TIEE

Teaching Issues and Experiments in Ecology - Volume 2, August 2004

EXPERIMENTS

Pollination Ecology: Field Studies of Insect Visitation and Pollen Transfer Rates

Judy Parrish
Biology Department, Millikin University
1184 West Main, Decatur, IL 62522
jparrish@mail.millikin.edu
(217) 424-6235, fax (217) 362-6408

Tiger Swallowtail (*Papilio glacus*) visiting Milkweed (*Ascelpias*), © Judy Parrish

Table of Contents:

CITATION:

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ABSTRACT:

Students will investigate questions related to the pollination ecology of the most common and accessible insect-pollinated flowers in bloom. Students will start with natural history observations to answer common questions such as how long does the flower stay open, what are its major visitors, and how often is it visited by likely pollinators. They may then follow up the class study with their own questions, such as whether flowers that are in large clumps are more likely to be visited than more isolated flowers, how far the most frequent visitors fly between visits, how likely is it that the next visit will be to the same species of flower, and whether self pollen grows through the style more slowly than pollen from a different individual. Common techniques in pollination studies such as determination of flowering phenology, visitation rates, and identification of visitors and of pollen carried on visitors will be used regardless of the question to be investigated. Spring beauties in the campus lawn, buckeye or crabapple flowers, horticultural plantings on campus, roadside goldenrods, or wildflowers in nearby natural areas should make it possible to complete this lab at almost any time in the growing season.

KEYWORD DESCRIPTORS:

Principal Ecological Question Addressed: How and why are animals attracted to flowers? How can animals and flowering plants act as selective agents upon each other, resulting in coevolution of a mutualistic relationship?

Ecological Topic Keywords: coevolution, pollination, floral phenology, mutualism

Science Methodological Skills Developed: natural history observations in the field, classification and use of dichotomous keys, sampling to estimate population size (of plants, flowers, pollen in loads), defining questions, formulating hypotheses, designing experiments, collecting and presenting data, microscope use, graphing summarized data, and development of equations to predict probability of visit, number of visits per flower, size of population

Pedagogical Methods Used: inquiry based learning emphasizing a specific set of techniques (see also guided inquiry); cooperative groupwork to generate and test hypotheses

CLASS TIME: Two hours, plus travel time if necessary, for the initial observations. An additional hour of planning and approval of projects to address student generated questions. At least one hour of lecture to precede the introduction of the lab.

OUTSIDE OF CLASS TIME: Pre-lab questions, one hour, two to four hours for the data collection for the class project, and two to four more hours to complete the projects from student generated questions. Flowering phenology questions require multiple short (ten minute) visits to the study site over the period the flowers are open, from one day for most composite flowers up to a week for flowers such as spring beauties.

STUDENT PRODUCTS:

- Prelab preparation of questions and definitions,
- Submission of responses to questions for further thought,
- Data set for compilation,
- Scientific research report on assigned portion of compiled class data and/or on the student-directed question addressed,
- List of five intriguing questions generated during observations, each with a testable hypothesis (may be part of the discussion section of the report on compiled data).

SETTING: Outdoors, using easily accessible plants such as spring beauties in the campus lawn, buckeyes or crabapples on campus, roadside goldenrods, or wildflowers in nearby natural areas. Landscape plantings on campus can also be used. Some lab/microscope work also necessary, as well as access to computers with graphics software.

COURSE CONTEXT: This lab activity is used in four different courses at Millikin University: (1) non-majors course, Local Flora, with 18 students, (2) freshman biology major's course, Attributes of Life, with five lab sections of 16 students each, (3) upper division summer Field Ecology course for 10-12 non-majors, and (4) upper division Plant Biology course for 12-16 junior and senior undergraduate students. Details of how the activity is used in each of these courses appear in the "Notes to Faculty: Uses of this Lab Activity in Different Courses at Millikin University" below.

INSTITUTION: Private, four-year, smaller comprehensive university

TRANSFERABILITY: Useful for non-majors in local flora and field ecology immersion classes, as well as junior and senior biology majors in upper level Plant Biology. Also adaptable for younger students, with more emphasis on observations. Additional comments appear in the "Notes to Faculty: Translating the Activity to Other Institutional Scales."

SYNOPSIS OF THE LAB ACTIVITY

WHAT HAPPENS:

Students observe animal pollinators on flowers and work to answer instructor-directed questions on topics such as which flowers are more attractive, probability of visitation, and types of visitors attracted. Students may then design an experiment or observations to test the hypothesis, analyze the data, and prepare a formal report on their findings.

LAB OBJECTIVES:

At the conclusion of this lab...

1. students will develop an appreciation for and understanding of the importance of mutualistic interdependence of organisms in the coevolution of structures and behaviors,

2. students will learn to identify common flowering plants, common insect pollinators/visitors to those plants, and common pollination syndromes evident in those common plants and pollinators,

3. students will compile, add to, and use a data base of flowering times, visitation rates, and a pollen reference collection usable by other classes,

4. students will learn to ask questions that generate testable hypotheses about pollination ecology, gain experience designing experiments to test those hypotheses, and analyze and present results in scientific format.

EQUIPMENT/ LOGISTICS REQUIRED:

- This handout and data sheets,
- Populations of flowering plants and visitors,
- Basic fuchsin gel,
- Dissecting needles for cutting and applying gel cubes, glass microscope slides, coverslips, and candle with matches or lighter (or dark paper and sunlight),
- Permanent markers for labeling slides; slide box and small insulated ice chest to keep prepared slides from melting,
- Microscopes with 100 power; counting grids,
- Insect nets,
- Ethyl acetate for stunning, or freezer,
- Hand lenses,
- Stopwatches or watches with second hand.

SUMMARY OF WHAT IS DUE:

From this lab, students should submit the following:

1. Responses to the following pre-lab questions due at the beginning of the first lab devoted to pollination,

   - Define the following terms: coevolution, mutualism, pollination syndrome, insect, phenology, diurnal, parasitism, angiosperm.
   - Differentiate between visitation and pollination, between pollination and fertilization, and between pollination and parasitism.
   - Draw and label a “typical” flower, and describe the major functions of each part.
   - Why do animals visit flowers? Describe the characteristics of animals that would make for a good pollinator. What rewards do the plants provide?
   - Do any visitors harm the flowers? Do any flowers harm their visitors?

2. Students will submit data collected by their groups which will be compiled and distributed,

3. Students will generate questions about the interactions they observed. These questions may be developed into testable hypotheses for student projects, or may be included as part of their discussion sections for a research-style report using the compiled data,

4. If student generated questions are investigated, a research-style report is required to present these data.

DESCRIPTION OF THE EXPERIMENT

INTRODUCTION:

Why are flowers so pretty? It is of little benefit to a wild plant to be admired. Why have plants put so much energy into the structure of flowers and production of nectar and other rewards? Of course, the flower's purpose is to result in sexual reproduction. Berenbaum (1995) states that "Sexual reproduction is just as important for plants as it is for animals when it comes to generating genetic variation, but plants have a singular disadvantage compared to animals when it comes to sex: they can't just get up and find themselves a mate. " Plants must rely on pollen vectors, from wind to insects to birds, to transport their pollen to another individual. Those visitors must be attracted to the same species repeatedly to bring about pollination. Visitors must cause pollen transfer for flowers to be successful. Usually this means that the visitor must be attracted, collect pollen accidentally by brushing floral parts, or purposefully collect pollen to take back to a nest, and then visit another flower of the same species and brush up against the stigma, effecting pollination. Flowers can attract pollinators by providing ample nectar of the right composition, and by advertising this nectar by deep shape and recognizable floral patterns, by providing excess pollen as food, or by providing shelter or a place to raise (and feed) young - or by at least looking as if they do (Faegri and van der Pijl 1971). We human observers have tastes that are somewhat similar to those of the birds and bees when it comes to floral attractiveness (although we vary from carrion beetles and flies, as we do NOT consider the smell of rotting meat attractive). Our perception of color differs from that of non-vertebrate pollinators. Bees don't see colors at the red end of what we consider the visible light spectrum, but they do see colors of ultraviolet. Many flowers have ultraviolet markings that act as nectar guides that cue insects on where to find floral rewards (Barth 1991, Buchmann and Nabhan 1996, Proctor et al 1996).

In some cases, there are many species of plants, or many flowers of the same species, open at the same time, resulting in a shortage of possible pollinators and competition among the plants for visits (Mosquin 1971, Waser 1983, Caruso 2000). This may result in differences in flowering time to reduce competition for pollinators (Frankie 1975, Anderson and Schelfhout 1980) or in changes in floral structure (Waser 1983, Fishman and Wyatt 1999, Medel et al 2003). In other cases, pollinators must compete with each other, as the floral rewards are in short supply (Pleasants 1981, Pyke 1982, Thomson 2004).

Natural selection has favored those flowering plants that are most attractive to pollinators, and those pollinators best able to get floral rewards. Millions of years of coevolution between flowering plants and their pollinators, with each participating species population acting as a selective agent on the other, have resulted in overwhelming biodiversity of both insects and flowering plants (Stebbins 1983). "The shapes and colors of the flowers, their scent, their location on the stalks, the season and daily schedule of their pollen and nectar offerings, as well as other qualities we admire but seldom understand, are adjusted precisely to attract particular species of insects; and those specialists in turn, whether beetles, butterflies, bees, or some other group, are genetically adapted to respond to certain kinds of flowers" (Wilson 1999). The mutualistic relationships that develop between a flowering plant , which benefits by cross-fertilization and its most effective pollinator, which benefits from an enhanced food source, are also influenced by other species populations. There may be herbivores and nectar thieves that visit flowers to acquire rewards, but do not disperse pollen (Irwin and Brody 1999, Maloof and Inouye 2000). We see the results of reciprocal selective pressures, with adaptations of the flower to restrict nectar acquisition to a visitor with the ability to move its pollen effectively, and adaptations of the insect to acquire nectar in long, curved tubes. Other organisms that interact with both flower and pollinator may complicate the system, resulting in apparent or real maladaptations (Thompson et al 2002).

The most frequent visitor to a flowering plant is not necessarily the one that is the most effective pollinator. Natural selection will result in the flower adapting to the pollinator that is most likely to bring about effective pollen transfer for fertilization. Floral color, shape, placement, timing, and reward will be selected, with the most attractive flower getting the most effective visits, producing more viable seed, and leaving more offspring in future gene pools. The most likely pollinator can often be deduced from floral characteristics, with flowers that attract the same type of pollinator converging in morphology into what we call pollination syndromes (Table 1, Key 2) but it usually takes careful observation to sort out specific pollination relationships. Most flowers attract several different kinds of pollinators, with several different attractants which may not be consistent with the "syndrome."

MATERIALS AND METHODS:

Study Site(s): Outdoors, anywhere there are plants in flower - ranging from spring beauties in the lawn, to flower beds on campus, to insect pollinated trees like crabapple or buckeye.

Overview of Data Collection Methods and Analysis.

Example Assignment - Natural History Observations.

The class might be divided into three groups, one to estimate visitation rates, one to determine the phenology (seasonal and/or daily timing of flowering) of the flowering species (Ohio buckeye in this case), and one to collect visitors and determine how much and what kind of pollen is carried. Alternatively, any one part could be used. Once you have these kinds of data about the most likely flowering plants and visitors for the time of your class, you may want to provide students with these data and go straight to the student-generated questions. Many of the techniques are described in Kearns and Inouye (1993).

Group 1 - Visitation Rates

Equipment Needed:

* stopwatches or watches with second hand,
* marking tape or flags,
* “eyeball ID,”
* hand lenses,
* thermometer,
* data sheets,
* tape recorder or partner to record visits so observer’s eyes can remain on flowers.

Procedure:

1. Each person in the group should find a branch (buckeye) or a plot (spring beauties) with receptive flowers (pollen is being shed, and/or stigmatic surface is visible and sticky). Mark the branch or plot with marking tape (buckeye) or flags (spring beauties).

2. Carefully examine the flowers. Use the key to pollination syndromes (sets of traits of flowers thought to attract and/or accommodate pollen vectors, and sets of traits of animals that allow them to exploit flowers with those traits) to predict visitors by floral morphology.

3. Count the number of individual OPEN flowers on the branch to be observed, or in the marked plot. Count only the flowers you will observe - and record

visits on only the flowers selected or in the plot.

4. Observe the marked branch or plot for three observation periods of exactly 10 minutes, counting the number of visits by each category of visitor (categories include honeybees, bumblebees, small bees, flies, butterflies, beetles, and birds).

5. Note the ambient air temperature in ^{o}C.

6. Return to the same branch or plot and make three ten-minute observations at additional assigned time (later in the day, or earlier on another day - assigned times will cover two hour blocks - 0800 to 1000, 1000 to 1200, 1200 to 1400, 1400 to 1600, and 1600 to 1800 hr),

7. Note the temperature in ^{o}C

8. Calculate the average number of visits per category of visitor per flower per observation period.

9. Is the most frequent visitor the one you expected from the pollination syndrome key? If not, why might there be a difference in what the flower appears to attract and what actually visits most frequently?

10. What were each of the visitors doing at the flower? Does that activity promote pollen transfer?

11. Turn in your observations to your instructor so that they may be compiled. Pick up the compiled observations from all groups,

12. Estimate the number of visits that can be expected per flower at each observation time.

Report Form: Visitation Rates - Group 1, In-class Observation

Time of Day________ Temperature - Degrees Celsius________

Number of flowers observed_________ Number of minutes observed____________

Category	Observation 1	Observation 2	Observation 3	Mean
Honeybees				
Bumblebees				
Small bees				
Flies				
Butterflies				
Beetles				
Other				
Total				

Report Form: Visitation Rates - Group 1, Additional Assigned Observation

Time of Day________ Temperature - Degrees Celsius________

Number of flowers observed_________ Number of minutes observed____________

Category	Observation 1	Observation 2	Observation 3	Mean
Honeybees				
Bumblebees				
Small bees				
Flies				
Butterflies				
Beetles				
Other				
Total				

Key 1 - Major Insect Visitors to Flowers (based on Central Illinois)		
1	Wings not visible, or hard wing covers concealing wings....	2
2	No wings, narrow area between thorax and abdomen....	Ants
2'	Hard wing covers conceal flight wings, form line down middle of back, chewing mouthparts....	Beetles
1'	Wings visible....	3
3	One set of filamentous wings, eyes large and obvious (careful - some Syrphid flies mimic bees)....	Flies
3'	Two sets of wings....	4
4	Both sets of wings often colorful, covered with scales....	5
5	Antennae with knob-like ends, wings usually folded when at rest....	Butterflies
5'	Antennae with feathered ends, no knob, wings often open at rest....	Moths
4'	Wings membranous, usually clear....	Bees and Wasps, 6
6	Thorax and abdomen joined by narrow “waist,” abdomen often pointed....	Wasps
6'	“Waist” not as marked, body usually hairy....	Bees, 7
7	Pollen carried on “belly”....	Megachilid leafcutting bee
7'	Pollen carried mainly on leg....	8
8	Usually small (~5-10mm), black or metallic green, short tongued....	Halictids, “sweat bees” Andrenids
8'	Long tongue, usually over 12 mm....	9
9	Spur on hind leg, abdomen often appears striped....	Anthophorid digger bees
9'	No spur, body robust, usually over 20mm, yellow and black, eyes not hairy....	Bumblebees *Bombus*
9"	No spur, golden brown color, 12-15mm, hairy eyeballs(!)....	Honeybees *Apis mellifera*

Key 2 - Dichotomous key to pollination syndromes

1	Flowers small, inconspicuous and usually green or dull in color, petals reduced or absent....							Wind
1'	Flowers conspicuous, usually with white or colored petals....							2
	2	Flowers regular in shape, radially symmetrical....						3
		3	Flowers purple-brown or greenish in color, often with strong odor of rotting fruit or meat, little floral depth....					4
			4	Flowers purple-brown, sometimes with a "light window"....				Flies
			4'	Odor day or night, dull color....				Beetles
		3'	Flowers with little odor, or sweet odor....					5
			5	Flowers with deep corolla tube....				6
				6	Flowers red, open in day, little or no odor, no nectar guide, nectar plentiful....			Hummingbirds
				6'	Flowers not pure red, usually sweet odor....			7
					7	Flowers yellow, blue, or purple, corolla tube not narrow, but sometimes needing forced opening, often with nectar guides....		Long tongued bees
					7'	Flowers red, purple or white, corolla tube or spur narrow, usually lack nectar guide....		8
						8	Flowers purple or pink, diurnal, upright, with landing area....	Butterflies
						8'	Flowers white or pale, pendant, open or producing odor at night....	Moths (in some areas, bats)
			5'	Flowers more dish-shaped, reward accessible, yellow, or with abundant pollen....				Bees, Flies, small moths
	2'	Flowers irregular in shape, bilaterally symmetrical....						9
		9	Flowers red, little or no odor....					Hummingbirds
		9'	Flowers with odor, usually with nectar guides....					Bees

Table 1. Pollination Syndromes: Characteristics of flowers and the vectors that shape them.

Vector	Characteristics of Flower	Characteristics of Vector
Wind	Inconspicuous, green or dull in color, petals reduced or absent, abundant and in canopy	Abiotic
Beetles, flies	Dull colors, dark red, strong, spicy odor, or odor of rotting flesh, flat shape May have light window (flies)	Good sense of smell Some lay eggs in rotting flesh
Bees	Often blue or yellow, with landing platform Often have markings that act as nectar guides, sometimes in UV spectrum Reduced numbers of floral parts Often irregular in shape May have deep tube or spur for nectar	Good sense of vision, smell Often have body hairs Can perceive depth, "count" petals Do not see true red – see UV
Moths	Open at dusk or night, emit sweet odor at night Often dull or white Long corolla, no landing platform	Most active at night Strong sense of smell Have long proboscis for nectar acquisition
Butterflies	Open in day, emit some odor in day Landing platform Long corolla tube, narrow May be blue, purple, red, yellow May have nectar guide	Active in day Have long, thin proboscis for nectar acquisition Can see red Alight on blossoms
Hummingbirds	Red, large flowers with deep nectar tube and abundant nectar Little or no fragrance Open in day No landing platform No nectar guide	Vision much like human – see red Long bill and tongue, large body Little sense of smell Intelligent – remember and return to flowers with abundant reward Active in day Approach flower and hover

Group 2 – Phenology

Equipment needed:

* Colored bell wire, embroidery floss
* Flags to mark plot
* Hand lens
* Aluminum tags, or colored plastic toothpicks
* Permanent marker
* Field guide to flowering plants

Procedure:

1. Carefully examine flowers. Determine if some are shedding pollen, with stigmatic surfaces not receptive (functionally male), or if pollen is all shed, but stigmatic surfaces are open and sticky (functionally female), or if both stamens and pistils are functional, or if both are finished. Note presence of a scent or nectar. Note color patterns, and presence of nectar guides.

2. Mark at least 15 individual flowers with numbered small tags, with colored floss, or with colored toothpicks. Note the phase or phenological state of each. The group should decide on criteria to describe each phase (see examples for spring beauty and partridge pea).

3. If population size estimates are required, randomly select at least 10 one meter squared plots and count number of individual plants of the target species are in flower.

4. Return to collect data on phenological phase of marked flowers and population in flower at four additional assigned times or days. Observations of class members should continue for at least 5 days, or at 4 times of day on one day if the flowers are short-lived.

5. Turn in observations to your instructor so that they may be compiled. Make sure to pick up compiled data.

6. Determine the sequence of each phase of floral function, the average longevity of each phase, and how long the flower is open for visitation.

Phase Key must be designed or modified depending upon what species of flowering plant is the target.

For Spring Beauty: Key to Phases: (from closed bud to flower finished)

A- Flower enclosed in bud
B- Flower open, streaked pink, stamens very pink and erect
C- At least 2 stamens appear less pink, pistil obvious
D- Stamens folded back against petals, style clearly splits into 3 stigmas
E- Ovary swollen, petals wilting

Date and Time of Observations:

Observation	Date	Time
1	________	_______
2	________	_______
3	________	_______
4	________	_______
5	________	_______

Phase at Each Observation

Flower #	Phase-A	Phase-B	Phase-C	Phase-D	Phase-E
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					

Group 3 – Visitors

Equipment needed to collect insects:

* Aerial nets,
* Kill jars with ethyl acetate, OR freezer,
* Insect pins and boxes,
* "Eyeball ID" for insects,
* Field guide to insects.

Equipment needed to collect pollen:

* Basic fuchsin gelatin (recipe below),
* Dissecting needle,
* Microscope slides, coverslips,
* Candle and matches, lighter, or black paper and warm sunshine,
* Marking pen,
* Microscope with 100x.

Procedure:

1. Individuals with allergic reactions to bee stings should not participate in this portion.
2. Keep the basic fuchsin gelatin out of the sun, in a small ice chest. If phenol was added, do not touch the gel.
3. In an area away from where the visitation observations are underway, collect as many visitors to flowers as you can with an insect net.
4. Use ethyl acetate to kill or stun insects.
5. Use a dissecting needle to cut a 0.5 x 0.5 cm cube of glycerin fuchsin jelly. Holding the cube of jelly on the dissecting needle, wipe pollen from the body of the visitor. Make separate slides for jelly wiped on mouthparts, abdomen, and legs of the insect (or other body parts as observed). Carefully remove all visible pollen from the body section. If the specimen is a bee with a pollen load, crush the load so that pollen grains are identifiable.
6. Place the cube on a glass slide, put on a coverslip, and gently melt over a candle or lighter, or place on dark surface in the sun.
7. Label the slide with a Sharpee - date, time, type of visitor, body part.
8. Make a reference slide from the flowering species for comparison. Wipe a cube of the jelly on an anther that is shedding pollen. Label it with date, time, species, and phase of flower
9. Examine your slides under a microscope at 100X. Count the number of pollen grains of the target species (buckeye or spring beauty) AND the number of non-target species pollen grains. Identify non-target species if possible. Start at one corner of the coverslip and systematically move the slide back and

forth so that the field of view covers the slide the same way that an eraser would erase a blackboard.

10. If pollen grains are too numerous for a complete count, sample the slide by counting 10 fields of view. Estimate how many fields of view there are (this will differ depending on the magnification you are using). Then multiply the average number of pollen grains of both target and non-target species counted in your 10 fields by the number of fields to estimate the number of pollen grains on the slide.

11. Calculate the proportion of target species pollen in the pollen load or jelly wipe that is from each part of the body. If non-target pollen is identified, calculate the proportion of each species pollen on the slide.

Group 3, Visitor - Target Flower Species:

Visitor Species	# Pollen Legs - Target	# Pollen Legs - Nontarget	# Pollen Body - Target	# Pollen Body - Nontarget	# Pollen Mouth - Target	# Pollen Mouth - Nontarget
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
10.						
11.						
12.						
13.						
14.						
15.						
16.						

Additional Instructions for All 3 Groups:

Turn in the report form from your section of the investigation. Pick up the compiled data from all groups and answer the following questions. After examining the data, develop five questions the data raise and testable hypotheses for each of those questions.

In addition, you may be asked to choose one of those hypotheses to to design and carry out an experiment to test. If so, use the Format for Written Research Report below to write your lab report.

Format for Research Report - should include the following sections:

* TITLE
  - One sentence summary of the paper - should be concise and informative. Include the appropriate taxonomic information about the organism.

* INTRODUCTION
  - State the purpose of the study and enough background material to demonstrate the significance of the study.
  - This is where your hypothesis and predictions go.
  - You should also refer to relevant published articles that pertain to your study (what have other researchers learned in investigating similar topics or processes?). Start with general background and work to your specific project.
  - Citation of sources should take place within the body of the paper, right after the information cited from that source. In science, it is important to know WHO said it and WHEN it was said, so put the author and year in parentheses (Parrish 2004). At least three non-textbook, published and peer-reviewed sources are required for the report.

* METHODS AND MATERIALS
  - Summary of setting of study (date, time in military format - 1400, not 2:00 p.m., cloud cover, temperature, environment of room, etc.), equipment and materials used, information about the organism(s) studied, experimental design and procedures used, and statistical methods.
  - This section should allow other researchers to repeat your experiment.

* RESULTS
  - All data and statistics. Do not include raw, unsummarized data in this section. Raw data should be placed in an appendix. Data should be summarized and analyzed (means, standard deviation, etc.) and presented as visually as possible. Well constructed graphs are clearer than tables for most data.
  - Graphs and drawings are figures, and are numbered consecutively.
  - Tables are also numbered consecutively (separately from figures).
  - Refer to the figures and tables within a narrative, describing the trends. In other words, walk the reader through your results. The opening paragraph should state the overall

trends found in the data - but do not say SIGNIFICANT difference unless you did the statistics to test for significance. The following paragraphs should give the specific findings that support the overall trend. Do not say "See tables for results" or "Figure one shows that..." or (See Figure 1). Say "There were more herbivores than primary producers (Fig. 1)."

- The tables and figures should have headings which are clear and complete enough that they can stand alone and the reader can extract the meaning without reading the text.
- Any explanation, interpretation, or BECAUSE statements should not appear in the results, but in the discussion.

* DISCUSSION

- This is the meat of the report. You should interpret your results, place them in context, and provide supporting references.
- Compare your results to your hypothesis. Avoid statements not supported by your data.
- You should also write about possible errors in the design and implementation of the study. Alternative explanations for the results should also be considered here, as well as alternate hypotheses.
- Compare your results to those of other scientists, and cite their work. Start with the specific (your work) and then go to the general, big picture.
- The final paragraph should be your conclusions from the study. What are the main points you want the reader to understand? Your conclusions should be forceful and memorable.

* LITERATURE CITED

- Every article cited in the body of the paper - and none that are not cited - should appear in this section.
- Citations are by alphabetical order of the last name of the first author listed in each paper, then by date if you cite more than one article by the same author.
- Different journals use different formats for the literature citations, as are shown in McMillan. To be consistent, we will use journals such as *Ecology* for format direction,

  Juenger, T., and J. Bergelson. 1997. Pollen and resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. *Ecology* 78: 1684-1695.

  You should have at least three references. Textbooks and encyclopedias DO NOT COUNT!!!! (but do cite them if you use them). Web articles may count ONLY if they have an author - and ONLY ONE of your three required sources may be from the web. Use current journal articles as much as possible. The quality of your introduction and discussion depends upon good use of literature.

IN ADDITION: PROOFREAD AND USE SPELL CHECK!!!!!

- Points will be deducted for errors in grammar, spelling, punctuation, and format.

Preparation of Basic Fuchsin Gelatin and Pollen Slides:

A jelly containing stain to make a semi-permanent microscope slide of pollen, as suggested by Beattie, A. J. 1971. *Pan-Pacific Entomologist* 47: 82.

Use the following ingredients:

* distilled water, 175 ml,
* glycerin, 150 ml,
* gelatin, 50 g,
* crystalline basic fuchsin as desired - enough to make solution the “color of a fine claret,”
* crystalline phenol, 5 g - important in humid environments, may be left out if gelatin can be refrigerated. Don’t touch gelatin if phenol is used.

Procedure to Make the Fuchsin Gelatin:

1. add the gelatin to the distilled water in a beaker and heat until the gelatin dissolves,
2. add the glycerin,
3. add phenol, if desired,
4. add basic fuchsin crystals a few at a time until the solution is the color desired. Too light will not stain the pollen, but too dark may mask details of the pollen,
5. filter the solution through glass wool or cheesecloth,
6. pour into sterile containers such as petri plates that can be covered. If phenol is not used, refrigerate the plates and slides. They will keep about a month without refrigeration.

Preparation of Pollen Slides

1. keep the prepared slides, and the unused jelly, out of the sun, and cool enough not to melt,
2. with a dissecting needle, cut a small cube of the jelly out of the petri plate,
3. brush the cube of jelly against an anther containing pollen, or on the insect body part,
4. place the cube containing the pollen sample on a glass slide,
5. place a coverslip on top of the cube of jelly,
6. gently heat the slide over a candle flame until the jelly melts. Do not overheat, or scorch the slide. If it is warm and sunny, the jelly may be melted by placing the slide on a dark surface in the sun instead of using the candle flame. This will make a semi-permanent, stained specimen,
7. using a permanent marker, label the glass slide (date, species sample was collected on).

Questions for Further Thought and Discussion:

1. Describe the phenology of the target species. Are there color changes in floral display? If so, can you think of any advantage to the flower for the change? To the pollinator?

2. Draw a graph to display the average visitation rate by all categories of visitors over daily time. Are flowers more likely to be visited at certain times of day?

3. Draw another graph to display the average visitation rate by all categories of visitor at different temperatures. How does this graph compare to the time of day graph?

4. Calculate the probability of visit to each flower by any possible pollinator. You will need data from the phenology group to know how long the flowers are open for visitation, as well as average visitation rates, or rates for specific time periods.

5. Which visitors carry the most pollen? Is that pollen likely to be transferred to another flower of the same species? If more than one species of pollen is present, is it in the same place on the visitor's body?

6. Which visitors are likely to be the most effective pollinators? Why? Which visitors are probably not pollinators?

7. What attributes of the plant population (e.g. density, patch size, identity of neighbors) might raise or lower the predicted visitation rate?

*** Note: Answers to many of these questions and numerous other comments by the contributing author can be found in the "NOTES TO FACULTY" section below.

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Links:

Pictures and descriptions of flowers adapted for various types of pollination (wind, beetles, flies, bees, birds)
www.cas.vanderbilt.edu/bioimages/pages/pollination.htm
see also bioimages.cas.vanderbilt.edu/

Dichotomous key to most likely pollinator
www.ns.purchase.edu/biology/bio1560lab/pollination.htm

Images of 96 flowers and descriptions of their pollination
www.biology.vanderbilt.edu/BIO/review.html

Descriptions of pollination syndromes, and images
www.ftg.org/EduProfDev/Birds_Bees.html

Test of the predictability of pollination syndromes
www.umsl.edu/~biology/Bourne/resVulgare.html

Source references to Pollination biology: means of attraction, coevolution, and diversification
www.biology.vanderbilt.edu/BIO/262sourcespollination.html

Descriptions and comparisons of Proteaceae that are pollinated by different vectors - wind, birds, rodents, and insects
protea.worldonline.co.za/p12pol.htm

Another site on the Proteaceae
protea.worldonline.co.za/p12pol.htm

Introduction to pollination biology, pollination syndromes. Laboratory instructions for three session investigation and presentations.
www.brynmawr.edu/biology/102_Lab03/PollinBioLabs.doc

koning.ecsu.ctstateu.edu/Plants_Human/pollenadapt.html

www.ecobooks.com/pollinat.htm

www.mobot.org/MOBOT/Research/prosoeca/discussion.html

www.mobot.org/MOBOT/Research/prosoeca/prosoeca.html

www.actahort.org/books/437/437_1.htm

www.nmp.umt.edu/geograph/edlund/g446/meeuse.html

Tools for Assessment of Student Learning Outcomes:

Guidelines for Assessment:

Each student's grade will be based on 15% from the answers to pre-lab questions, 15% for group data collected, 10% for answers to questions for further thought, and 10% for questions and testable hypotheses generated. The other 50% will be from the individual research-style papers submitted (Ten points for each section - Introduction, Methods and Materials, Results, Discussion, and Literature Cited). See "Formal Report Scoring Sheet." A practical exam will test knowledge of flower parts and species of flowering plants and insect visitors.

Formal Report Scoring Sheet:

Section	**Possible Points**	**Points Earned**	**Comments**
Introduction Background Justification Literature used Hypothesis clear	10		
Methods - repeatable, clear	10		
Results			
Tables &/or figures properly drawn & labeled DATA PRESENTED ONCE Pertinent results presented	8		
Narrative - describes trends	1		
No explanation (only in discussion)	1		
Discussion			
Results compared to hypothesis	3		
Explanations, alternative explanations	2		
Literature used correctly	2		
Demonstrated understanding of coevolution	2		
Strong conclusion	1		
Literature Cited			
At least 3 primary sources	6		
Proper format	4		
TOTAL	50		

Tools for Formative Evaluation of this Experiment:

An extensive discussion on Evaluation appears in the Teaching section of the TIEE website: http://tiee.ecoed.net/teach/teach.html.

NOTES TO FACULTY

Comments by Contributing Author - Judy Parrish

Challenges to Anticipate and Solve:

Allergic reactions: Students should be asked about allergies to bee stings and pollen. Those with potential negative reactions should be assigned to tasks that reduce their risks.

Weather: Especially in the spring, there are days when this activity will not work because the insects will not be active if it is raining, too cloudy, and/or too cool (under 10oC). A back-up plan should be in place for an indoor activity that can be alternated with this one. However, it is still possible to do the floral assessments.

Comments On the Lab Description:

Introducing the Lab to Your Students.

I usually begin with at least one 45 minute lecture on pollination ecology and coevolution of mutualism in the classroom portion of my courses. On the day of lab, the approach differs in different classes. For all, we start with careful observation of the flowering species chosen and its visitors. For the freshman biology majors, we then divide into three groups (visitation, phenology, and visitors) to start the natural history observations, to be compiled and followed up on, OR we start with group observations and brainstorming questions. Students then decide on a question to investigate in a group of about 3.

Comments On the Activities in the Lab.

Since temperature, wind, and how sunny the day is all greatly influence insect activity, it is good to have a back-up activity that could be substituted for the pollination observations.

Suggested Back-up Activity:

Willing, R. P. 2000. A Simulated Pollination Exercise. Pages 469-473, *in* Tested studies for laboratory teaching, Volume 21. S. J. Karcher (ed.). *Proceedings of the 21st Workshop/Conference of the Association for Biology Laboratory Education (ABLE*), 509 pages. (www1.union.edu/~willingp/ABLE/POLLEN.html)

Facilitating Development of Student Questions:

Students are sometimes stymied when asked to ask questions (even though they were too good at it when they were five!). Feinsinger et al (1997) suggest the following guidelines for questions:

- The question chosen should be answerable within the time allotted.
- Questions of “How? Which? How many? and Where?” are likely to be answerable. The “Why?” questions may be more natural and more beguiling, but are rarely answerable directly through hands-on investigation. The other questions are likely to generate many “Whys?" for reflection, however!
- Questions must fit the following criteria:
  - Intriguing,
  - Well-defined,
  - Testable,
  - Elements must be measurable and controllable.
- COMPARISON-TYPE questions will be easiest to address, given time and equipment.
- Compare things that common sense and prior knowledge suggest will be different, or where finding no differences should be interesting. Do NOT choose questions that have obvious answers, or you won’t want to waste your time.
- Choose a question that you find somewhat tantalizing - neither too obvious, nor too tedious in methodology.

Hypothesis: Develop an hypothesis - a tentative explanation for what we observe

- Must be TESTABLE and FALSIFIABLE.
- Should be specific - not “temperature will affect visitation rates” but “increased temperatures will increase visitation rates.”
- CAN DISPROVE, BUT CAN NEVER PROVE TRUE!!!! There may be other explanations.

Investigation: Design an experiment to test the hypothesis

- Define variables:
  - Dependent variable - response to treatment,
  - Independent variable - manipulated factor - should be only one in order to isolate effect of variable on response,
  - Controlled variables - all other factors that might influence response should be held constant,
- Outline procedure:
  - Treatment level,
  - Replication,
  - Record carefully steps to be performed.
- Determine controls - independent variable held at an established level, or omitted.
- Predict outcome based on hypothesis - If...Then...

Examples of questions students have investigated:

A. SHORT TERM STUDIES

1. Do small insect visitors visit more flowers on one plant than do larger visitors? If so, what effects would this have on cross pollination?
   TECHNIQUES: “Eyeball ID” of visitors, visitation observation, data analysis.
2. Which visitors are the most “faithful” to the target species (i.e. visit only that species)? What effects would faithfulness, or floral constancy, have on the amount of pollen “wasted?"
   TECHNIQUES: “Eyeball ID,” following visitors, collection of pollen load in staining gel, microscopic analysis of pollen loads, sampling of pollen on the slide, creation of pie-graph of load for each species of visitor.
3. How far between target species plants do the pollinators move for their next visit?
   TECHNIQUES: “Eyeball ID,” following visitors, estimation of distances between target species plants visited, counting numbers of flowers visited on each plant.
4. Where is most of the pollen carried on the insect visitors?
   TECHNIQUES: Collection and preserving of insects, careful swabbing of body parts with staining gel, sampling of pollen on the slide,
5. Do flowers in large patches attract more visitors than isolated flowers or flowers in small patches?
   TECHNIQUES: Mark and observe flowers in patches of different sizes, “Eyeball ID,” visitation observations,
6. Do flowers with larger petals attract more visitors than flowers with smaller petals?
   TECHNIQUES: Clip petals to reduce size (nail clippers) of experimental

group, and observe flowers of different sizes, “Eyeball ID,” visitation observations.

7. Do flowers near an artificial display of similar flowers attract more visitors than more isolated flowers?
TECHNIQUES: Set up control and experimental plots with and without artificial flowers of similar size, color, and shape as target species, “Eyeball ID,” visitation observations.

B. EXTENDED STUDIES

1. Do flowers that have not been visited last longer than visited (and possibly pollinated) flowers?
TECHNIQUES: Preparation of exclosure to prevent visitation, hand pollination, phenology observations.
2. Do flowers in large populations produce more seeds than more isolated flowers?
TECHNIQUES: Locate sites with high and low populations of target species, collect seed, calculate percentage of seed set,
3. Are flowers more likely to be visited at certain times of day?
TECHNIQUES: Visitation observations throughout time flowers are open, compilation of class data for diurnal (daily) flowering and visitation profiles.
4. What is the relationship between size of active pollinators and ambient temperature?
TECHNIQUES: Sorting visitors into 3 size classes, observe flowers on several different days at about the same time, measurement of air temperature, visitation observation, compilation of class data.
5. What is the relationship between size of active pollinators and ambient temperature?
TECHNIQUES: Sorting visitors into 3 size classes, observe flowers on several different days at about the same time, measurement of air temperature, visitation observation, compilation of class data.
6. What is the relationship between the relative time of flowering of an individual in the population (early, middle, or late), probability of visitation, and seed set?
TECHNIQUES: Mark flowers open at 3 different times within the flowering period for that target species, visitation observations, seed collection, calculation of seed set, compilation of class data.
7. Do pollen grains from different individuals germinate and grow at a faster rate than self-pollen in self-compatible species? Does temperature affect rates of germination and/or growth of pollen?
TECHNIQUES: Hand pollination, stigma dissection and staining with basic fuchsin gel, microscopic examination of style.

Comments On the Uses of This Lab Activity in Different Courses at Millikin University.

This lab activity is used in four different courses at Millikin University. In a spring semester non-majors course, Local Flora, with 18 students, we do one two-hour lab at a park across the street when spring beauties are in flower following a two hour lecture and discussion on pollination, including showing David Attenborough's "Birds and Bees" video from the Private Life of Plants series. Pairs of students spend ten minutes watching a patch of flowers, and then brainstorm questions. We get back together as a group and share "favorite" questions, then choose one or two to work on as a class, form a testable hypothesis, and plan an investigation to test it. The question chosen usually deals with which flowers are more likely to be visited, comparing color, patch size, patch position in sun or shade, or flower height. The brainstorming and planning take about 30 minutes, and the investigation takes another 30 minutes. Each pair of students can get in two ten-minute observation periods, which results in a sample size large enough for statistical comparisons. We compile the data, and then students write a scientific report on the class-generated question and experiment.

The lab activity is used differently in our spring freshman biology major's course, Attributes of Life, with five lab sections, each with 16 students. The lab is preceded by one or two lectures on coevolution of plants and pollinators and a pre-lab assignment. On lab day, in each lab, students are divided into three groups to examine the natural history of pollination of spring beauty, crabapple, or buckeye flowers. One group assesses flowering phenology, one group estimates visitation rates, and one group examines visitors for pollen, all using the sample experiments included with this module. Data are compiled from all five labs and posted for all to use. Some years students are asked to write a paper estimating probability of visitation of a flower by the visitors most likely to be effective (based on pollen load composition). Data from all three groups are necessary to know how long the flower is open, which visitors are most frequent, and which are likely to carry pollen. Students are required to come up with a list of questions generated by their observations and those of the class as part of their discussion sections. In some years, a second lab period is devoted to designing and carrying out an investigation of a question generated by the student, and then the write-up is of the student-generated project rather than about the class data. When we have a data from two or more years, such as for spring beauty and buckeye flowers, we plan to present the natural history information in an introductory lecture/discussion on the site rather than spending the two-hour lab collecting more of that data. We can then start the brainstorming and student-generated hypothesis testing.

In our Field Ecology course, which is a summer immersion experience at Lake Shelbyville, Illinois, for 10-12 non-majors, we include the lab activity much like it is used in Local Flora. However, students have a full day to complete the investigations of questions they generate, and can further develop hypotheses in a two-day, individual follow-up project.

In Plant Biology, a fall course for 12-16 junior and senior undergraduate students, students work in pairs on a prairie species. One three-hour lab is devoted to observing visitation, examining flowers, acquiring pollen samples, generating questions, developing hypotheses, and designing an experiment to test the hypothesis chosen. Projects are discussed and approved, and investigations are undertaken outside of class. A formal scientific report is required.

Comments On Questions for Further Thought:

Students often mix seed dispersal and pollen dispersal - for example, when I ask them to bring me a flower with wind dispersed pollen, nearly all bring me a dandelion. It is important to stress the timing and source of pollen versus seeds.

Students also often have difficulty with mathematical manipulations - even with figuring the number of visits per flower observed during a ten-minute period. It is often better to lead them through the development of a formula to figure rates than to present them with one (as in the example in section 6C).

The last several questions for further thought, and the questions generated by students, make good group discussion material.

Comments On the Assessment of Student Learning Outcomes:

Extensive notes on course assessment are in the Teaching Resources sector of TIEE under the keyword "Assessment"

Comments On the Evaluation of the Lab Activity:

Extensive notes on how to conduct formative evaluation are in the Teaching Resources sector of TIEE under the keyword "Formative Evaluation" and in an Essay on Evaluation of Course Reforms

Comments On Translating the Activity to Other Institutional Scales:

Since transportation is not necessary, this outdoor activity can be adapted for much larger groups on large campuses. Landscape beds can be used, or trees with large, attractive flowers, like redbud, crabapple, hawthorn, and buckeye.

This activity can be useful for non-majors in local flora and field ecology immersion classes, as well as junior and senior biology majors in upper level Plant Biology. Also adaptable for younger students, with more emphasis on observations and not pollen removal from insects.

Different approaches have been effective, including:

- Natural history observations as major part of lab, generating list of questions.
- Natural history observations as one lab, with spin-off projects to address questions raised in follow-up lab.
- Natural history information given about system chosen, with student-generated questions addressed in experiments designed and carried out in lab.

ACKNOWLEDGMENTS:

The major part of the idea for this lab activity was inspired by a workshop at the ESA meetings in Albuquerque, New Mexico by Alan Berkowitz and Kathleen Hogan, 1997 on Schoolyard Ecology for Elementary School Teachers (SYEFEST), and by Peter Feinsinger's work with Berkowitz, Margutti, Grajal, and Oviedo. Much of the design of the activity was done as a module that was a part of a National Science Foundation grant (DUE #9653676) to Millikin University, "Creating Linkages Through Institution Wide Reform of the Science Curriculum." Dean Mauri Ditzler and Clarence Josephson, of the Millikin Chemistry Department, and Marianne Robertson of Biology, encouraged the development of the module. This submission has benefited from comments by TIEE Editors and an anonymous reviewer.

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