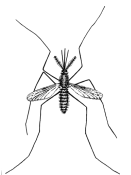
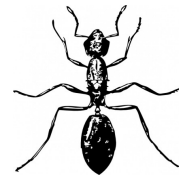
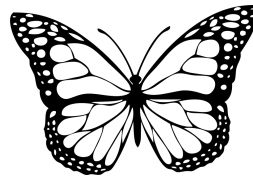
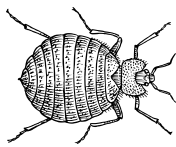
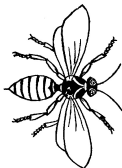


Wolbachia & Reproductive Parasitism

Teacher Guide



The *Wolbachia* Project

1 Arthropod Identification

2 DNA Extraction

3 PCR

4 Gel Electrophoresis

5 Bioinformatics



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The *Wolbachia* Project: Discover the Microbes Within! was developed by a collaboration of scientists, educators, and outreach specialists. It is directed by the Bordenstein Lab at Vanderbilt University.

<https://www.vanderbilt.edu/wolbachiproject>

UNIT AT A GLANCE

Overview:

Students will use manipulatives, such as Cheerios and Froot Loops, to illustrate *Wolbachia*-induced reproductive phenotypes. Teachers will use **Slides** to present facts about *Wolbachia* infections and **Guided Discussion Questions** to explore the associations between *Wolbachia* and arthropod hosts. As a result, students will unravel the mystery of this host-microbe symbiosis and investigate its relevance to human health.

Goal:

This unit is designed to introduce students to *Wolbachia*, its role as a reproductive parasite, and its relevance to ongoing vector control efforts.

Learning Objectives:

Upon completion of this unit, students will understand that:

- *Wolbachia* is an obligate intracellular endosymbiont.
- *Wolbachia* resides in the reproductive tissues of its host where it may act as a reproductive parasite.
- *Wolbachia* is maternally transmitted.
- *Wolbachia* induces cytoplasmic incompatibility to rapidly spread through a population; this is currently being applied in worldwide vector control efforts.

Teaching Time:

- 45-60 minutes

Unit Components:

- *Wolbachia* & Reproductive Parasitism: Teacher Guide
- *Wolbachia* & Reproductive Parasitism: Slides
- *Wolbachia* & Reproductive Parasitism: Student Activity Sheet
- *Wolbachia* & Reproductive Parasitism: Student Notes
- *Wolbachia* & Reproductive Parasitism: Word Search & Coloring Page

Materials Needed for In-Class Discussion:

- *Wolbachia* & Reproductive Parasitism: Student Activity Sheet
- *Wolbachia* & Reproductive Parasitism: Slides
- *Wolbachia* & Reproductive Parasitism: Guided Discussion Questions (pages 5 & 6)
- Two different manipulatives per student (i.e., 15+ Cheerios and 35+ Froot Loops) or two pens (black/colored)
- Internet connection ****Only if watching vector control videos*

INSTRUCTIONS FOR IN-CLASS DISCUSSION

PREPARATION

1. Distribute the following to each student:
 - **Wolbachia & Reproductive Parasitism: Student Activity Sheet**
 - Two sets of manipulatives (i.e., 15+ Cheerios and 35+ Froot Loops) or two different pens (black/colored).
2. Display the **Wolbachia & Reproductive Parasitism Slides** on a white board or print a copy for each student/group.
3. The **Wolbachia & Reproductive Guided Discussion Questions** (pages 5 & 6) are provided for your reference.

CLASS DISCUSSION

1. *Slide 2: Reproductive Parasitism*
 - Ask the class to identify each arthropod in the left panel of the activity sheet.
 - Walk through Slide 2, reading one statement at a time. Instruct students to record the expected number of male and female offspring for each arthropod. Label the reproductive phenotype.
 - Refer to the **Guided Discussion Questions** on page 5. Give students hints along the way and encourage class discussion to reach the answers.
2. *Slide 3: Key to Genetic Crosses*
 - Review symbols on Slide 3
3. *Slide 4: Cytoplasmic Incompatibility*
 - Ask the class to identify the arthropods in the right panel of the activity sheet.
 - Walk through Slide 3, reading one statement at a time. Instruct students to record the expected number of *Wolbachia*-infected vs. uninfected for each cross.
 - Refer to the **Guided Discussion Questions** on page 6. Give students hints along the way and encourage class discussion to reach the answers.

RECOMMENDED VIDEOS

Population Replacement Strategy

- World Mosquito Program: <http://www.eliminatedengue.com/our-research/wolbachia>

Sterile Insect Technique

- Debug: <https://debug.com/>

Genetic Engineering

- Vanderbilt Research: <https://news.vanderbilt.edu/2017/02/27/new-tool-for-combating-mosquito-borne-disease-insect-parasite-genes/>

SUMMARY

The **Wolbachia & Reproductive Parasitism: Student Notes** includes fill-in-the-blank notes that can be completed as a follow-up class exercise, assigned as a take-home activity, or used for student assessment.



GUIDED DISCUSSION QUESTIONS: REPRODUCTIVE PARASITISM (Slide 2)

STUDENT ACTIVITY SHEET: LEFT PANEL

Yellow fever mosquitoes are usually uninfected; therefore, their offspring develop normally.

- What is the expected Female:Male ratio? 5:5
- If scientists want to study *Wolbachia*-infected individuals and need negative controls, how could they make them uninfected? Antibiotic treatment

In ladybugs, or lady beetles, *Wolbachia* can induce male-killing.

- What is the expected F:M ratio? 5:0. All males die; this typically happens at the embryo stage.
- If scientists want to study a male-killing strain in the lab, how could they propagate the line without males? They could treat a subset of the population with antibiotics. Uninfected individuals will resume normal reproduction and produce sons. These sons could then be mated to the male killing females.

In roly polies, or pill bugs, *Wolbachia* can induce feminization.

- What is the expected phenotypic F:M ratio? 10:0. Although half are genetically male, they will look, function, and reproduce as females.
- What is the difference between genotype and phenotype? The genotype is the genetic code (DNA) responsible for a particular trait. The phenotype is the visible or expressed trait.

In clover mites, a type of spider mite, *Wolbachia* can induce parthenogenesis.

- What is the expected F:M ratio? 10:0. Females reproduce asexually.
- What is a benefit of parthenogenesis? Females can reproduce without the effort of finding a mate.
- What is a disadvantage of parthenogenesis (and asexual reproduction, in general)? Lack of genetic diversity. The population could bottleneck. Without any incoming mutations, it may not be able to adapt to changes in the environment and/or may not be able to fight off pathogens.

SUMMARY QUESTIONS

- Now look over your results. What is the impact of *Wolbachia* on these arthropod hosts? Kills males; generates more females in the population. *Wolbachia*-infection is transmitted to offspring.
- Why would *Wolbachia* prefer, or select for, females? It is maternally-transmitted.
- *Wolbachia* are obligate intracellular endosymbionts, which means they live within the cells of other organisms. Based on their host interactions, where would you expect to find *Wolbachia*? Which types of cells? *Wolbachia* reside in reproductive tissues (i.e., testes and ovaries).
- Now switch gears to human biology. Can you think of a cellular component that is maternally-transmitted? Mitochondria. *Wolbachia* is an Alphaproteobacterium; mitochondria share a common ancestor with Alphaproteobacteria.



GUIDED DISCUSSION QUESTIONS: CYTOPLASMIC INCOMPATIBILITY (Slides 3 & 4)

KEY TO GENETIC CROSSES

If *Wolbachia* is maternally-inherited, which of the following would pass along the *Wolbachia* infection to offspring?

- Infected male
- Infected female
- Uninfected male
- Uninfected female

STUDENT ACTIVITY SHEET: RIGHT PANEL

Uninfected male x Uninfected female

- What is the expected observation? 5 uninfected (Cheerios/black pen)

Uninfected male x Infected female

- What is the expected observation? 5 infected (Froot Loops/colored pen)

Infected male x Uninfected female

- What is the expected observation? No offspring

Infected male x Infected female

- What is the expected observation? 5 infected (Froot Loops/colored pen)

SUMMARY QUESTIONS

- Now look over your results. What is the impact of *Wolbachia* on this population? The majority, 2/3 of offspring, are now infected
- What do you expect to happen to this population over multiple generations? *Wolbachia*-infected flies will displace uninfected flies
- Which cross is “incompatible”? Infected males x uninfected females
- *Wolbachia* is maternally-transmitted and not found in the sperm. Therefore, what could be an explanation for this incompatibility? We now know that *Wolbachia* “modifies” the sperm. Only an infected female can “rescue” the sperm.
- In mosquitoes, some strains of *Wolbachia* prevent the transmission of RNA viruses (such as Zika, dengue, and chikungunya). Applying what we’ve learned about cytoplasmic incompatibility, how could this be applied to disease control? Scientists are releasing *Wolbachia*-infected mosquitoes across the world, including California and Florida, to combat mosquito-borne diseases. They can utilize the biology of *Wolbachia*-induced cytoplasmic incompatibility to either crash a population (Sterile Insect Technique) or rapidly spread the *Wolbachia* infection through a population (Population Replacement Strategy).
- Would you rather use pesticides or release *Wolbachia*-infected mosquitoes into your community? Why? Answers will vary. The main arguments against pesticides are ecosystem balance (killing mosquitoes will impact their predators) and preservation of pollinator species.



RECOMMENDED VIDEOS: CURRENT APPROACHES TO VECTOR CONTROL (Slide 5)

Population Replacement Strategy

World Mosquito Program: <http://www.eliminatedengue.com/our-research/wolbachia>

Sterile Insect Technique

Debug: <https://debug.com/>

Mosquito Mate: <https://mosquitomate.com/how-it-works/>

Genetic Engineering

Vanderbilt Research: <https://news.vanderbilt.edu/2017/02/27/new-tool-for-combating-mosquito-borne-disease-insect-parasite-genes/>

DISCUSSION QUESTIONS

- How does the Population Replacement Strategy differ from the Sterile Insect Technique? *Sterile insect technique* releases *Wolbachia*-infected, incompatible males that are unable to successfully reproduce with local mosquitoes, thus crashing the population. *Population replacement* doesn't crash the mosquito population. Rather, it replaces the local population with mosquitoes that are unable to transmit human viruses.
- Which approach would you prefer in your backyard? *Opinion*
- What are the benefits/caveats for each approach? *Variable answers*
- What are the long-term consequences of each approach? *Variable answers*
- How might these *Wolbachia* techniques be applied beyond vector control? *Variable answers* including: agricultural pest management, controlling invasive species, tick-borne diseases, etc.

SUMMARY

The ***Wolbachia* & Reproductive Parasitism: Student Notes** contains a fill-in-the-blank summary. Complete this as a class exercise, assign as a take-home activity, or use for student assessment.



Wolbachia & Reproductive Parasitism

40%	reproductive	rescue	mosquito
heartworm	Zika	parthenogenesis	mutualists
maternally	filarial	mitochondria	genotype
antibiotics	phenotype	eukaryotic	sterile insect technique
endosymbiont	feminization	modifies	male-killing
population replacement strategy		cytoplasmic incompatibility	

Use the word bank to complete the statements below.

- Wolbachia* is an obligate, intracellular endosymbiont. It lives within the cells of its eukaryotic host.
- Wolbachia* infects approximately 40% of all arthropods.
- Wolbachia* resides in reproductive tissues, such as ovaries and testes.
- Like mitochondria, *Wolbachia* is maternally-transmitted.
- Wolbachia* infections can be treated with antibiotics.
- Genotype is the genetic code (DNA) responsible for a particular trait whereas phenotype is the visible or expressed trait.
- Wolbachia* induces four reproductive phenotypes:
 - male-killing: Males die during embryogenesis.
 - parthenogenesis: Females reproduce asexually.
 - feminization: Genetic males develop as phenotypic females.
 - cytoplasmic incompatibility: *Wolbachia*-infected males can successfully mate with only females harboring the same infection.
- In cytoplasmic incompatibility, *Wolbachia* modifies the sperm. Only a female with the same *Wolbachia* infection can rescue the incompatibility.
- Some strains of *Wolbachia* inhibit the transmission of mosquito-borne diseases, such as Zika, dengue, and chikungunya virus.
- In sterile insect technique, *Wolbachia*-infected male mosquitoes are released to crash a local population.
- In population replacement strategy, *Wolbachia*-infected mosquitoes are released to rapidly replace the population with mosquitoes that are unable to transmit human viruses.

Extension: Beyond arthropods, *Wolbachia* also infect filarial nematodes. Here, they act as mutualists and are required for successful host development and reproduction. Therefore, a veterinarian might treat heartworm with antibiotics to target the resident *Wolbachia* infection. Understanding this host-microbe symbiosis is critical for treating human diseases such as elephantiasis and river blindness.