Pollen Microscopy to Reveal Pollinator Diet Breadth

Grade Level: 5-12

Duration:

Prep time: 1 hour

Activity duration: 2.5 hours

PA Standards:

- BIO.A.1.2. Describe relationships between structure and function at biological levels of organization.
- BIO.B.4.2.2. Describe biotic interactions in an ecosystem.
- 4.5.7.D. Explain how biological diversity relates to the viability of ecosystems.
- 4.5.10.D. Research practices that impact biodiversity in specific ecosystems.

NGSS Practices

The bolded practices below are included in these lessons:

1. Asking questions
2. Developing and using models
3. **Planning and carrying out investigations**
4. **Analyzing and interpreting data**
5. Using mathematics and computational thinking
6. Constructing explanations
7. Engaging in argument from evidence
8. Obtaining, evaluating, and communicating information

OBJECTIVES

Students will be able to understand the fitness implications of pollinator diet breadth for (a) pollinators and (b) plants.

Students will be able to discover the intricacies of pollen morphology.

Students will be able to develop field observation and sampling skills.

MATERIALS

Compound light microscopes
(1 for each pair of students)

Microscope slides + cover slips

Glycerine jelly stained with safranin-O or basic fuchsin (mounting medium)

- 7 g gelatin powder (from grocery store)
- 42 mL water
- 49 mL glycerin
- Safranin red or basic fuchsin (or try other stains)
- Preparation**:**
  1. dissolve gelatin in warm water
  2. mix in glycerin
  3. add stain to achieve strong but not opaque color
  4. Pour into petri dish and allow to harden
  5. Using razor blade, cut into 3x3x3mm cubes.

Pins (for holding glycerin jelly cubes during pollen swabbing)

Dry ice + cooler

Small collecting jars and Paper bags

Plant and insect field guides or online identification resources (e.g. bugguide.net, discoverlife.org, iNaturalist.org)

Pencils and paper for sketching, notes, specimen labeling

Laboratory gloves (for steps involving the use or preparation of stained glycerin jelly)

Hot plate (ideally a low temperature hot plate designed for slide preparation; if a regular hot plate, use at lowest temperature setting)

BACKGROUND

Pollinating insects visit flowers to collect pollen and/or nectar for food. In the process of serially visiting flowers, they incidentally transport pollen within and between flowers. When this process succeeds in delivering pollen from male anthers to female stigmata, *pollination* has been achieved. If the pollen thus delivered is compatible with the receiving stigma, the receiving flower may become successfully fertilized, facilitating fruit and seed production. While pollen-stigma compatibility is a complex subject, for the purposes of this lesson we will make the generalization that pollen of the same plant species as the receiving stigma ("conspecific") is compatible, while pollen of a different species ("heterospecific") is incompatible.

In any given locale, there will be a variety of flowering plants and flower-visiting insects. This raises important questions about how pollinators choose which plants to visit, how plants "select" which pollinators to attract, and what the consequences of these relationships are for the evolutionary fitness of plants and pollinators and the ecological function of plant-pollinator networks. For the pollinator, there is a trade-off between the flexibility of visiting multiple plants species and the efficiency (and, in some cases, safety) of specializing on few plant species. Similarly, for plants, there is a trade-off between the security of having many pollinator species to depend on and the efficiency of attracting a specialized subset of the pollinator community that will more reliably transport only conspecific pollen.

Pollen grains have intricate microscopic architecture that varies characteristically (though sometimes cryptically) between plant species. In this lesson, students will use a combination of field sampling and microscopy to investigate the diet breadth of a focal pollinator and the pollen present on a focal plant.

PROCEDURES

Field sampling (30 min + transport time)

Schedule field work for a clear day with temperatures above 60F. Any location with a variety of flowering plants will serve well as a field site.

Working in pairs, students will spend 15-30 minutes (duration is flexible, and can be adapted to fit the needs of the teacher and students) observing interactions between insects and flowers. Halfway through this period, students will select one interaction to focus on. Once the students have selected one interaction to focus on, the students need to: (1) collect the flower-visiting insect by trapping it in a labeled collection jar and (2) collect all or part (the visited flower at minimum) of the plant from which the insect was collected. If possible, students should attempt to identify their focal insect and plant in the field while the specimens are fresh, though identification can be revisited in the lab. The insect will be euthanized by placing the collection jar in a cooler with dry ice, and the plant will be stored in a labeled paper bag. To ensure that insects are humanely killed, leave them in the cooler for 5 minutes with the jars closed, then open the lids of the jars and leave them in the cooler for another 30 minutes. The initial 5 minutes will chill and immobilize the insects; opening the jars then allows CO2 from the sublimating dry ice to kill the insects.

Microscopy (45 min)

If insect and plant specimens have not yet been identified, students, with the help of instructors, should identify the specimens to the best of their ability, achieving at least a provisional taxonomic determination for the purposes of the lesson. Below are some good resources for aiding identification:

Web resources

- Bugguide (https://bugguide.net): A great resource for North American arthropod identification; well-curated photographs, up-to-date taxonomy, high level of expert involvement.
- iNaturalist (https://inaturalist.org): iNaturalist is an app and a website that lets users upload images as time- and location-stamped observations. These get added to a global database of observations and can be viewed on an interactive map. iNaturalist features a machine learning algorithm that tries to match new observations with confirmed identifications of previous observations. For some taxa, including plants, this feature works very well. Once uploaded, observations can be viewed by the whole iNaturalist community, and identification can be crowd-sourced. **Note: registration is required to upload to iNaturalist, and registered users must be 18 or older; thus, the teacher will have to create an account for the class and upload photos through that account.**
- DiscoverLife (https://www.discoverlife.org/mp/20q): Interactive online keys for more involved identification. Guides are available for both plants and insects.

Books

- Field Guide to the Flower Flies of Northeastern North America (Skevington et al. 2019)
- The Bees in Your Backyard: A Guide to North America's Bees (Wilson and Carril 2015)
- Field Guide to Wildflowers: Northeastern and North-Central North America (Peterson and McKenney 1998)

To create pollen slides, students will swab their insect and flower, respectively, with a cube of glycerin jelly held on the end of a pin. When

swabbing the insect, focus on the head, legs, and belly, since these are the areas where pollen grains are most likely to be found. When swabbing the flower, focus on the anthers and stigma. After each specimen has been swabbed (with separate glycerin jelly cubes), prepare a slide for each specimen by depositing the glycerin cube on the slide, adding a cover slip, and gently heating until the glycerin jelly melts and the cover slip settles onto the surface of the slide. Slides should be pre-labeled so that the insect and flower samples do not get confused.

Give students ample time to study their slides under the microscope. Encourage them to make sketches of the pollen types they see. It may be helpful for students to browse the Global Pollen Project to familiarize themselves with the range of pollen forms (https://globalpollenproject.org/). After they have familiarized themselves with the pollen grains in their samples, they are to count the number of unique types found on each slide, and identify which of these types occur on both the insect and flower slides. Students can summarize their findings with a data sheet following this template:

	INSECT	FLOWER
Identity of Specimen		
# Unique Pollen Types		
# Shared Pollen Types		

Discussion (45 min)

With the help of the instructor, students will collate their results into a shared spreadsheet, ideally displayed on a screen that everyone can see at once. The discussion can be guided by the following questions:

- Describe the different pollen types you saw. Compare sketches and have students view each other's specimens. Encourage discussion of the diversity and intricacy of pollen morphology.
- Have students compare their pollen specimens with reference specimens from the Global Pollen Project (https://globalpollenproject.org/) to see if they can find any tentative matches. Note: these should not be considered positive IDs, but working hypotheses to facilitate discussion and comparison across samples.
- Did you find the same pollen on your insect and your flower?
- Do you think your insect was functioning as an effective pollinator of your flower?
- Which had more different types of pollen, your flower or your insect?
- What can you conclude about the degree of specialization of your insect? Of your flower?
- When looking at our shared data set, do any patterns emerge based on the identity of the insect and flower, respectively? For example, are flies tend to be more or less specialized than bees? Do open flower forms (e.g. Asteraceae) tend to have more pollen types than closed flower forms (e.g. Fabaceae)?

Modifications

The lesson could be strengthened by incorporating the preparation of pressed plant specimens. This would involve the use of a plant press, which can easily be made from scrap wood, cardboard, and newspaper. Instead of immediately bringing fresh plants to the lab after the field collection stage of the procedure, students would prepare pressed

plant specimens in the field, and these could be stored indefinitely. This would relieve the temporal constraint of having to do field sampling and lab work in the same day while adding the additional learning outcome of teaching students how to preserve plant specimens.

If one wishes to avoid sacrificing the insects, the procedure could be modified by anesthetizing the insects rather than euthanizing them. To do this, insects would be placed in the dry ice cooler with the jars closed for just long enough to immobilize the insects. They would then be removed and quickly swabbed for pollen, and the glycerin jelly cube alone would be returned to the lab. After warming up, insects should revive completely with no permanent harm.

REFERENCES

A thorough discussion of the microscopic techniques used in this lesson can be found in:

Kearns, C. A. and Inouye, D. W. 1993. Techniques for Pollination Biologists. University Press of Colorado, Niwot, CO.