Updated: February 2023

The *Wolbachia* Project

Discover the Microbes Within!

Introduction to *Wolbachia*

Wolbachia & Reproductive Parasitism

Project Guide

The *Wolbachia* Project

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.

https://www.wolbachiaproject.org

The word search was created with The Teacher's Corner at: https://www.theteacherscorner.net

Introduction to Reproductive Parasitism

Recommended Background Video

I Contain Multitudes: Mosquitoes Might SAVE Lives, Thanks To Bacteria
https://www.pbs.org/video/mosquitoes-might-save-lives-thanks-to-bacteria-ufusya/

From PBS Digital Studios, Ed Yong talks with Dr. Scott O'Neill about the use of *Wolbachia* to combat mosquito-borne diseases. This 9-minute video introduces *Wolbachia*-induced reproductive parasitism and discusses *Wolbachia*'s ability to block the transmission of viruses.

Class Activity

Work with your class or in a group to complete Figure A (page 8) and answer the questions below.

For the purpose of this activity, assume that the average clutch size for uninfected individuals is 10 with an even distribution of 5 females and 5 males.

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

1. FIGURE A: Record the number of male and female offspring. Label the phenotype.
2. DISCUSSION QUESTION: If scientists want to study *Wolbachia*-infected individuals and need negative controls, how could they make them uninfected? (Hint: How are bacterial infections treated?) _____
   _____
   _____

In ladybugs, or lady beetles, *Wolbachia* can induce male killing. In this case, male offspring do not survive.

3. FIGURE A: Record the number of male and female offspring. Label the phenotype.
4. DISCUSSION QUESTION: *Wolbachia*-induced male killing could lead to extinction of its arthropod host. Alternatively, how might it benefit the host? (Hint: Refer to the introduction of this paper for guidance - https://doi.org/10.1371/journal.ppat.1007936)
   _____
   _____
   _____

In roly polies, or pill bugs, *Wolbachia* can induce feminization. In this case, genotypic males develop into phenotypic females.

5. FIGURE A: Record the number of male and female offspring. Label the phenotype.
6. DISCUSSION QUESTION: What is the difference between genotype and phenotype?
   _____
   _____
   _____

In clover mites, a type of spider mite, *Wolbachia* can induce parthenogenesis. In this case, females can produce daughters without mating.

7. FIGURE A: Record the number of male and female offspring. Label the phenotype.
8. DISCUSSION QUESTION: What is a disadvantage of parthenogenesis (and asexual reproduction, in general)? _____
   _____
   _____

Figure A Summary Questions

9. Review the results. What is the impact of *Wolbachia* on these arthropod hosts?
   _____
   _____
   _____

10. Why might *Wolbachia* prefer, or select for, females? (Hint: How are mitochondria inherited?)
    _____
    _____
    _____

11. *Wolbachia* are obligate intracellular endosymbionts, which means they live within the cells of other organisms. Based on the host interactions described above, in which body system would you expect to find *Wolbachia*?
    _____
    _____
    _____

Introduction to Cytoplasmic Incompatibility (CI)

Recommended Background Video

Cytoplasmic Incompatibility Genes and Applications
https://youtu.be/tgUFKHm0eu4

From NSF Science Now, Dr. Seth Bordenstein discusses cytoplasmic incompatibility and the potential use of *Wolbachia* to control the spread of insect-borne diseases and agricultural pests.

Class Activity

*A fourth type of reproductive parasitism is called **cytoplasmic incompatibility (CI)**. Work with your class or in a group to answer the questions below.*

Empty circle = *Wolbachia*-uninfected
Filled circle = *Wolbachia*-infected

***Wolbachia* (-)** **Uninfected male** | ***Wolbachia* (+)** **Infected male** | ***Wolbachia* (-)** **Uninfected female** | ***Wolbachia* (+)** **Infected female**

1. If *Wolbachia* is maternally-inherited, which of the following would pass along the *Wolbachia* symbiont to offspring? _____
    a. Uninfected male
    b. Infected male
    c. Uninfected female
    d. Infected female

Use the following symbols to complete Figure B (page 8). Use only one symbol per box.

***Wolbachia* (-) Uninfected offspring**

***Wolbachia* (-) Infected offspring**

X

Incompatible Cross Offspring Die

2. Uninfected male x Uninfected female
    - This is a compatible cross. Because mom is uninfected, offspring are uninfected.
3. Infected male x Infected female
    - This is a compatible cross. Because mom is infected, offspring are infected.
4. Infected male x Uninfected female
    - This is an incompatible cross. Offspring are not viable.
5. Uninfected male x Infected female
    - This is a compatible cross. Because mom is infected, offspring are infected.
6. Label the phenotype.

Figure B Summary Questions

7. Review Figure B. Rather than a selecting for female offspring, what is the impact of *Wolbachia* on this population?

    _____
    _____
    _____

8. What do you expect to happen to this population over multiple generations?

    _____
    _____
    _____

9. Which cross is “incompatible”?

    _____
    _____
    _____

10. *Wolbachia* is maternally-transmitted and not found in the sperm. Therefore, what could be an explanation for *Wolbachia*-induced cytoplasmic incompatibility?

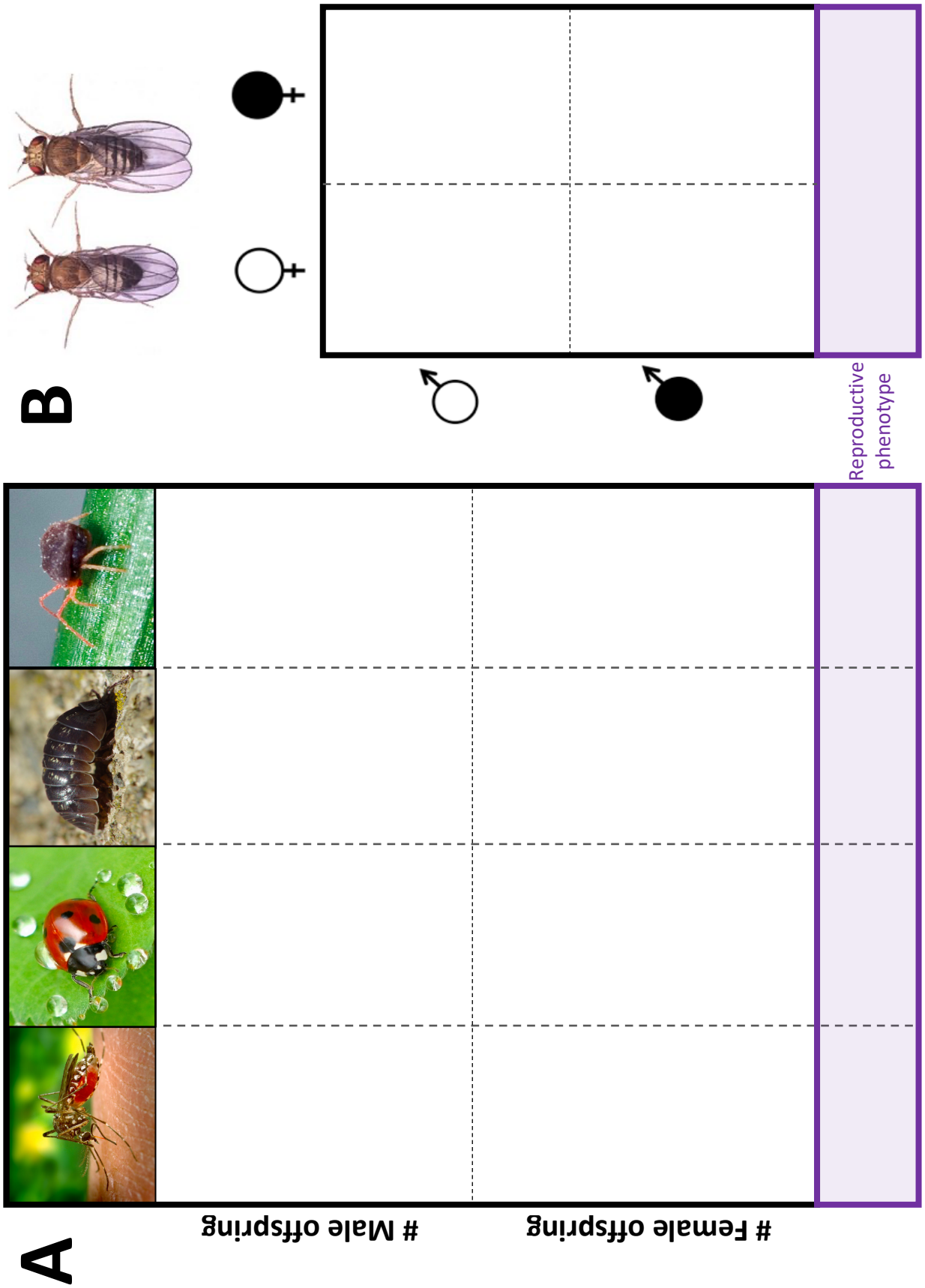
    _____
    _____
    _____

11. 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?

    _____
    _____
    _____

12. Would you rather use pesticides or release *Wolbachia*-infected mosquitoes into your community? Why?

    _____
    _____
    _____


B
Reproductive phenotype
A
Male offspring
Female offspring

Student Notes

40%	feminization	modifies
antibiotics	rescue	cytoplasmic incompatibility
endosymbiont	parthenogenesis	mosquito
reproductive	mitochondria	genotype
Zika	host	male-killing
phenotype		

Use the word bank to complete the statements below.

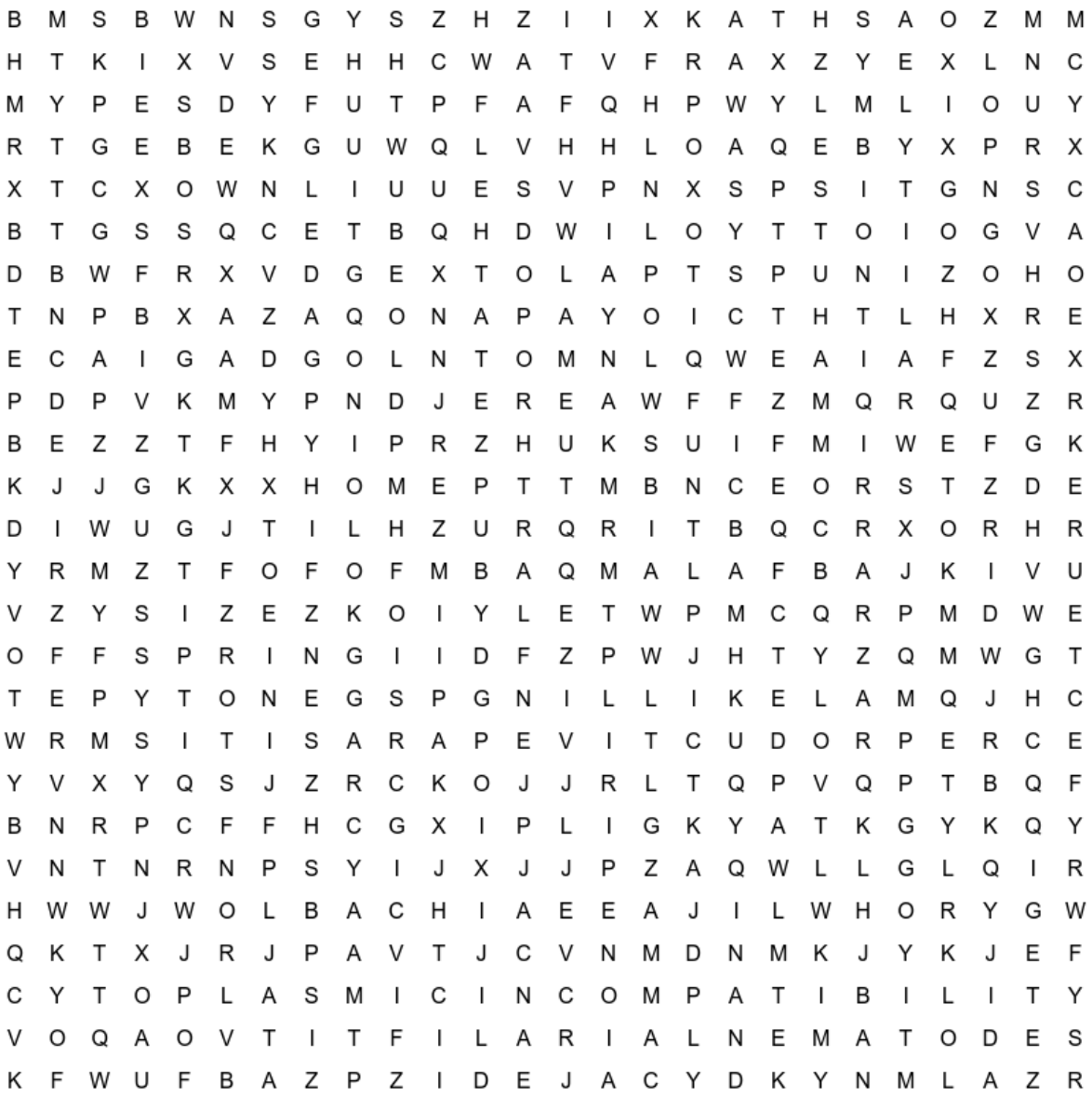
1. *Wolbachia* is an obligate, intracellular _____. It lives within the cells of its eukaryotic _____.
2. *Wolbachia* infects approximately _____ of all arthropods.
3. *Wolbachia* resides in _____ tissues, such as ovaries and testes.
4. Similar to _____, *Wolbachia* is maternally-transmitted.
5. *Wolbachia* infections can be treated with _____.
6. _____ is the genetic code (DNA) responsible for a particular trait whereas _____ is the visible or expressed trait.
7. *Wolbachia* induces four reproductive phenotypes:
   - _____: Sons of infected females are selectively killed.
   - _____: Females reproduce asexually.
   - _____: Genetic males develop as phenotypic females.
   - _____: *Wolbachia*-infected males are incompatible with uninfected females.
8. In cytoplasmic incompatibility, *Wolbachia* _____ the sperm. Only a female with the same *Wolbachia* infection can _____ the incompatibility.
9. Some strains of *Wolbachia* inhibit the transmission of _____-borne diseases, such as _____, dengue, and chikungunya virus.

Research the association of *Wolbachia* with nematodes to complete the following section.

heartworm	filarial	mutualists

Beyond arthropods, *Wolbachia* also infect _____ nematodes. Here, they act as _____ and are required for successful host development and reproduction. Therefore, a veterinarian might treat _____ 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.

Word Search



DNA
host
symbiont
Wolbachia
offspring

feminization
male killing
parthenogenesis
cytoplasmic incompatibility
reproductive parasitism

arthropods
genotype
phenotype
mutualists
filarial nematodes

Glossary

Clutch size: the number of offspring produced at one time.

Cytoplasmic incompatibility (CI): a reproductive phenotype in which sperm and egg are unable to produce viable offspring. In *Wolbachia*-induced CI, infected males modify the sperm and are incompatible with uninfected females. Only females with a similar *Wolbachia* symbiont can rescue the modification to produce viable offspring.

Endosymbiont: an organism that lives within another organism.

Feminization: a reproductive phenotype in which genetic males develop into phenotypic females.

Genotype: the genetic code (DNA) responsible for a particular trait.

Host: an organism in which (or on which) another organism lives.

Infected: harboring a microbial organism, in this case *Wolbachia.*

Male killing: a reproductive phenotype in which sons of infected females are selectively killed.

Offspring: the immediate descendants of an organism.

Parthenogenesis: a reproductive phenotype in which virgin mothers produce all female offspring from unfertilized eggs.

Phenotype: the visible or expressed trait of an organism.

Reproductive parasitism: the ability of a microbial symbiont, in this case *Wolbachia*, to influence the sexual reproduction of its host.

Symbiont: an organism living in close association with another, often larger, organism.

Uninfected: not harboring a microbial organism, in this case *Wolbachia*.