

INSECT IDENTIFICATION LAB

ACTIVITY AT A GLANCE

Goal: This lecture and lab introduce the students to the spectacular diversity and abundance of insects. The students are introduced to field work, insect collection and classification.

Learning Objectives: Upon completion of this activity, students will conduct field work to collect insects from local fauna, appreciate the ubiquity of symbiotic microbes in animals, understand how to use a taxonomic key to identify insects to Order, sort insects into “morphospecies” – similar looking species-, and prepare lab notes and specimens for molecular studies.

Assessed Outcomes:

- Ability to collect, organize and identify insects
- Knowledge of insect morphology
- Understanding of the relationship between *Wolbachia* and insects
- Ability with microscopes and lab materials

Teaching Time: 60-90 minutes (2 class periods)

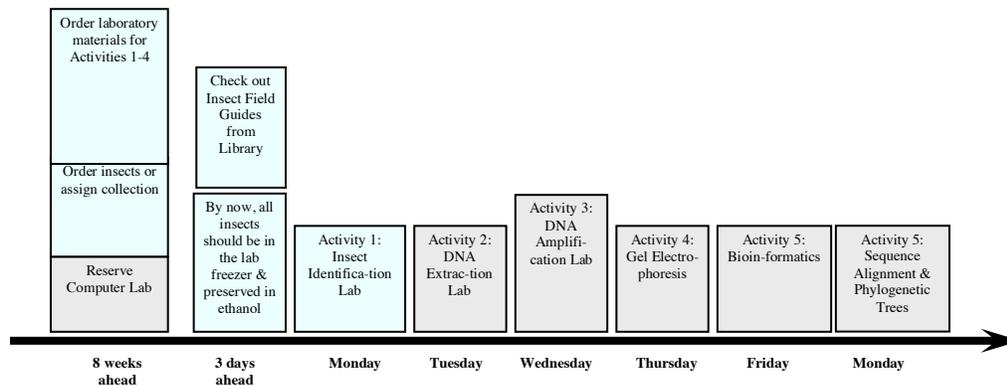
National Science Education Standards Addressed:

Science as Inquiry – Students develop scientific inquiry skills in posing scientific questions and predictions, taking ownership of their research, and using varied tools and evidence to characterize the insect samples that they chose to study.

Science and Technology – The students integrate field work with taxonomic classification in the lab to determine insect Order using a computerized key

Life Science – In this activity, students use field work, classification, and basic content on symbiosis to learn about evolution and ecology of insects, the interdependence of microbes and animals, and the organization of living systems including insect body structure, life cycle, and associations with microbes.

Timeline for Teaching *Discover the Microbes Within: The Wolbachia Project*



OVERVIEW

Insects are among the most diverse and abundant animals on earth. They represent 85% of all described animal species and there are an estimated 1,000,000,000,000,000,000 (10^{18}) insects alive on the planet any given day. If the services insects provide everyday (for free) disappeared suddenly, humans would soon disappear. Insects clean water, pollinate flowers that produce about 1/3 of the food we eat, breakdown waste and decompose plants and animals. Given their importance, why are insects so little appreciated and poorly understood? Probably the single largest factor is their small size. Most of the 1 million described species are less than 1/3" long. This entire world of small creatures exists literally under your feet.

In this activity, students will embark on an expedition to collect their own insects from the local insect fauna. You and the students determine where to collect and can discuss an interesting sampling scheme for determining which insect may or may not harbor symbiotic microbes. It could be the home, park, or habitats that differ in temperature, location, etc. Upon preservation in ethanol, students will sort the collections into *morphospecies*, (individuals that have similar morphology and are probably members of the same species), identify the common morphospecies to taxonomic order, and prepare vouchers and specimens that will be analyzed for microorganisms. Vouchers are “copies” of the insects the students will later test for microorganisms. These voucher specimens represent the only way the insect hosts can be linked to the bacteria that inhabit them. In addition, vouchers enable us to further explore new questions, such as:

- Why do species in some insect groups have few or no microorganisms associated with them, while insect species in other groups have microorganisms in every species so far examined?
- Is this pattern due to differences in their ecology, or is it that microorganisms successfully infected some hosts early in their evolutionary histories and not others?
- Suppose you find that a collection of ants nesting in soil have microorganisms, whereas the ants that live in the trees above do not. Does this indicate that soils in some way contribute to how microorganisms transmit themselves among species of ants? Can this hypothesis be supported if both soil-dwelling and tree-dwelling ants have microorganisms?

Without knowing the identity of the host these ideas may never be asked, and certainly could not be tested.

In the first part of this activity, students will select two sets of morphospecies, record observations, and prepare specimens for vouchering and DNA analysis. This will require analyzing samples carefully as they will notice that many species range in size from so small that they can barely be seen with the naked eye to very large. This will most likely lead to the general conclusion that similar-looking specimens collected in the same location have a higher likelihood of being the same species.

The second part of the activity requires students to identify insects to taxonomic Order. Because there are so many species (1-30 million estimated species), determining the scientific name of insects (genus/species) can be very difficult. For this purpose, vouchers are crucial to the exercise, as students will maintain a record of the organism and can revisit it throughout the rest of the module to properly identify the host. **It is essential to maintain a sample voucher or photovoucher for each “morphospecies” that will be screened for *Wolbachia pipientis*.**

MATERIALS

Per activity station for two students:

- | | |
|--|--|
| <input type="checkbox"/> Insect Collection | <input type="checkbox"/> Small squirt bottle of 80% ethyl alcohol |
| <input type="checkbox"/> Gloves, 2 pair | <input type="checkbox"/> 6 Glass vials (or baby food jars, film canisters) |
| <input type="checkbox"/> 2 Safety goggles | <input type="checkbox"/> Sharpie, extra fine point |
| <input type="checkbox"/> Bent probe | <input type="checkbox"/> Colored labeling tape |
| <input type="checkbox"/> Forceps | <input type="checkbox"/> Colored Pencils |
| <input type="checkbox"/> Scalpel | <input type="checkbox"/> 2 microcentrifuge tubes |
| <input type="checkbox"/> 2-3 Petri dishes (100 x 15 mm or 150 x 20 mm) | <input type="checkbox"/> Dissecting microscope |
| <input type="checkbox"/> Transfer pipette (eye dropper) | <input type="checkbox"/> Computer |

TEACHER PREPARATION

Insects may be collected as part of a field trip, student homework, or ordered from a biological supply company. Students will be more receptive to the study if they play an active role in the development of the activity, such as what environment to collect insects from. It is recommended that an order be placed to a supply house or collection assignment announced at least 3 weeks prior to the start of this activity.

Field Work and Sample Collection:

1. Capture a live specimen:
 - a. Use nets for flying insects
 - b. For other insects, place cup or margarine container over the insect and let it crawl in. Safely pick it up and cover with lid or paper towel.
2. Immediately place insect in 80% or greater ethanol. (You may also use rubbing alcohol = isopropyl)
3. Place in freezer until ready to use in school lab.
4. Collect at least 2 insects of the same species
 - a. One to use
 - b. One to store as a “voucher” – you can also use digital image
5. Label with date, location, and your name.

Lab Preparation:

Each activity station should include one full set of materials. Place computers and insect field guides near the stations where microscopes are being used for insect identification. If there isn't enough for the entire class, create a common area for insect identification reference materials.

ACTIVITY PROCEDURE

Review the activity with your class and encourage them to formulate a hypothesis before starting the activity. Hand out student activity sheets and mini-reports. Students should work in groups of two and follow the protocol outlined on the student activity sheet. Note that student teams are encouraged to briefly review their observations with you before storing specimens and preparing vouchers.

Follow up option: Groups of two students can be asked to make short presentations of their identified samples and Mini-Report data. This can be done in a single class period or for a few minutes each period over several of the lab periods.

EXTENSIONS

1. *Digital Imaging.* Use of digital image is strongly recommended using a cell phone or camera. Digital imaging provides a permanent record of the insect that can be stored, shared, and presented. Pictures can be taken directly through the oculars of most dissecting microscopes.
2. *Insect Collection.*
 - a. Each student should preferably take ownership of their experiment by collecting insects (two-three individuals of the same species) from around their home or school.
 - b. One alternative is that insects can be ordered from a biological supply company. This has the advantage of the teacher knowing the identity as students familiarize themselves with the interactive key. The down side of this is that the students do not explore on their own the diverse shapes, sizes and species of insects around them everyday.
 - c. As an alternative, identified insects purchased from a supplier can be used in a laboratory before the lab when they sort and identify specimens they collected themselves. This may increase student confidence of their identification skills and in the use of the interactive key.
3. *Magnification.* Many insects can be identified with non-prescription glasses sold for enlarging small print. These glasses are inexpensive and widely available and may be a useful substitute for at least some students when there are fewer microscopes than total students.
4. *Additional Specimens.* Multiple specimens per morphospecies can be tested (rather than just one per species) to see the infection level of that species. Or, for positive morphospecies, the next exercise could be to screen 20-40 samples of that type, to see the infection level.
5. *Ecology.* Does ecology or distribution influence the presence or absence of endosymbiotic bacteria in insects? Categorize your morphospecies into ecologically relevant groups (perhaps combine the results for the entire class). Groups can be based on what they eat (herbivores, predators, pollinators), whether they have wings or are wingless, body size (large vs. small), etc. Do species in one of your groups differ in the percentage that test positive for microorganisms? Similar comparisons could be done for collections from different locations, different habitats (aquatic vs. terrestrial), etc.

Insect Identification Lab – Student Activity Sheet

Name: _____

Hypothesis: Formulate a hypothesis about the environment you expect to collect insects from and the types of insects you predict to find there.

INTRODUCTION

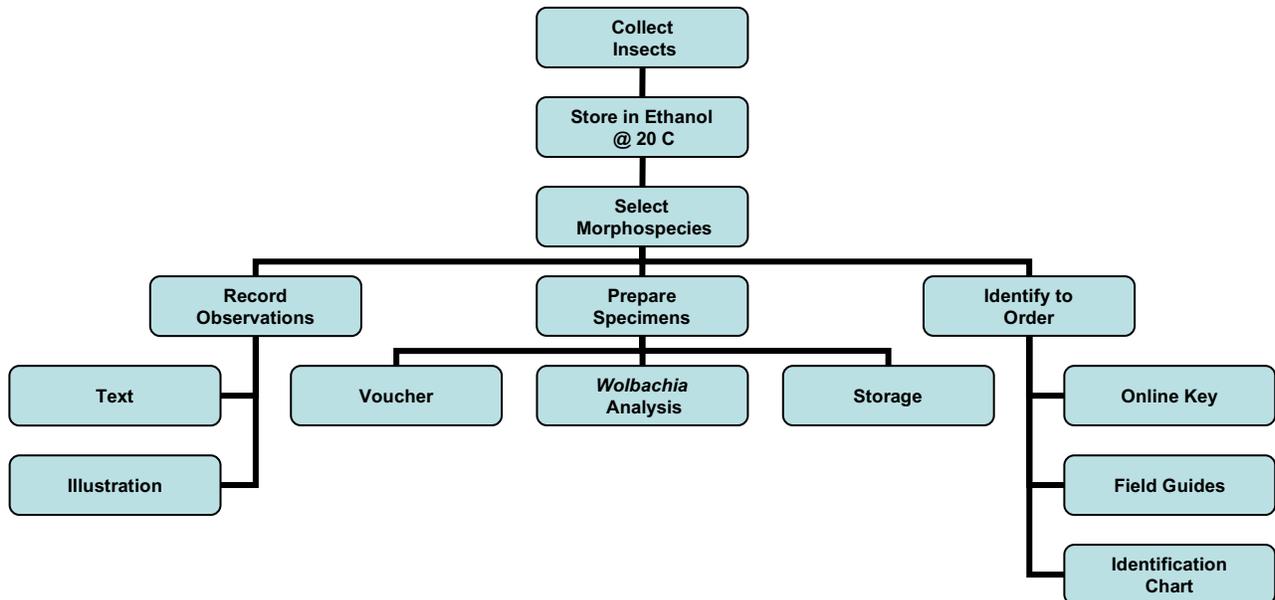
In this activity, you will:

1. Identify insects to Insect Order using an on-line identification key.
2. Use a microscope to sort insects into *morphospecies*.
3. Select specimens from a set of morphospecies for screening by PCR.
4. Prepare a *voucher* for a set of morphospecies.

MATERIALS

- Insect Collection
- Gloves
- Safety goggles
- Bent probe
- Forceps
- Scalpel
- 2-3 Petri dishes
- Eye dropper
- Computer
- Ethyl alcohol 80% in squirt bottle
- 4 Glass vials (or baby food jars)
- Sharpie, extra fine point
- Colored labeling tape
- Colored pencils
- 2 microcentrifuge tubes
- Dissecting microscope

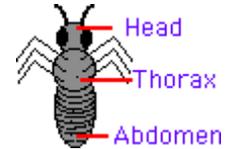
Insect Identification Flow Chart



Procedure 1: Identify Morphospecies and Prepare Specimens

Select two sets of morphospecies.

1. Place a Petri dish on the stage of your dissecting scope and carefully squirt enough ethanol into the dish to properly cover insects. Ethanol preserves DNA for future molecular studies, such as PCR. (*When not being used, insects should be stored in the freezer.*)
2. Using forceps, place one insect from your collection container into the Petri dish with ethanol. Use a pipette (dropper) to wash your insect, making sure it is submerged in ethanol throughout the procedure.
3. Examine the three main body regions of the insect: Head, thorax, and abdomen. In general, on the head of most insects are eyes, mouthparts, and antennae; on the thorax are the wings and the legs; and on the abdomen are the reproductive organs. These regions will help you to eventually classify your insects into groups.
4. Repeat Steps 2 and 3 for the rest of your collection. All insects can be added to the same Petri dish.
5. While keeping the insects submerged in ethanol sort similar looking specimens into groups of morphospecies. Individuals that have similar morphology and are probably members of the same species.
6. Choose 2 different morphospecies to be further analyzed for the presence of *Wolbachia*. In general, it is best to use smaller species because they are easier to dissect and preserve better. Larger insects rot more quickly, thus degrading DNA and making it more difficult to examine bacterial endosymbionts.
7. If possible, select 3 representatives from each of these morphospecies, resulting in a total of 6 specimens. One will be used for the *Wolbachia* study, one for vouchering, and one will be stored back in the freezer.
8. Using the tweezers, place the rest of your insect collection aside back in the collection container. You will no longer use them.



Record Observations.

1. On the back of the *Mini-Report* (or on a separate sheet of paper) record a written observation of your three morphospecies. Include specific notes on the head, thorax, and abdomen.
2. Carefully draw your three morphospecies. Make your sketches BIG and use colored pencils to correctly portray the color.
3. Check your work with your teacher.

Discover the Microbes Within: The *Wolbachia* Project

Prepare specimens for vouchering and *Wolbachia* study.

	Voucher Specimen	Specimen for <i>Wolbachia</i> Analysis	Third Specimen
Morphospecies 1	Glass Vial	Microcentrifuge Tube	Glass vial
Morphospecies 2	Glass Vial	Microcentrifuge Tube	Glass vial

1. Fill two glass vials halfway with ethanol.
2. Make labels for each vial on colored tape with Sharpie. Record your names, the date, and the voucher number as below.
 - a. Voucher # = initials/year/morphospecies1 or 2

J. Doe, R. Smith 5/13/07 Voucher: #JDRS2007-01
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3. Wash the insects with ethanol again. Using forceps keep one individual of your morphospecies in the Petri dish and transfer the other two specimens of the same morphospecies into the glass vials.
4. Record voucher label on *Mini-Report* below.
5. Label a microcentrifuge tube with the corresponding voucher number (e.g., #JDRS2007-01).
6. The remaining individual of each morphospecies will be examined for *Wolbachia* in the next lab. The amount of material necessary to analyze for *Wolbachia* DNA is about equal to that of an entire fruit fly. If the insect you will examine is large, remove the abdomen and use forceps or a scalpel to obtain a “fruit fly-sized” amount. Use of the abdomen is most important because *Wolbachia* are concentrated in the reproductive organs. For smaller insects the entire body can be used. Store abdomen or whole insect in the labeled microcentrifuge tube.
7. Repeat steps 1-6 for the remaining morphospecies.
8. Clean up your lab area.

Procedure 2: Insect Identification

1. Set up a dissecting microscope and computer. Log on to:
http://pick4.pick.uga.edu/mp/20q?guide=Insect_orders
2. Identify specimens to Order by reviewing the guidelines below.
3. Complete the *Mini-Report* on the classification of your insect.

The online key allows you to look at the wings and several other features. You do not have to answer all questions – only those you are confident in! Click on the pictures with your mouse to view a more detailed explanation. After answering several questions, click on any of the ‘**Search**’ buttons to narrow down the list of Orders that have the characteristics you selected. This list is displayed on the left. Next, click on the ‘**Simplify**’ button in the upper left menu column to eliminate unimportant characteristics from the question list. The new list will show only the characteristics that might help you further identify your organism from others, if necessary. Proceed to answer more questions and use the Search and Simplify buttons to determine which Order your insect is from.

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*Insect Identification Summary*

Recall that insects have three main body regions;

- The *head* features conspicuous eyes, mouth, and antennae
- The *thorax* contains wings and legs
- The *abdomen* contains the reproductive organs, where many of the endosymbiotic bacteria live.

Definition of insect features can be found in the left menu column after clicking on the black and white pictures under each question.

Of course, it is common for there to be exceptions to the body plan. Many insects lack wings or legs, some are eyeless, etc. These features allow us to classify them as groups. *Order* is the taxonomic grouping most people are familiar with, for example:

- Order Lepidoptera – moths and butterflies
- Order Coleoptera – beetles
- Order Hymenoptera – bees, wasps and ants
- Order Diptera – flies

Notice that most insect Orders end with the suffix “-ptera.” This word means wing; wings are one of the most conspicuous ways Orders of insects are differentiated.

## Discover the Microbes Within: The *Wolbachia* Project

### Insect Identification Lab Mini-Report

Name \_\_\_\_\_

Date \_\_\_\_\_

1. Attach your observations from Procedure 1.
2. Complete the classification chart for each of your morphospecies.

| Scientific name<br>(Order) | Common name | Copy Voucher Label                                             | Notes                                                                   |
|----------------------------|-------------|----------------------------------------------------------------|-------------------------------------------------------------------------|
| <i>Ex. Hymenoptera</i>     | <i>Wasp</i> | <i>J. Doe, R. Smith<br/>5/13/07<br/>Voucher #: JDRS2007-01</i> | <i>Collected in the<br/>Sippewissett Salt Marsh,<br/>Woods Hole, MA</i> |
|                            |             |                                                                |                                                                         |
|                            |             |                                                                |                                                                         |

3. Research the habitat and life of your identified morphospecies. Record notes in the following chart.

|                                      | Voucher 1<br># _____ | Voucher 2<br># _____ |
|--------------------------------------|----------------------|----------------------|
| Common name                          |                      |                      |
| Habitat<br><i>(trees, soil, etc)</i> |                      |                      |
| Diet                                 |                      |                      |
| Life cycle                           |                      |                      |
| Geographic Location                  |                      |                      |
| Interesting Facts                    |                      |                      |

4. What hypothesis did we research in this lab?
  
  
  
5. Why did you choose this field of study?
  
  
  
6. What did you expect to find and were your predictions supported?

