**Activity Set A: Background for Inquiries**

**Investigating Pollen in Plant Reproduction**

**Summary:** Students gain background knowledge for inquiry investigations through dissecting flowers and/or cones and observing pollen grains through compound microscopes. In addition, students conduct either a guided or open inquiry investigation by growing pollen tubes. Through these activities, students become familiar with the role pollen plays in the reproductive system of seed plants. They understand the importance of pollen transport and learn that pollen germinates and grows into the female plant structures to deliver sperm to the ovule. Students communicate with their online mentors about these subjects.

**Learning Activities:**
1. Activity 1: Plant Dissections – dissect flowers and/or cones to understand plant anatomy
2. Activity 2: Collect and Examine Pollen – observe pollen under magnification
3. Activity 3: Growing Pollen Tubes – a guided or open inquiry investigation

**Skills:**
1. Use of dissecting microscopes or hand lenses
2. Organism dissection
3. Use of compound microscopes for pollen observation
4. Culturing microscopic organisms (pollen)
5. Design and conduct an investigation based on a research question
6. Communicate ideas, questions, observations, conclusions

**Concepts**
- Sexual reproduction is part of the life cycle of most plants.
- Sperm cells are contained in the pollen grains of angiosperms and gymnosperms.
- Pollen from outcrossing plants is moved from plant to plant by wind, animals, and water.
- Pollen viability depends on many factors.
- Plants have diverse forms and structures, and diverse reproductive strategies.
- There is a diversity of pollen forms.

**Evidence of Understanding:**
- Students identify that pollen is produced in anthers of angiosperms and “male” cones in gymnosperms, and that it lands on the stigma of angiosperms and the “female” cones in gymnosperms.
- Students become familiar with the diversity of pollen types, and suggest probable means of pollen transport.
- Students describe the function of the pollen tube and contrast pollination and fertilization.

**Juicy Questions for Discussion and Investigation:**
- How do plants create pollen?
- Where on a plant do we find pollen?
- Is all pollen the same?
- How big is pollen?
- How does pollen reach an ovule?
Activity 1: Plant Dissection – Where’s the Pollen?

This lab focuses on exploring general plant anatomy in relation to reproduction. Plants are quite diverse, and their anatomy can be as well.

Suggested timing:
- **Day 1:**
  - Dissect a simple perfect flower (with both male and female parts) such as a tulip, daffodil, or lily. These flowers will largely conform to the idealized drawings of plant anatomy in biology textbooks, and to the flower anatomy worksheet offered here.
  - Complete the flower anatomy worksheet together.
- **Day 2:**
  - Dissect different types of flowers and cones that offer a range of diversity in types of plants and flowers.
- **Allow time for posting online to mentors.**

Materials per group:
- Selection of flowers (including catkins) from store or outdoors, ready to shed pollen
- Selection of “male cones” ready to shed pollen
- Dissection probe, pin, or toothpick
- Dissecting tweezers
- Single edged razor blades
- Scissors
- Pen or pencil
- Dissecting microscope and/or hand lens

Safety note: Take precautions with using sharp edges and points. Take precautions against plant or pollen allergies. Check with students who know they may have allergies, and have other students handle the pollen. Be sure to have students wash hands thoroughly after working with plants and pollen.

Notes for Collecting Flowers
If you collect local wildflowers, use a local flowering plants guide to identify flowers. Be sure not to pick rare flowers. Do not pick any flowers in a preserve where collecting is prohibited.

Florists will often contribute their older flowers to you if they know it’s for education. Suggested flowers that can be gathered from a florist (or grocery store floral section):

- Alstroemeria (Peruvian lily)
- Lilies, tulips, daffodils
- Roses (small spray roses work well)
- Snapdragons
- Gladiolus
- Gerberas, asters, sunflowers, mums
- Many others
Plant Dissection – Introduction

Most people know many things about pollen: for example, it makes some people sneeze, it’s needed for reproduction in plants, bees harvest it for food, and levels of pollen are reported on the news. Where is pollen produced? Think about what you already know about pollen, and discuss it with your team members. Also, post online to discuss with your online mentors what you predict and what you discover.

Before you begin, think about the following questions and write your answers below.

1. Make a prediction about where you think pollen is produced in the plants you are investigating:

2. Predict ways you think the different types of plants will differ:

3. Predict ways you think the different types of plants will be similar:

4. Share your thoughts and predictions with your PlantingScience mentor online.
Dissection Procedure

1. Examine flowers or cones from several plants. Focus on the location of the pollen-producing structures in each.

a. If one of your plants is a cone, cut it in half longitudinally and examine its structure. The male cones are smaller and feel papery when compared to the female cones. Sketch each cone in the spaces on the next page and show where the pollen is produced.

b. Sketch each flower on the next page, showing the location of the different structures, and their relative sizes. Carefully open the flowers, perhaps using forceps and probes, in order to locate the pollen producing anthers. You can cut the flower in half longitudinally by either using scissors, or by inserting a dissecting needle at the base of the flower and carefully lifting it up through the petals and other structures. Use the dissecting microscope and/or a hand lens to more easily see the structures. Show where you think the pollen is produced.
2. Label the structures on the diagram of dissected cones below. Did you find any of these structures on the cones you dissected?

Male cone

Female cone
3. Label the structures on the diagram of a dissected flower below. Did you find any of these structures on the flowers you dissected? (Note that flowers have many different forms, and they certainly don’t all look like the diagram below.)

**Flower Anatomy**
4. Consider the way each of the plants above is likely to be pollinated. Communicate with your online mentors about these questions.

a. Do you think any of your plants self-pollinate? Which ones? What is your evidence?

b. Which of your plants do you think is most likely to be pollinated by wind? What is the evidence for this?

c. Which of your plants is most likely to be pollinated by an animal? What evidence do you have for this?

d. What characteristics of the female plant structures (female cone or pistil) might help collect pollen?
Labeled Pine Cone Structures

Male cone

Scale
Pollen sac
Pollen

Female cone

Scale
Ovule
Egg
Labeled Flower Anatomy

1. leaf
2. stem
3. sepal
4. petal
5. pistil (or carpel)
6. stamen
5a. ovary
5b. style
5c. stigma

6a. filament
6b. anther
6c. pollen

ovule(s) inside ovary or ovaries
Flower Dissection Tip Sheet

Flowers are incredibly diverse, and it would be good to review flower types so as to recognize floral anatomy as it appears in different flower types.

Be sure to look at one individual flower. For instance, evening primrose or spiderwort tend to have obvious individual flowers (see below). But a clover head is in fact a cluster of many tiny flowers. Flower heads in the sunflower family (sunflowers, asters, mums, daisies, dandelions) are also composed of many tiny little individual flowers. They often look like one flower because the outside flowers in the head have a petal (the ray flowers), and the little flowers in the middle don’t have any petals (disk flowers).

Top row – clear individual flowers. Evening primrose (left), and spiderwort (right).

Middle row – clusters of flowers. Verbena (left), and white clover (right).

Bottom row – composite flowers. Sunflower (left), and daisy fleabane (right). Note the ray flowers on the outside have petals, and the disk flowers inside do not.

Some flowers have multiple pistils rather than just one, and there are many different types of stamens. Sometimes petals are not colorful and showy, but rather leaves or sepals are. Many flowers are not showy at all, especially wind-pollinated flowers. Catkins such as those found in oaks, elms and birches, are flowers and they’re easy to overlook. Likewise, ragweed has inconspicuous green flowers. Grasses are largely wind-pollinated, and their flowers are not showy. What do your flowers look like? How do the different parts appear in the flowers you observe?
Activity 2: Collect and Examine Pollen

Pollen collection and identification are skills used in many careers – meteorology, medical technology, medicine, plant breeding, forensics, archeology, anthropology – to name a few. Here you will collect pollen from known species of plants, examine it and if you wish, store some for future use. Discuss with your online mentors your investigation.

Materials per group:
• Selection of flowers (including catkins) from store or outdoors, ready to shed pollen
• Selection of “male cones” ready to shed pollen (if available)
• Four microscope slides with cover slips
• Dissection probe, pin, or toothpick
• Scotch tape
• Four glassine envelopes or plastic sandwich bags to store pollen for future use
• Marking pen
• Compound microscope (best if high power is at least 400X)

Collecting Pollen
Making 4 slides:

1. Choose one of your plants, and sprinkle pollen onto one slide.
   a. You might simply shake the flower or cone over the slide.
   b. You might remove a stamen and brush its anther lightly over the slide.
   c. You might also use the dissecting probe or pin to agitate the flower or cone over the slide to loosen pollen
   d. If pollen is clumped up, try to break it up and spread it around

2. Place a cover slip on top.

3. Tape the cover slide down on 2 edges, with tape covering edge but not the whole cover slip

4. Label the slide with the name of the plant.

5. Repeat for your other three specimens.

Saving pollen for future use:

1. Label a glassine envelope or plastic bag with the name of the plant.

2. Shake the flower or cone, or remove the stamens and brush them against the inside of the envelope/bag to collect pollen.

3. Repeat for your other specimens.

4. Store labeled envelopes/bags for later use in pollen tube investigation.
Examining Pollen Slides

1. View one of your pollen slides with the microscope at low power. Use the coarse focus to get as clear an image as possible. Adjust the light levels up or down to get a good image (it’s often better to use less light).

2. Step by step, increase the power of the magnification by turning the objective lenses. Each time, focus with the fine focus to get as clear an image as possible.

3. Using the fine focus, view the pollen grain through its depth of field, or vertical height. You can get a sense of the three-dimensional features by slightly changing the focus so that you start with viewing the top surface of the pollen grain, and then lower, and then to the bottom near the slide surface itself.

4. Sketch the features of the pollen and label your drawing with the name of your plant.

5. Take a photo of these or scan them to post and discuss with your online mentors.

Plant 1 ___________________________   Plant 2 ___________________________

Plant 3 ___________________________   Plant 4 ___________________________
Tips on Viewing Pollen Using a Light Microscope

In order to get a sense of the three-dimensional texture of the surface of a pollen grain, scientists use a technique called called “LO Analysis.” LO stands for light/obscuritas (=dark), that is, comparing the light and dark surfaces while you change the depth of field, or vertical focus. For instance, use the fine focus to start with viewing the top surface of the pollen grain, and then lower, and then to the bottom near the slide surface itself.

If you gradually change the focus on a grain starting from the top and moving down, you will notice changes in what is in focus showing as changes in light and dark. A pit on the surface will initially appear dark and then become light. Alternatively, a bump or projection will appear light and then become dark.

Look at the series of pictures below of a stained Chenopodium-type pollen. Number 1 shows the focus at the top of the pollen surface. Each progressive photo shows a focus slightly lower. Are the dark spots in photo 1 pits or bumps?

![Series of pictures showing changes in focus on a pollen grain.](image-url)
Activity 3: Growing Pollen Tubes

Once pollen lands on a flower’s stigma or on a female cone, how does the sperm reach the ovule? Remember that the sperm cells are inside the pollen grain, and the ovule is inside the ovary. There are some barriers to overcome!

If all conditions are right, pollen grains germinate, just like a seed germinates and starts to grow. It will grow a tube that contains sperm cells, called a pollen tube, which grows toward the ovule to deliver the sperm.

What kind of conditions do you think are favorable for the growing of pollen tubes? Do you think this will change by species? Do you think conditions will be different for plants that produce pollen at different times of year? In different climatic conditions?

This lab focuses on growing pollen tubes. Discuss with your online mentor your experiment, your results and your conclusions.

Materials per group:
- Four different types of fresh pollen
- Four microscope well slides with cover slips
- Four sterile petri dishes (glass or plastic)
- Paper towels or filter paper or coffee filters – one for each petri dish
- Four plastic drinking straws – the type that bend
- Scissors
- 10% sucrose solution (a few drops per slide)
  - Made with 10 g sucrose and 90 ml distilled water
- Sterile pipette
- Compound microscope

Using a Humidity Chamber
Pollen germinates on the tip of a stigma, or on a female cone, where there is a sticky substance that helps nourish and moisten the pollen. For our purposes, we will make a humidity chamber, and use a sucrose solution to incubate pollen grains for a length of time. The humidity chamber will look like the photo below.

Pollen will be placed on a microscope well slide, a couple drops of sucrose solution will be added, and then it will be placed inside a covered petri dish with a wet filter paper / paper towel at the bottom to increase the humidity level. The straw segments serve to keep the slide above the paper towel. While the pollen is incubating, there will be no cover slip so that the pollen has full access to oxygen for cellular respiration.
Formulating Inquiry Questions and Extensions

The following questions are the types of questions scientists investigate about pollen tubes. The growth of pollen tubes is necessary for fertilizing the ovule, and there are many different types of questions to research about pollen tubes. Discuss possible research questions and experimental design with your team members and your online scientist mentors.

- How long does it take to grow a pollen tube? (A matter of minutes to hours to days)
  - Does this vary among species? Among plant families?

- What is the germination rate of different types of pollen?
  - For instance, if you estimate on your microscope slide 100 pollen grains, but only 5 pollen grains are germinating pollen tubes, your germination rate would be 5%.
  - Would germination rate differ among different plant species?
  - Would germination rate differ in different conditions?

- Does the amount of sucrose (sugar) affect pollen tube growth? Consider varying the percent of sucrose in solution (for instance, 5%, 10%, 15%, 20%)

- Do different micro-nutrients affect pollen tube growth? Scientists have identified some chemicals, such as calcium, magnesium, nitrogen, and boric acid that may affect pollen tube growth. The following solutions are recommended for experimentation by the scientists Shivanna and Rangaswamy (1992):
  - Solution A: 10% sucrose (100 g in 900 ml water)
  - Solution B: 10% sucrose, 100 mg/L boric acid, 300 mg/L calcium nitrate
  - Solution C: 10% sucrose, 100 mg/L boric acid, 300 mg/L calcium nitrate, 200 mg/L magnesium sulfate, 100 mg/L potassium nitrate

- Does temperature matter while pollen tubes are germinating? Consider culturing them at different temperatures.
  - Why would this matter? Imagine what time of year the pollen tube grows. A plant flowering in early spring might have more cold tolerance than a plant flowering in mid-to late summer.

- How long do pollen tubes grow for each species? Measure them and find average lengths per species, and compare these values among species.
  - Why would lengths matter? Remember that the pollen tube needs to span the length of the pistil. If the style on a particular plant is long, it would make sense that the pollen tube would be long.
Growing Pollen Tubes Procedure

Prepare the humidity chamber:

1. Trace the shape of the petri dish onto the coffee filter or paper towel.

2. Cut out the circle, cutting slightly inside the traced circle so it will fit inside.

3. Take a straw, pull the bendable part down, place it in the petri dish, and snip off a segment where the longer part of the straw meets the edge of the petri dish as shown in photo.

4. Place the bottom segment in between the bent straw as shown in photo. These straw segments will serve to keep the microscope slide up off the bottom of the petri dish.

5. Add enough water to the paper to moisten it completely, yet without pooled water.

Prepare microscope slides:

1. Label microscope slide with type of pollen and today’s date.

2. Place pollen on microscope well slide, inside the well.

3. Place 2-3 drops of 10% sucrose solution inside the well.

4. Place uncovered microscope slide in the humidity chamber, with slide sitting on top of the straws.

5. Place the petri dish cover on.

6. Store petri dishes for one hour to one day, being careful not to spill the sucrose solution within the well of the slide.

7. Do not allow the well to dry out, and do not allow the paper at the bottom to dry out.
Viewing Pollen Tubes

Once you’re ready to view pollen tube growth, lift your microscope well slide out of the petri dish humidity chamber, and place a cover slip over the well. Place the slide on the microscope stage, and see if you had any pollen tubes grow. Be sure to start with low magnification first to scan the slide, and then advance to higher magnification. Draw what you see below. Label the pollen type. Scan your drawings or take a photo, and post to your online mentor for discussion. Be sure to tell your mentor what you’ve done and discovered.
Pollen Tube Images (all at 400X)

Left: Pollen tubes of Korean clover (*Kummerowia stipulacea*)

Left: Pollen tubes of red clover (*Trifolium pretense*)

Right: Pollen tube of Peruvian lily (*Alstroemeria sp.*)
Fungal Hyphae

Be aware that fungal hyphae may also grow in the sucrose solution. Below are photos of goldenrod (Solidago canadensis) pollen with fungal hyphae also in the field of view. Try not to mistake hyphae for pollen tubes.

In the left-hand photo, the beginning of a pollen tube can be seen growing at about the 10:00 o’clock position of the pollen grain. Fungal hyphae cover the right and lower portion of the field.

In the right-hand photo, two goldenrod pollen grains are seen without pollen tubes, but with plenty of fungal hyphae. Note the jointed nature of the hyphae here. Pollen tubes do not have those joints or nodes.
Background Information

What is special about pollen and plants that produce it?

Plants that produce pollen are able to transport the sperm to the ovule without water! In the case of plants that don’t produce pollen such as ferns and mosses, the sperm from the male gametophytes have to swim to the egg of the female gametophytes. Pollen-producing plants do not have this limitation.

All plants have a life cycle referred to as Alternation of Generations. This means they cycle through a haploid (1n) gametophyte form, which produces gametes (egg and sperm). After fertilization, a diploid (2n) sporophyte form germinates, grows, and eventually produces haploid (1n) spores through meiosis.

This life cycle is very clear in ferns, for instance, see the C-Fern Inquiry Module in PlantingScience. In the case of pollen-producing plants, the gametophyte stage is extremely reduced and hardly visible. The female gametophyte is microscopic and resides inside the ovule. The male gametophyte is the pollen tube, which germinates from a pollen grain, and is also microscopic.

Plants that produce pollen also produce seeds. The two main types of seed-producing plants are gymnosperms and angiosperms.

Gymnosperms comprise the cone-bearing plants (pines, firs, spruces) as well as junipers and cedars. The name gymnosperm translates literally as “naked seed.” The seeds of gymnosperms do not develop within a fruit, like seeds from flowering plants do, and so are not as protected; hence, they are “naked” in comparison. Pollen is produced in the “male” cone and delivered to the “female” cone, which contains the ovule.

Flowering plants are the angiosperms (literally, “vessel seed”). Some flowers (like magnolias or lilacs) are quite showy and fragrant. Others (like grass or ragweed) are less noticeable. They all share a similar anatomy in that pollen is produced in the anthers on the stamens. Pollen is then delivered to the stigma of the flower, which is the opening to the ovary or ovaries, where the ovules are. The ovules ripen into seeds, and the ovary ripens into a fruit which contains the seeds. The ovary is considered the “vessel” in which the seed(s) ripens.

Rather than relying on water to deliver sperm to the eggs, gymnosperms and angiosperms rely on pollination and pollen tube formation for sexual reproduction.

Pollination is the process by which the pollen grain lands on the receptive (“female”) part of the plant.

Fertilization is the process by which the sperm fertilizes the egg, after the pollen tube has grown to it and delivered the sperm cell(s).
How does pollen move from plant to plant?

Pollen grains are carried from one plant to the next by air currents or animals (insects, birds, mammals). Gymnosperms generally rely on wind pollination, although there are some exceptions. Wind pollination occurs in about 18% of angiosperm plants, and is common in a few plant groups such as grasses. Corn, rice, oats and other grains that humans rely on for major foods produce small, highly specialized wind-pollinated flowers. Only a very few angiosperms that live in aquatic environments with submerged flowers such as sea grasses are water-pollinated. Animal pollination is the specialty of angiosperms.

The size, shape, structure, and number of pollen grains vary a great deal among plants. These pollen features, along with characteristics of the flowers, provide strong hints about a plant’s pollination mode. Pollen carried by wind is usually small (10-50 microns), lightweight, non-sticky, and produced in large quantities. Wind-pollinated flowering plants usually lack odor and showy petals, but have exposed stamens and large feathery stigmas.

The outer wall of the pollen grain is hard, serving as a hard protective coat during travel to the receptive part of a flower or cone. Because of the remarkable durability of the outer wall, pollen can stay around in the environment for a long time and is well preserved in the fossil record. Geologists examine pollen in soil cores to detect changes in vegetation types. Forensic palynologists examine pollen samples to solve crimes. Differences in pollen morphology allow these scientists to identify most plant families and a number of species by careful examination of pollen grains alone. For example, the pollen of pines and several other conifers has 1-3 air bladders to carry them aloft in the air. Pollen of members of the grass family (Poaceae) is round, smooth, and has a single circular aperture.

In wind-pollinated plants, and the few water-pollinated plants, currents determine where the pollen travels. In animal-pollinated plants, the movements of the animal visitors influence how much pollen is transferred and how far pollen is transferred. Movement of pollen from plant to plant promotes out-crossing and genetic diversity. Some plants, in contrast, are adapted for self-pollination.

What happens to a pollen grain when it reaches its destination?

When a pollen grain lands on the receptive (“female”) part of the plant and absorbs moisture, it germinates, forming a pollen tube. The pollen tube expands out of the aperture in the pollen grain and carries the sperm cells toward the ovule. In many angiosperm plants, the stigma has a glandular surface that secretes a sugary solution. Many pollen grains can land on the sticky stigma, and many pollen tubes extend down the length of the style. When one pollen tube successfully reaches the ovule and releases its sperm cells, the other pollen tubes stop extending. How does the pollen tube navigate toward the ovule? Cells near the ovule release species-specific proteins attracting the pollen tube. Other influences on pollen tube growth include temperature and calcium availability. In most angiosperms, the journey of the pollen tube to the ovule and the release of the sperm cells to complete fertilization is completed within 24 to 48 hours after pollination. However, in pines, one of the best-studied gymnosperms, the pollen tube does not reach the egg until approximately 15 months after pollination.
Additional Resources and References

**Prepared slides:** Order prepared microscope slides of flower structures, common allergens, pollen, and pollen tubes from Carolina Biological Supply Company: [http://www.carolina.com/](http://www.carolina.com/) (hint: search the site using the word “pollen”)

**Web Resources:** Some of the short videos are well worth showing the students if you can download them prior to class. Additional links to web resources are embedded in the lab activities.

- Plant Reproduction: The Pine Tree (Brittanica) (3-minute video): [http://www.youtube.com/watch?v=o6Se_9y68P0&feature=channel](http://www.youtube.com/watch?v=o6Se_9y68P0&feature=channel)
- Angiosperm Plant Pollination Mode Examples [http://www.cas.vanderbilt.edu/bioimages/pages/pollination.htm](http://www.cas.vanderbilt.edu/bioimages/pages/pollination.htm)
- Plant Reproduction: Methods of Pollination (Brittanica) (4-minute video): [http://www.youtube.com/watch?v=RuYrFwDuYn0&feature=channel_page](http://www.youtube.com/watch?v=RuYrFwDuYn0&feature=channel_page)
- Comparative plant morphology and dispersal methods: [http://www.life.illinois.edu/help/digitalflowers/](http://www.life.illinois.edu/help/digitalflowers/)
- Dr. Palanivelu’s University of Arizona Faculty page, with introduction to and videos of pollen tube growth [http://www.ag.arizona.edu/research/ravilab/lab%20introduction%20page.html](http://www.ag.arizona.edu/research/ravilab/lab%20introduction%20page.html) [http://www.ag.arizona.edu/research/ravilab/lab%20images&movies%20page.html](http://www.ag.arizona.edu/research/ravilab/lab%20images&movies%20page.html)
- Pollinator Partnership [http://www.pollinator.org/resources.htm](http://www.pollinator.org/resources.htm)
- Ecology Society of America’s Pollination Fact Sheet, with linked resources [http://www.esa.org/ecoservices/poll/body.poll.fact.html](http://www.esa.org/ecoservices/poll/body.poll.fact.html)
- TED Talk by Jonathan Drori: “Every pollen grain has a story” – with incredible visuals. [http://www.ted.com/talks/jonathan_drori_every_pollen_grain_has_a_story.html](http://www.ted.com/talks/jonathan_drori_every_pollen_grain_has_a_story.html)
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