INSECT IDENTIFICATION LAB

ACTIVITY AT A GLANCE

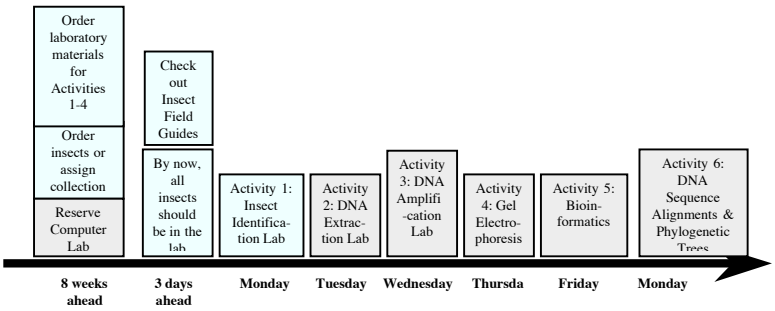
Goal:
To introduce students to the incredible diversity and abundance of insects and to prepare specimens for DNA analysis.

Learning Objectives:
Upon Completion of this activity, students will:

- *Describe* the diversity of insects collected in one location.
- *Identify* insects using taxonomic classification.
- *Sort* insects into "morphospecies" – similar looking species.
- *Prepare* specimens for molecular studies.

Teaching Time:
60-90 minutes (or 2 class periods of 45min each)

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



OVERVIEW

Insects are among the most diverse and abundant animals on earth because they divide habitats so finely. Some are found on one kind of animal (e.g., fleas on mammals), feed on one kind of plant, or eat eggs of only one other insect species! 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 1-20 million species (of which only 850,000 have names) are less than 1/3" long. This entire world of small creatures exists literally under your feet.

In this activity, students will work with a batch of insects that have been preserved in ethanol and stored in the freezer. 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 students will test for microorganisms and represent the only way the insect hosts can be linked to the bacteria that inhabit them. 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 a 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 three 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), determining the scientific name of insects 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 voucher or photovoucher for each "morphospecies" that will be screened for *Wolbachia pipientis*.**

MATERIALS

Per activity station for two students:

- Insect Collection
- Gloves, 2 pair
- 2 Safety goggles
- Bent probe
- Forceps
- Scalpel
- 2-3 Petri dishes (100 x 15 mm or 150 x 20 mm)
- Transfer pipette (eye dropper)
- Small squirt bottle of 95% ethanol
- 6 Glass vials (or baby food jars, film canisters)
- Sharpie, extra fine point
- 24-32 lb. Bond paper or labeling tape
- Colored Pencils
- 3 microcentrifuge tubes (USA Scientific 1415-9199)
- Dissecting microscope
- Computer

TEACHER PREPARTION

Insects may be collected as part of a field trip, student homework, or ordered from a biological supply company. It is recommended that an order be placed or collection assignment announced at least 3 weeks prior to the start of this activity.

Environmental 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 95% or greater ethanol. (You may also use rubbing alcohol = isopropyl)
3. Place in freezer & store at -20˚C until ready to use in school lab.
4. Collect at least 3 insects of the same species
   a. One to use
   b. One to store as a "voucher" – can use digital image
   c. One for later study comparing 18s ribosomal subunit of insect (Optional, *see Appendix B*).
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.

EXTENSIONS

1. *Digital Imaging.* Use of digital image is strongly recommended if a camera is available or ask students to bring their own cameras. Digital imaging provides a permanent record of the insect examined along with the voucher specimen. Pictures can be taken of insects directly through the oculars of most dissecting microscopes. To do this, turn off the flash and

experiment with the macro settings to adjust to the optimal quality and resolution.

2. *Insect Collection.*
   a) Each student should preferably take ownership of their experiment by colleting insects (at least two of the same species) from around their home or school.
   b) Insects can be ordered from a biological supply company rather than collected locally by the students. 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 the great diversity of shapes, sizes and species of insects around them everyday.
   b) 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 them selves. 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 5-10 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 in the world (tropics vs. temperate), different habitats (aquatic vs. terrestrial) and so on.

Student Activity Sheet Name:_____

Insect Identification Lab

Hypothesis: Formulate a hypothesis about the taxonomic diversity of insects you expect to be represented in your collection.

_____
_____
_____

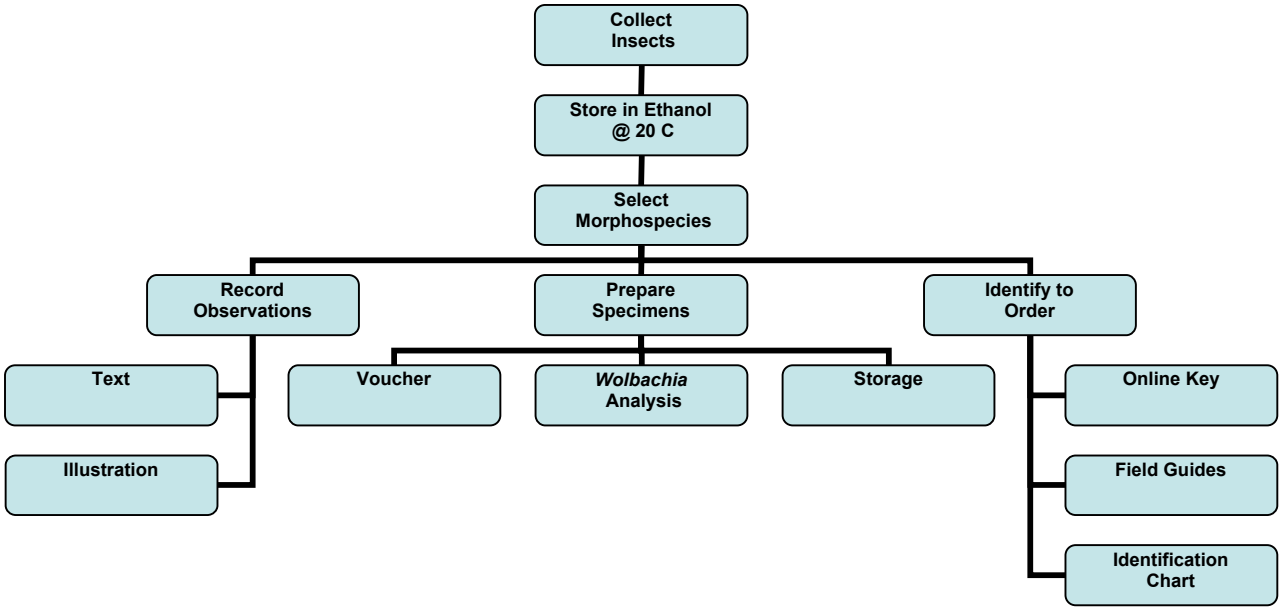
MATERIALS

- Insect Collection
- Gloves
- Safety goggles
- Bent probe
- Forceps
- Scalpel
- 2-3 Petri dishes
- Eye dropper
- 95% ethanol in squirt bottle
- 6 Glass vials (or baby food jars)
- Sharpie, extra fine point
- 24-32 lb. Bond paper or labeling tape
- Colored Pencils
- 3 microcentrifuge tubes
- Dissecting microscope
- Computer

INTRODUCTION

In this activity, you will:

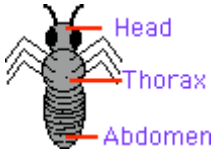
1. Identify insects to Order using an on-line identification key.
2. Use a microscope to sort insects into *morphospecies*.
3. Select specimens from a set of morphospecies for screening by PCR.
4. Prepare a *voucher* for a set of morphospecies.



Procedure 1: Identify Morphospecies & Prepare Specimens

Select 3 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 & 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 3 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 9 specimens. One will be used for the Wolbachia study, one for vouchering, and one will be stored back in the freezer.
8. Using the eye dropper, 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.

Prepare specimens for vouchering and Wolbachia study.

	Voucher Specimen	Specimen for *Wolbachia* Analysis	Third Specimen
Morphospecies 1	Glass Vial	Microcentrifuge Tube	Glass vial
Morphospecies 2	Glass Vial	Microcentrifuge Tube	Glass vial
Morphospecies 3	Glass Vial	Microcentrifuge Tube	Glass vial

1. Fill two glass vials halfway with ethanol.
2. Make labels for each vial on bond paper with Sharpie. Record your names, the date, and the voucher number as below.
    a. Voucher # = initials/year/morphospecies1, 2, or 3

J. Doe, R. Smith
5/13/07
Voucher #: *#JDRS2007-01*

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 two 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.

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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.

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.

The online key allows you to look at the wings and several other features of the legs, mouths and abdomen. You may click on the **Search** button anytime in the process of answering questions, so only answer the questions that you understand. Click on the picture to view a more detailed explanation. As you narrow down, the groups that have the characteristic or characteristics you select are displayed on the left. Clicking on the **simplify** button will eliminate unimportant characteristics from the list and show only the characteristics that might help you further identify your organism from others, if necessary.
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Name _____
Date _____

Insect Identification Lab
Mini-Report

1. Attach your observations from Procedure 1.

2. Complete the classification chart for each of your morphospecies.

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

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

	Voucher 1 # _____	Voucher 2 # _____	Voucher 3 # _____
Common name			
Habitat (*trees, soil, etc*)			
Diet			
Types of human interaction			
Geographic Location			
Interesting Facts			

4. (a) What was the purpose of this lab? (b) Was your hypothesis supported?