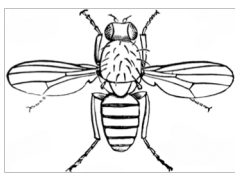
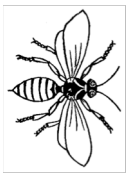
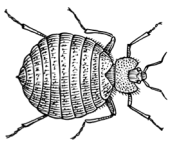
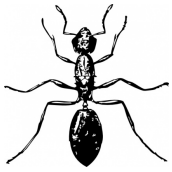
The Wolbachia Project

*Discover the Microbes Within!*

# *Wolbachia* & Reproductive Parasitism

**Teacher Guide**



Discover the Microbes Within! The *Wolbachia* Project was developed by a collaboration of scientists, educators, and outreach specialists. It is managed by the Bordenstein Lab with support from the Vanderbilt Microbiome Initiative and the National Science Foundation.

**The *Wolbachia* Project**
https://my.vanderbilt.edu/discover/

**Vanderbilt Microbiome Initiative**
https://my.vanderbilt.edu/microbiome/

# 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 Infected 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.

*Answer Key*

# *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.**

1. *Wolbachia* is an obligate, intracellular **endosymbiont**. It lives within the cells of its **eukaryotic** host.
2. *Wolbachia* infects approximately **40%** of all arthropods.
3. *Wolbachia* resides in **reproductive** tissues, such as ovaries and testes.
4. Like **mitochondria**, *Wolbachia* is **maternally**-transmitted.
5. *Wolbachia* infections can be treated with **antibiotics**.
6. **Genotype** is the genetic code (DNA) responsible for a particular trait whereas **phenotype** is the visible or expressed trait.
7. *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.
8. In cytoplasmic incompatibility, *Wolbachia* **modifies** the sperm. Only a female with the same *Wolbachia* infection can **rescue** the incompatibility.
9. Some strains of *Wolbachia* inhibit the transmission of **mosquito**-borne diseases, such as **Zika**, dengue, and chikungunya virus.
10. In **sterile insect technique**, *Wolbachia*-infected male mosquitoes are released to crash a local population.
11. 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.

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