

## Chapter 12

### Why are reports of ant pollination rare? A field and lab exercise using the scientific method

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## Introduction

This laboratory exercise addresses questions concerning the conditions necessary for an animal to be a successful pollinator of a plant. Students are presented with the following fact: Ants are among the most abundant insects on earth, yet less than 20 well-documented cases of ant pollination exist among the many types of insect pollination (Peakall, et al., 1991). First, we ask students to generate hypotheses of what makes any animal (ant, bee, or other) a good pollinator and then we ask students to generate hypotheses on why there are few reports of ant pollinators. These hypotheses can be lumped into two categories: those that relate to the quantity of visits an animal makes to flowers and those hypotheses that relate to the pollination quality of each individual visit to a flower by an insect (e.g., how much pollen is deposited and is it all intraspecific pollen?). Field work consists of gathering data to address both quantity and quality questions, such as whether ants visit flowers at all, whether and how far they move among flowers while foraging, and if they appear to contact reproductive parts of flowers. In the laboratory, we test two hypotheses regarding the pollination quality of an ant visit. Students are told of the hypothesis proposed by Beattie et al. (1984) that ants have glands on their bodies that secrete antibiotic substances that reduce pollen viability. Using cell-culture plates, students set up an experiment to test if pollen grains that have contacted ants have a lower germination rate over time than pollen that has not contacted ants. Pollen is germinated in a simple pollen germination media (Scott, 1995), and students measure germination rates and observe the growth of pollen tubes. Ants used in the previous experiment as well as any ants collected outside off of flowers are squashed in a simple basic fuchsin gel on microscope slides. The gel, designed by Beattie (1971), selectively stains the pollen and allows students to see whether pollen is sticking to the bodies of ants (and to see whether it is true that ants' bodies don't have any hairs as students may suggest!). This exercise is a particularly good way to get students to generate hypotheses, become familiar with plant reproduction, and cleverly design ways to test their hypotheses. Furthermore, students should be able to apply their thoughts on ants to other insects, and to consider what conditions are necessary for mutualism to exist. Hopefully, students will walk away with an appreciation of the fact that mutualisms depend both on the magnitude of effect of an individual of one species on another as well as the abundance of interspecific individuals interacting (e.g., ants may have a small effect but can be pollinators when they are in high abundance). Although this laboratory is best when the field component is possible, the indoor component to this lab is designed to make the exercise successful even if it is raining.

### Overview of Ant Pollination

Ants are generally regarded as unlikely pollinators (Holldobler and Wilson, 1990). They are small unspecialized insects whose movements are usually restricted to short inter-plant distances.

## Ant Pollination

Moreover, secretions from glands on ant bodies (e.g., the metapleurial gland) of some ant species generally reduce pollen viability (Beattie et al., 1985). Nonetheless, pollination by ants has been documented for a small number of plant species (Table 12.1), and Hickman (1974) proposed that the low energetic or water costs of attracting ants to flowers might select for ant-pollination in dry or hot habitats. Recently, studies in the Mediterranean mountains and arid lands of Spain and France have suggested that flower-visiting ants are relatively more common in this region and act as important pollinators for plant species visited at high frequencies (Gómez and Zamora, 1992; Gómez et al., 1996).

Numerous hypotheses have been proposed to explain the global rarity of ant pollination, but no general conclusions have yet been reached. Several non-exclusive explanations all concern the low rate of cross-pollen transfer by ants. The walking (rather than flying) foraging behavior of ants and the tendency of ants to consistently return to the same food source may prevent movement of pollen from one plant to another. Yet another hypothesis is that pollen does not adhere as well to the surfaces of ant bodies as to the bodies of other insects. These various hypotheses will be explored in this laboratory exercise (See Appendix C for a summary of hypotheses).

**Table 12.1.** Some ant-pollinated species (not a complete list!).

Species	Common Name	Location Studied	Source
<i>Alyssum purpureum</i>	none given	Mediterranean	Gómez et al., 1996
<i>Arenaria tetraquetra</i>	none given	Mediterranean	same
<i>Diamorpha smallii</i>	Small's stonecrop	North & South Carolina	Wyatt and Stoneburner, 1981
<i>Hormathophylla spinosa</i>	none given	Mediterranean	Gómez and Zamora, 1992
<i>Paronychia pulvinata</i>	alpine nailwort	alpine Colorado	Puterbaugh, 1998
<i>Polygonum cascadenense</i>	Cascade knotweed	Western Cascades of Oregon	Hickman, 1974
<i>Retama sphaerocarpa</i>	none given	Mediterranean	Gómez et al., 1996
<i>Sedum anglicum</i>	none given	Mediterranean	same

## Materials

### Overview of all materials

#### *Items for catching & keeping ants*

- Jar to keep ants in
- Flat dish (such as pie plate) for students to sort through dirt to find ants
- Microfuge tubes (any size ok, 1 mL good) where students to put the ants they catch
- A vacuum collector to catch ants (optional; directions in Appendix)

#### *Items to enable analysis of pollen*

- One dissecting and one compound microscope per pair of students
- One cell culture plate per pair of students (preferably with 48 wells but can be more - no need to be sterile. I wash and use these over and over.)
- One pair of forceps per student or per pair of students
- Microscope slides
- Coverslips (preferably glass)
- Wooden clothes pins (to allow students to hold slides over flame without burning themselves)
- 70% ethanol to rinse pollen off of forceps (handy to have in squirt bottle)
- Alcohol lamps containing alcohol (1 per every 5-10 students is good)
- Tally counters for keeping track of the number of pollen grains
- A piece of graph paper with 1 grid line per mm. Photocopies of this graph paper for students (cut these to a size that can go under the cell culture plate while it is under the microscope); graph paper can be photocopied onto transparent overhead projection film so that transmitted light may be used to observe pollen grains.

#### *Items for 1000 mL pollen germination media (from Scott, 1995)*

- Latex/vinyl gloves for students to wear
- Protective goggles for students to wear
- Volumetric flask (1 L) or other container to hold pollen germination media (or smaller if you make less)
- Sucrose (100 g; table sugar from the grocery store)
- Boric acid,  $H_3BO_3$  (0.1 g)
- Calcium nitrate,  $Ca(NO_3)_2 \cdot 4H_2O$  (0.3 g)
- Distilled water (1 L or so)

#### *Items for 1 Petri plate of basic fuchsin gel (probably more than you'll need) (modified from Beattie, 1971; also see Kearnes and Inouye, 1993)*

- Latex/vinyl gloves to wear (just for the one or few individuals making the gel)
- Protective goggles to wear (just for the one or few individuals making the gel)
- Glass Pyrex or Kimax covered Petri dish
- Hot plate
- One packet of Knox gelatin (approximately 7 g/packet)
- Distilled water (at least 25 mL)

## Ant Pollination

- Glycerin (glycerol) (at least 35 mL)
- crystalline basic fuchsin stain (pararosanilin)/color of claret (less than 0.1 g)
- Optional: 1 g crystalline phenol may be added as preservative, we omit this

### *Items for field work*

- Lab notebooks/clipboards
- Stop watch or watch with a second hand
- Fluorescent dusts for marking ants; two companies that make vast quantities of there are:
- Radiant Color Company ([www.radiantcolor.com](http://www.radiantcolor.com)) - Series R-103-G
- Day-Glo Color Company ([www.dayglo.com](http://www.dayglo.com)) - product Arc Yellow (orange) A-16 (these companies will sometimes provide free samples)
- Toothpicks for applying dust to ants
- Optional: Refractometer for measuring sugar concentration of nectars

## Notes for the Instructor

### Break Students into Groups

Other ways exist to organize this laboratory, but we break students up into groups and assign them one or a few hypotheses to try to address. We try not to tell students how to address the hypotheses, and leave it up to the students to think creatively. However, here are a few examples for the benefit of the instructor. For example, we might have one group focus on the hypothesis that pollen does not attach to the body of ants and that ants groom off pollen. We might have a second group focus on whether ants visit flowers in the first place and whether they are too small to contact the reproductive parts of plants, and a third group focus on whether the foraging behavior of ants (in particular how quickly and distantly they move) might preclude them from cross-pollinating.

For the size question, the first group may measure ants and measure flower morphology to see whether an ant can contact both stigma and anther simultaneously (of course even if ants can not, they could still pollinate.) To address the question of whether pollen sticks to ants, students can take anthers and dust ants with them and then bring them into the lab to determine if any pollen stuck to the ant. By examining a dusted ant over time, they can test whether ants groom off the pollen. The third group can measure distances that they see ants move, and how fast they see ants move. They can measure the distances between flowers to see if the distances ants move are further or shorter than the distances between most flowers. The third group can also see whether ants tend to go from a single food source to their nest and back, and if the ants appear not to go from food source to food source as might be necessary for cross-pollination. Hopefully, your students will come up with even better and more creative ideas that those suggested here! Note: *Data can be gathered even if students do not find any ants on flowers!*

### Avoid Fire Ants

FOR SAFETY REASONS, FIRE ANTS SHOULD BE AVOIDED BY STUDENTS. Fire ants refer to several species of highly aggressive ants that were introduced to the United States. Fire ants have a painful sting, and a small number of people are highly allergic to their venom. Worker fire ants range in size from 1.5 to 4 mm and are reddish brown with a darker abdomen, and nest in mounds. The range of the ants is primarily in the southern US including the states of Texas, Florida,

Oklahoma, Arkansas, Georgia, Alabama, Mississippi, South and North Carolina, Tennessee, California, Louisiana, New Mexico, and Nevada. Some of these states have their own web sites on the fire ant problem. More general web sites with information on and pictures of fire ants are at the following addresses (these sites will also link you with other sites):

<http://www.ceris.purdue.edu/napis/pests/ifa/index.html#sites>  
<http://fireant.tamu.edu/index.html>

### **Collecting Ants in Case of Bad Weather**

Field exercises on insects are great, but bad weather can cause pollinators to become inactive. We suggest that a couple of days before the laboratory and when the weather is good, go out with a spade and collect some "emergency" ants. We suggest NOT using these ants unless the weather is such on the date of the laboratory exercise that the students are unable to get their own ants. To collect ants: dig up some ants with their dirt (you don't need a queen so this should be an easy task), and stick them in a jar with holes in the lid (so the ants get some air and will still be alive the day you do the exercise - but the holes need to be smaller than the ants or they will escape!). Leave at least a couple inches between the jar lid and the dirt level (you can leave more room). You'll need to get enough ants for each pair of students to have about three or four ants each. You can use *any* type of ant you find; though it seems most relevant to collect flower-visiting ants.

### **Pollen Germination Media**

Make a fresh batch of the pollen germination media given by Rodney Scott, 1995, in the Proceedings 16<sup>th</sup> Workshop/Conference of the Association for Biology Laboratory Education (ABLE). The recipe is reprinted in Appendix B.

### **Pollen Staining Gel**

The pollen staining gel needs to be made at least several hours before lab, and can be made several weeks sooner. One batch can last many years, depending on how many students need to use it. We make this ourselves and do NOT ask students to make it. The recipe is in the Appendix. Students should wear gloves when handling the gel (and so should you).

### **Data on Other Insects**

This laboratory exercise could be expanded to include other insects. Some data on other insects can be found in Herrera (1987).

## Ant Pollination

### Ant Vacuum

Students may find it hard to collect ants. The instructions for creating an ant vacuum are included in the Appendix (Figure 12.2).

### Ant Diagram

A diagram of an ant indicating the approximate location of the metapleurial gland is found in Appendix A.

## Student Outline

If you sit and watch a single patch of flowers for an extended period of time, you will notice that flowers of one plant species often get visited by a diverse array of insects. Flowers of one plant species may be visited by more than one species of bee or may even be visited by insects of more distantly related taxa: beetles, flies, and bees. One question that interests pollination biologists is whether all those insects that visit flowers are really pollinators. Other intriguing questions are, "What features of an insect make it more likely to be a pollinator?" and "What characteristics make a pollinator most effective at pollinating?" Because many of our native and endangered plant species depend on insect pollination to reproduce, these questions are important to conservation biologists. Farmers are interested in these questions because some of our crops are insect pollinated (radishes, pumpkins, fruits trees, etc.). These questions fascinate evolutionary biologists because there exist over 200,000 flowering plant species that appear to have arisen in the last 2 million years, and animal-mediated pollination may have played a role in speciation.

Today, we will consider these questions in the context of one particular plant-animal interaction: ants that visit flowers, and flowers that are visited by ants. Two eminent ant biologists: Bert Hölldobler and E. O. Wilson (1990) have written that, "about one-third of the entire animal biomass of the Amazonian *terra firme* rain forest is composed of ants and termites, with each hectare of soil containing in excess of 8 million ants and 1 million termites . . . Although comparable biomass measurements have not yet been made elsewhere, it is our subjective impression that the eusocial insects, ants foremost among them, are comparably abundant in most other principal habitats around the world." Even though ants are so abundant, fewer than 20 plant species have been conclusively documented by biologists as ant-pollinated. In contrast, many thousands of plant species are pollinated by other insects such as bees and wasps and flies. Why do you suppose that ant pollination is so scarce? Numerous hypotheses have been proposed to explain the global rarity of ant pollination, but no general conclusions have yet been reached.

One hypothesis that was proposed by several Australian researchers is that ants are rarely pollinators because they secrete substances from their bodies that kill the pollen (Beattie et al., 1985, 1984). One explanation for why ants secrete such substances is that ants live close to soil and may be exposed to a lot of bacteria and fungi; glandular secretions may inhibit unhealthy growth of bacteria or fungi on the body of the ant. One way to test the hypothesis that the ants secrete pollen-killing substances is to compare pollen that has not been exposed to ants with pollen that has been exposed to ants. If the hypothesis is valid, pollen exposed to ants should have a lower germination rate than pollen that is not exposed to ants.

The purpose of today's lab is to test the hypothesis that ant secretions kill pollen. Additionally, you are expected to come up with other plausible hypotheses as to why ant pollination is scarce. You will be given an opportunity to creatively design experiments and collect data to evaluate your hypotheses. As you work through this laboratory exercise, notice that we are focussing on a specific question, "Why is ant pollination rare?" but keep in mind that our question falls into a much bigger context, "What characteristics have been important in the evolution of insect pollination?" and "What characteristics of behavior or morphology influence the effectiveness of a pollinator?" We will not answer these two bigger questions today, but we will contribute to a growing body of data gathered by many researchers throughout the world that collectively help to give insight into these larger questions. Scientists frequently use this approach to focus their research: find a small question that is one that can feasibly be investigated, but choose a question that will help in a small way to answer a larger, more important question.

### Questions

*Please answer following questions 1-5 before coming to lab:*

1. What conditions are necessary for an animal to pollinate a plant?
2. Is every insect or animal that visits a flower necessarily a pollinator? Explain.
3. Hölldobler and Wilson have written that "about one-third of the entire animal biomass of the Amazonian *terra firme* rain forest is composed of ants and termites, with each hectare of soil containing in excess of 8 million ants and 1 million termites... Although comparable biomass measurements have not yet been made elsewhere, it is our subjective impression that the eusocial insects, ants foremost among them, are comparably abundant in most other principal habitats around the world." Even though ants are so abundant, fewer than 20 plant species have been conclusively documented by biologists as ant pollinated! Yet many thousands of plant species are pollinated by other insects such as bees and wasps and flies. Why do you suppose that ant pollination is so scarce? Why aren't there more ant-pollinated plant species? List as many hypotheses as you can for why ant pollination is rare.
  - A. *Ants secrete antibiotic substances from their bodies. These secretions kill the pollen that comes into contact with the ants and result in ants being less likely to be pollinators than other insects that do not secrete such substances.*
  - B.
  - C.
  - D.
  - E.

Feel free to add more!
4. Are any pair of the hypotheses you listed above mutually exclusive? [In other words, if one of the above hypothesis is correct does that mean that another hypothesis must be incorrect? If two hypotheses can both be correct simultaneously then they are not mutually exclusive.]
5. Organize your hypotheses by type:

## Ant Pollination

Which hypotheses relate to the BEHAVIOR of ants?

Which hypotheses relate to the MORPHOLOGY of ants?

Which hypotheses relate to factors OTHER than behavior or morphology?

6. For the first half of the laboratory period, your task is to work with your student group to use your creativity and intelligence to collect data that will evaluate the hypotheses you have been assigned. [In other words, there are no directions on how to do this, you must figure it out yourself.] Below is a list of equipment that you have available to you to help you address your hypotheses. For the second half of the laboratory period, you should pair up with one other student to follow the directions provided on how to test the hypothesis that ants secrete pollen-killing substances.

### Equipment Available to You:

*Ant vacuum:* The ant vacuum will help you collect ants from outside.

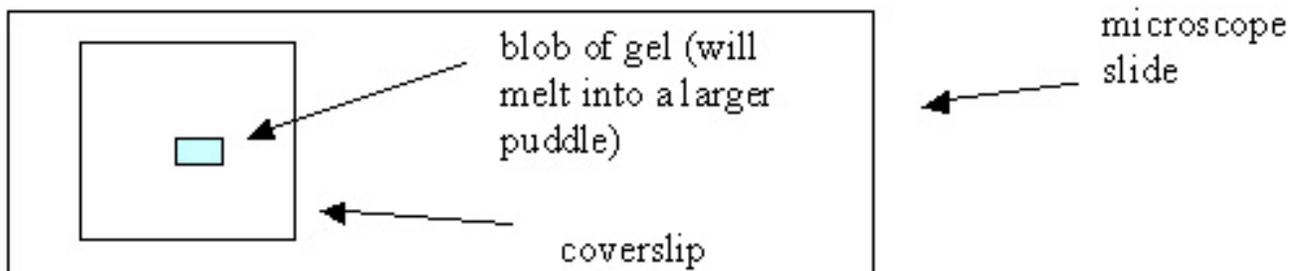
*Notepads and clipboards:* One of the most important parts of designing a field experiment is to set up a consistent data sheet that will ensure that if more than one person works to address the same hypothesis, you will collect similar data. A sample data sheet is provided in the materials section, but you are encouraged to design your own data sheets.

*Ethanol in squirt bottle:* For rinsing forceps, etc. to clean off any pollen.

*Fluorescent powder:* With a toothpick, you may be able to mark ants. To do this, pick up a small amount powder with the toothpick and gently dab a foraging ant on the rear end. Be careful, if you touch the ant too hard, she may run away. Catching the ant first with the vacuum, marking and releasing it, is generally not advisable since the ant often will not return to foraging.

*Basic fuchsin gel:* Whole ants that have been caught can be squashed in this gel on a microscope slide. The gel makes it easier to find pollen because the pollen stains red. You can also take a small bit of unmelted gel, dab it against an insect, and then melt and examine the gel alone (without the insect) to see if contains pollen.

To use the gel, remove a small blob of basic fuchsin gel and place it on a glass microscope slide. By a small amount, we mean an amount that is the size of the square below (in area) and a few millimeters thick (Figure 12.1).



**Figure 12.1.** Diagram of microscope slide with basic fuchsin gel.

Holding one end of a slide with a clothespin, gently move the other end back and forth over the flame of an alcohol lamp. Do not put the slide near the wick of the lamp, but hold it a centimeter

or more above the wick. After moving the slide over the flame a few times, you may want to hold it away from the flame and watch as the heat transfers from below the slide to the top of the slide and the gel begins to melt. Try not to boil the gel. Once the gel becomes a small melted puddle, grasp an ant and place it in the gel and relatively quickly, add the coverslip to the slide. The larger the ant, the more difficult it is to lay the coverslip flat. You may wish to break the ant up into different parts and stain each separately. In addition to the method just described, the blob of gel can be rubbed against an insect and then examined for pollen. However, be sure that the gel is really pollen free before touching an insect (what control could you use to show this?).

*Refractometer:* (may not be available) The amount of sugar in a solution (such as nectar) can be determined with this instrument.

*Diagram of ant*

*Stop watch*

*Your eyes and ears:* Information can be gathered just by watching and paying attention, but design your data sheets well so that you are sure to know what to look for and to keep good records of what you see.

### **Directions for testing hypothesis #1:**

*Antibiotic secretions from the bodies of ants reduce the viability of pollen carried by ants.*

Every student should attempt to test this hypothesis. The data will be summarized at the end of the lab.

1. Ideally, collect whole anthers of Petunia, Jewel Weed, cultivated *Impatiens*, or other plants from the field or greenhouse (or from plants that are dug up and brought in the night before).
2. Put at least one anther in each of two to four wells in a cell culture plate. For each pair of wells used, one of the wells should be randomly chosen to receive an ant.
3. Gently drop a live ant into the well designated to receive the ant and leave it there for 5-10 minutes. Gently picking the ant up with your fingers is one way to move a live ant. If it is possible to get the ant into the well without touching it, that is even better! Once the live ant is in the well, cover the well to prevent the ant from escaping. Cover the ant-free well as a control.
4. After 5-10 minutes has past (record the time), remove the ant from the well. This ant can also be used to answer the question of whether pollen sticks to the bodies of ants (hence data can also be gathered to address other hypotheses if it is raining).
5. Add about 0.3 mL of pollination media to the control and ant-treated wells.
6. Immediately after adding the pollen germination media, look at the wells under the dissecting scope to determine whether any grains already appear to have germinated (They should not have germinated yet, but you need this baseline to show that germination occurred.). A piece of graph paper can be put below the cell culture plate, and you can count the grains within a given area. If the graph paper doesn't work, try to count 50 grains and keep track of how many are germinated. A tally counter may help. Count the total number of grains (germinated and ungerminated) in a given area and the total number of germinated grains in the same area. Dividing the latter by the former will give an estimate of percent germination.

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7. Repeat the procedure above every 10-20 minutes for a total of about 3-4 readings. The timing between readings will depend on the species of pollen you are using. Petunia pollen grows more slowly than does pollen from Jewel Weed. Record your readings in Tables 2 and 3 below.
8. Optional: A chi-square test can be used to compare exposed and unexposed pollen germination statistically.

**Table 12.2.** Laboratory observations on pollen germination.

Cell culture well:	Time 0 (0 minutes)		Time 1 (___ minutes)		Time 2 (___ minutes)	
	Total Grains	Germinated Grains	Total Grains	Germinated Grains	Total Grains	Germinated Grains
Without an ant (control)						
With an ant (treatment)						

Total Grains = germinated + ungerminated grains

**Table 12.3.** Summary of the reading taken at Time 2.

Cell culture well:	Proportion Germinated = Germinated Grains / Total Grains
Without an ant (control)	
With an ant (treatment)	

Keep this data to share with the rest of the class.

Note: Beattie et al. (1984) suggested that the metaplural gland is particularly responsible for secreting substances that reduce pollen viability. Not every ant species has functional metaplural glands. Determining whether the ant species you are using has or does not have functional metaplural glands is unfortunately beyond the scope of this lab. If you find no difference between the pollen that contacted ants versus the pollen that did not contact ants, consider using this as a topic of discussion.

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## Appendix A Data Sheets

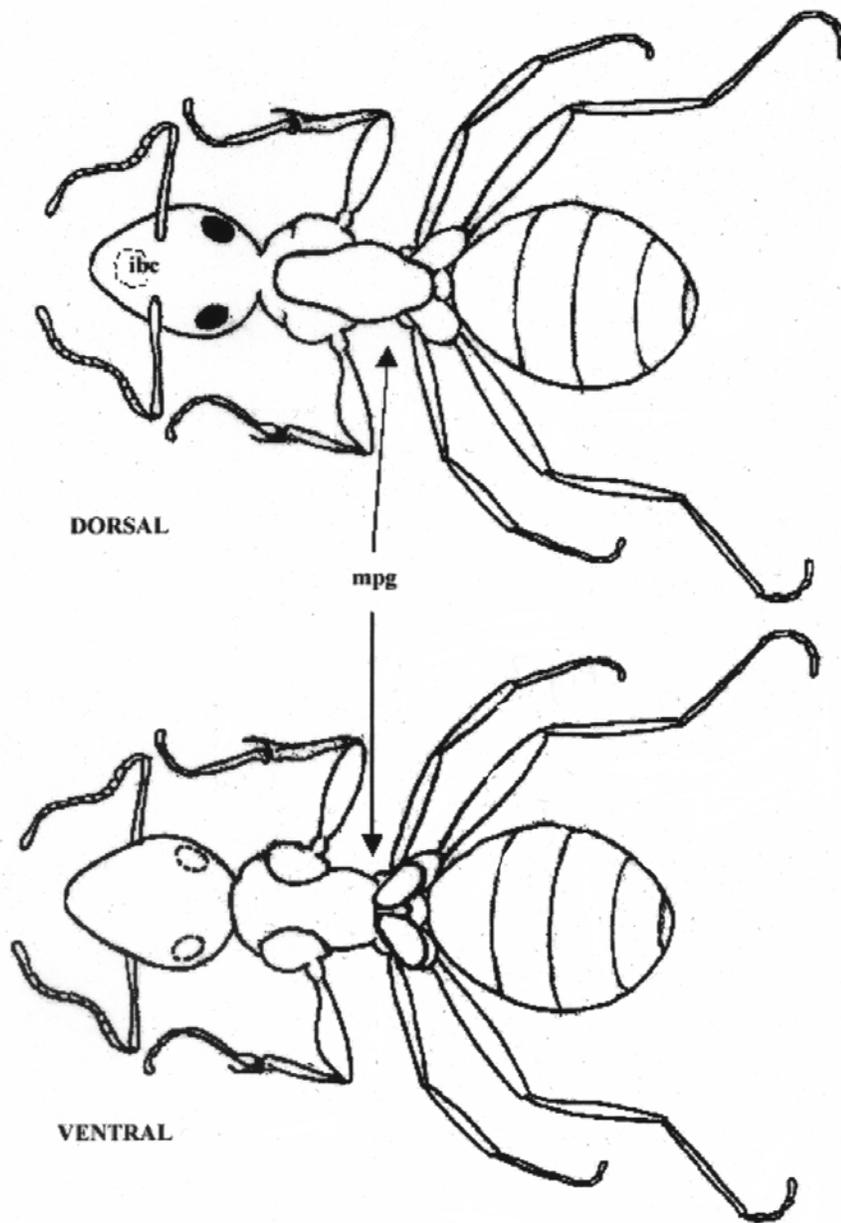
### *Sample Data Sheet*

Field Observations

Total Number of Ants Found on Flowers: \_\_\_\_\_

Total Number of Species of Plants with Ants on Flowers: \_\_\_\_\_

ANT	DESCRIPTION OF PLANT	TIME SPENT WATCHING ANT (MINUTES)	NOTES ON ANT BEHAVIOR <b>Did ant move among flowers? What did ant appear to be doing? Maximum distance moved between flowers?</b>
example: #1	orange-flowered jewel weed	5 minutes	stayed in flower entire time; appears to be collecting nectar
example: #2	yellow composite	10 minutes	stayed on flower for 3 min. - crawled down stem and didn't return to a flower
etc.			



**Figure 12.2.** Diagram of ant. You might consider indicating where on the ant you have observed pollen grains. You may notice an abundance of pollen in the infrabuccal chamber (*ibc*). Take note of how close the pollen you observe on the ant is to the approximate location of the metaplural gland (*mpg*). The closer to the gland, the more likely the pollen will contact the secretions.

## Appendix B Recipes

### Recipes

#### *Pollen Germination Media*

100 g of sucrose, 0.1 g of  $\text{H}_3\text{BO}_3$ , and 0.3 g of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  made to 1,000 mL with distilled water (weigh carefully)

We use plain old table sugar for the sucrose. Rather than putting a preservative into the media, we have the students make it fresh the day of the lab (or we make it ourselves the day before) and dispose of left-overs. The media can be kept in the refrigerator for short periods of time (a week). However, it is a tasty media and well-liked by microorganisms so it will go bad quickly if you don't add a preservative or don't use it quickly. I do not filter the media (I just shake well!). If you have students make the media themselves, ask them to think about what the sugar is for and why it is so important to weigh carefully. [...takes energy for little pollen grains to grow tubes many times their size and the osmotic pressure of the solution needs to be right or the pollen grains will burst or shrink...]

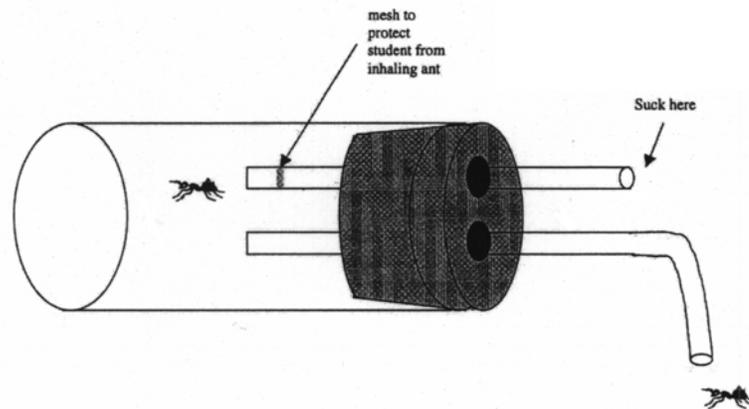
#### *Pollen Staining Gel*

The pollen staining gel needs to be made several hours before lab. One batch can last many years, depending on how many students need to use it. We make this ourselves and do NOT ask students to make it. The recipe is in the Appendix. Students should wear gloves when handling the gel (and so should you).

Sprinkle one packet of Knox gelatin onto the surface of 25 mL distilled water in a glass Pyrex or Kimax Petri dish (or in a 600 mL beaker). Allow gelatin to absorb water (~ 1 min.). Place the covered dish on a hot plate at a setting of 4 (~ 160°C) just until mixture clears. Carefully remove the dish from the hot plate and gently stir in 22 mL glycerin and 0.005-0.01 g crystalline basic fuchsin (also 0.1g phenol if desired). Beattie (1971) suggests the standard gel be the color of claret. Re-cover the dish and allow the gel to cool at room temperature until the gel is solidified. Seal the dish with Parafilm, invert the dish, and refrigerate it for long-term storage (until it molds).

#### *Ant Vacuum*

An ant collector can be made with a vial and a two-holed stopper; two tubes are inserted into the stopper (Figure 12.3). A little cloth mesh is stuck on to the vial end of one of the glass tubes; students point the other glass tube at an ant and suck on the mesh-containing tube.



**Figure 12.3.** Ant vacuum.

### Appendix C Summary of Hypotheses

Some additional hypotheses that students may come up with for "why reports of ant pollination are rare:"

1. Researchers have not looked hard enough for ant pollination, and it is actually more common.
2. Ants do not visit flowers often enough.
3. Ants do not move sufficient distances quickly enough to cross pollinate flowers.
4. Ants are too small and do not contact the reproductive parts of most flowers.
5. Ants do not seek nectar but instead are chewing insects that destroy rather than pollinate flowers when they do visit flowers.
6. Ant bodies are smooth and pollen does not stick to the body of ants.
7. Ants groom very frequently so any pollen is removed from ants' bodies by their grooming behavior.
8. The foraging pattern of ants is such that they usually go from one flower to their nest and rarely from one flower to another, reducing the likelihood of cross-pollination.