.5 M
KCl		Make 20 ml	0.5 M
MgSO ₄		Make 20 ml	0.1 M

Equipment and Supplies needed

Microscopes: dissecting and light microscopes. Slides, coverslips
 ImmEdge Pen (to make a square with hydrophobic barrier on a slide)-optional
 Forceps, 35 mm petri plates
 pH meter

Tests

- i). Test pollen grains from one flower for their ability to germinate and extend a tube.
 ii) Test nutrient requirement: E.g. a) complete medium, **and** b) no sucrose, , **or** c) no calcium.

Complete medium for pollen germination and tube growth in vitro

	stock	Vol/20 ml	% final	Conc
sucrose	powder	2 g	10%	294 mM
Boric Acid(H ₃ BO ₃)	1%	200 ul	0.01%	1.5 mM
Ca-Nitrate.4H ₂ O	0.5 M	120		3 mM
KCl	0.5 M	120		3 mM
MgSO ₄	0.1 M	200		1 mM
DI water		Final 20		

Adjust pH to 7.5 with 1M KOH. (~ 4 ul is sufficient, though this depends on the source of the DI water.)

Nutrients:

1. Prepare the test solutions 20 ml/each. Each group will need a) complete medium, and a medium lacking one nutrient. Each group will need 4 petri plates.
2. Take 2 tiny (35 mm) petri plates. Dust pollen over the bottom of the plate.
3. Add drops of the control solution onto the pollen of one dish, and a test solution in the second dish. Look at the dishes using the scanning (4X or 10 x) objective and find a spot where there is a lot of pollen. [or Use dissecting scope]
4. Examine at low magnification. Change to the low power objective (10X) and watch the pollen. After about 15 minutes, you should see pollen tubes starting to form. The first evidence of this is a small bump on the side of a pollen grain.
5. Examine at higher magnification. When germination starts, take out a sample and place on a slide with a coverslip so you can look at it with 40x. If you see a pollen grain germinating, center it in your field of vision and change to the high-power objective (40X) to watch the tube grow. You can probably see the cytoplasmic streaming within the tube. [Many pollen tubes will break apart (lyse) because the tube membrane is too thin to hold the cell together in this sucrose solution. However, it would not lyse if it were growing in a flower's style.]
6. Analyze results
 - a. Determine % germination. (Time at 30 min). In a field, count the number of total pollen grains, and the number of those showing emerging tube. (total count >20) Express results as % grains germinated at __ specified time.
Put in a table showing % Germination
 - b. Measure tube lengths of pollen tubes. (Time at 1- 1.5 h)
Measure the relative length of tubes (count 20 tubes for controls, and 20 for a treatment).
*Hint: Take pictures. Save and measure according to pixels with Image J.
Record each length and show range of lengths. Indicate frequency of each length.
7. Discussion: Share results of all groups with the lab section. One student from each group will give a 5 min oral report of the results to the entire class. Discuss: What do results show and why.

Report

Test medium	Germination	% Germination	Tube length	Frequency of tube length
a. Complete	e.g. 14/20			
b. no sucrose				
c. no calcium				

Discuss & include in your report:

1. What do results show? How does the absence of sucrose, nitrate, calcium or B affect pollen germination and tube growth. How do you interpret the results?
2. What purpose(s) does sucrose serve? Give at least 2 distinct examples. In the plants, where does the sucrose in the flower come from?
3. What is a likely reason for NO_3^- requirement?
Propose a likely fate of nitrate from its uptake to its incorporation into a macromolecule. Show specific reactions.
4. Pollen tube growth is fairly fast, and reaches the ovule in a few h or less. Why?

Ref.

Taiz & Zeiger 2006 or 2010. 'Plant Physiology' book (Sinauer) chap. 16

Fig. 2. Image from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates and WH Freeman

Moctezuma E (2008). Manual for Plant Biology Lab (U. Maryland, College Park)