**Journal reading:** Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye

**Introduction:** Flowers are objects of beauty. They occur in an enormous variety of shapes, colors and scents. We give them as gifts, use them as decoration and plant them in gardens. Yet, it is important for us, as ecologists, to remember that flowers do not exist solely for our enjoyment. Flowers are the products of a complex relationship between angiosperms (flowering plants) and their pollinators.

The relationship between a plant and insect pollinators is a mutualism. Plants benefit because the pollinators help them to reproduce successfully. The pollinators benefit from the relationship because plants provide them with food (pollen and/or nectar).

One big difference between plants and most animals is that plants can't move around. This means that plants can't go cruising around, looking for mates. Instead, flowers attract insects that move the sperm-carrying pollen grains to a spot on other flowers where, if they are lucky, the sperm may have the opportunity to fertilize an egg of a different individual (see below).

The Sawtooth Sunflower (*Helianthus grosseserratus*) we will be investigating in this lab is somewhat unusual. They are members of the family, Asteraceae. This plant family includes common plants like sunflowers and daisies. They are unusual because what appear to be flowers are actually groups of flowers called inflorescences. The petal-like structures around the edge of the aster are ray flowers. The
Ray flowers may be brightly colored and/or marked with UV nectar guides (see below), which attract pollinators to the central disk flowers that actually produce the pollen and eggs (see below).

The pollinators we will be investigating are bees. It is natural for us to assume that, in general, animals sense the world in more or less the same way that we do. But, as ecologists, we must be especially sensitive to differences in the way other organisms interact with their environments. In the case of bees, it turns out that while they see many of the colors that we see, with the exception of red, they can also see colors that we do not see, in the UV spectrum. Correspondingly, many flowers have markings called nectar guides that are only visible in the UV spectrum. Therefore, a flower that looks solid yellow or white to humans may look pretty fancy to a bee (see below).

For the next two weeks we will explore the relationship between plants and their pollinators. During this time it will be useful to consider how each organism benefits from the mutualistic relationship, and what motivates each organism’s behavior.

Week 1: Initial Observations and Measurements
Methods:
Part I: Follow that BEE.
Before forming hypotheses and setting up experiments, it is crucial for us to understand our study organisms. Today, we will be investigating various types of bees and their mutualistic relationship with...
Helianthus grosserratus. You may also notice butterflies and yellow jackets (wasps) pollinating the flowers.

Find a quiet spot, away from other students, where you can view flowers easily. Being a pollination biologist requires a lot of patience. When a bee arrives at a flower in your study area, make note of how long the bee stays on the flower (count the seconds), how far the bee flew to get to the flower (use yardstick) and whether the next flower it visits is on the same plant. Continue following the bee for as long as possible, taking note of the time spent, distance traveled and flower visited for each stop. Design a simple table on which you can record your results. Continue making observations for 15 minutes.

Now that you are familiar with the behavior of the bees, we can use tools to learn more about the relationship between the pollinator and the plant. Today we have two such tools available for your use:

**Part II. Day-Glo Biology: Pollen Carryover in a Bee-Sunflower System**

1. Fluorescent powder dyes can be used to simulate pollen to investigate the movement of pollen from flower to flower. This technique can be used to see how far bees move pollen as they visit flowers. Before you get started, consider the advantages and disadvantages for each member of the mutualism of:
   A) moving pollen short vs long distances
   B) moving pollen from flower to flower on the same plant vs moving pollen to different individuals of the same or different species

2. Formulate a set of alternative hypotheses, concerning the amount of pollen deposited by a bee as it moves from flower to flower. If a bee picks up pollen at Flower 1, would you expect it to deposit more or less or the same amount of Flower 1's pollen on Flower 2 (the next flower it visits) or Flower 3 (the third flower it visits)?

3. Use a small paintbrush to dust fluorescent dye onto the center of one or some of the flower heads of a plant. Be careful to mark flowers of the sort you see bees visiting frequently. Use the dye liberally. It may take a while for the bees to come back to your marked flowers, so you may want to go for a walk, or mark some other flowers using technique 2 (below), or figure out what kind of data you want to collect from this experiment - and come back in 20 minutes or so.

3. Once you have bees visiting the dyed flowers, you can follow the bees, removing each new flower that they visit, and placing it carefully into an individual, marked plastic bag. These flowers should now be marked with fluorescent dye, so handle them carefully.
Continue to collect flowers until you have as many good trials as possible. Discuss among members of your group how you can know when you have collected enough data. Before leaving the field, make sure that you show your data to your lab professor and justify the amount of data you have chosen to collect.

**IMPORTANT: PLEASE REMOVE ALL DYED FLOWERS BEFORE LEAVING THE STUDY SITE. PLACE THEM IN A PLASTIC BAG FOR DISPOSAL IN THE LAB.**

4. At the end of lab, take the flowers back to the lab/darkroom and illuminate with a blacklight to reveal the amount of dye being moved from flower to flower by each bee.

5. Figure out a good method of categorizing the amount of pollen on each flower head (rate them from 1-5, or whatever is practical).

6. Produce a graph for your Lab Report that illustrates the amount of pollen moved from flower to flower by the bees.

7. Were you able to document a pattern in the amount of pollen deposited, as the bee moved away from the source flower?

### Part 3. See Like a Bee

1. We can also alter flowers, in an attempt to make them more or less attractive to bees. This can give us clues about what flower characteristics are most important, from a bee’s point of view. Consider how changing a flower’s attractiveness might influence bee behavior. Design a series of alternate hypotheses that you could test by altering the way flowers appear to bees. Make a list of some characteristics of the pollinators that you could measure, once you have altered the attractiveness of the flowers.

2. Use markers, paint and/or scissors or scents to alter the attractiveness of some flowers. Test only one change at a time, so that you can analyze your results with simple statistical tests.

3. Collect relevant bee visitation data.

**IMPORTANT: PLEASE COLLECT ALL MARKED/ALTERED FLOWERS, BEFORE LEAVING THE STUDY SITE. PLACE THEM IN A PLASTIC BAG FOR DISPOSAL IN THE LAB.**

4. Were you able to document a significant change in bee visitation behavior by changing the appearance of the flowers? Produce a graph (or series of graphs) and run statistical tests of data. Include these in your Lab Report.

Before leaving for the day, discuss options for your week 2 project with your lab partners and professor or lab peer teacher.

**Week 2: The Research Project:**
For the second week of the lab, design an experiment, using the techniques you learned in Week 1 that will test some aspect of pollination biology. You should have this plan ready before you arrive to lab for week 2.
**Part I: Planning the Project**

Examine your data from Week 1. Were you able to reach conclusions using the data you collected? Would collection of additional data help you distinguish between your hypotheses more clearly?

If you were able to answer the questions from Week 1 adequately, or if you just want to do something else, here are some suggestions:

1) Try working on another species of plant.
2) If you are more interested in insects, consider comparing pollinator behavior (distance flown, amount of pollen moved, etc.) exhibited by different types of bees. Are some bees better pollinators, from the plant’s point of view, than others?
3) Are there additional ways of altering flowers attractiveness in ways that make them more/less attractive to bees? What characteristics might bees and other pollinators be sensitive to?
4) Ignore all of the above and design your own lab from scratch. Just make sure you check with your professor in the early stages of planning, so that any materials you might need can be provided and to double-check your experimental design.

**Journal Club Readings**

1) Flower constancy in honey bee workers (*Apis mellifera*) depends on ecologically realistic rewards
2) Floral signposts: testing the significance of visual ‘nectar guides’ for pollinator behavior and plant fitness
3) Predation risk makes bees reject rewarding flowers and reduce foraging activity
4) Floral signal complexity as a possible adaptation to environmental variability: a test using nectar-foraging bumblebees, *Bombus impatiens*
5) Trade-off between travel distance and prioritization of high-reward sites in traplining bumblebees

**Image Sources:**
Pollination and UV Flowers:
Composite Flower