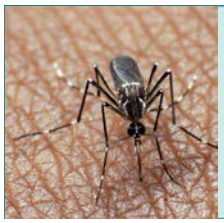

Genetically Modified Mosquitoes

hhmi | BioInteractive

Scientists at Work
Educator Materials

OVERVIEW

This document provides background information, discussion questions, and responses that complement the short video "Genetically Modified Mosquitoes" from the *Scientists at Work* series. The *Scientists at Work* series is intended to provide insights into the daily work of scientists that builds toward discoveries. The series focuses especially on scientists in the field and what motivates their work.

This 8-minute 35-second video is appropriate for any life science student audience. It can be used as a review of the nature of science or to enhance a discussion on transgenic technology. Classroom implementation of the material in this document might include assigning select discussion questions to small groups of students or selecting the top five questions to discuss as a class.

KEY CONCEPTS

- Researchers genetically modified mosquitoes to help prevent the spread of a virus.
- Male GM mosquitoes pass on a lethality gene to the offspring when they mate with non-GM females in the wild.

CURRICULUM CONNECTIONS

Standards	Curriculum Connection
NGSS (2013)	HS-LS1.A, HS-LS3.A
AP Bio (2015)	3.A.1
IB Bio (2016)	3.1, 3.5
Vision and Change (2009)	CC1, CC2, CC3

PRIOR KNOWLEDGE

Students should

- be familiar with the idea that genes code for proteins and that genetic information can be passed to offspring.
- have some understanding of transgenic technology and what it means for an organism to be genetically modified.

BACKGROUND INFORMATION

Mosquitoes can transmit pathogens that cause many human diseases, such as malaria, yellow fever, dengue fever, chikungunya, and Zika fever. Many of these diseases can be physically devastating and even fatal. For example, according to the World Health Organization (WHO), there are over 200 million new cases of malaria per year worldwide, resulting in over 400,000 deaths, most of them children under the age of 5.

Zika fever is caused by a virus transmitted to humans primarily by the bite of *Aedes aegypti* mosquitoes. Symptoms in infected human adults are typically mild, but if the virus infects a pregnant woman it can be transmitted to the developing fetus and affect brain development, causing a condition called microcephaly. To reduce the number of *A. aegypti* mosquitoes that may carry Zika virus, researchers at a biotechnology company called Oxitec have produced genetically modified (GM) *A. aegypti* mosquitoes that when released into the wild, mate with wild mosquitoes, and any offspring produced die before becoming adults.

Oxitec scientists first developed their GM mosquito line (called OX513A) in 2002, by inserting genes from other organisms into the mosquitoes' genomes (Phuc, *et al*., 2007). The OX513A GM mosquito line has two important genes:

1. a "fluorescence gene" from a colored marine coral (*Discosoma* sp.) that causes mosquito larvae to glow red under fluorescent light; and
2. a "lethality gene" that consists of a combination of DNA sequences from the bacterium *E. coli* and from the herpes simplex virus, and causes mosquito larvae to die unless they receive an antidote.

The fluorescence gene is used to identify GM mosquitoes. The lethality gene, which is more accurately called *tetracycline transcriptional activator variant* (or *tTAV*), encodes a protein that blocks transcription of several other genes that are essential to mosquito development. GM mosquito larvae that produce the tTAV protein die before reaching maturity. However, the tTAV protein cannot prevent the transcription of other genes when it is bound to the antibiotic tetracycline. Therefore, tetracycline acts as a repressor of the lethality gene, or, in other words, its antidote. In the lab, the GM mosquito larvae are reared in water containing tetracycline and develop normally into adult mosquitoes. When adult GM mosquitoes are released into the wild and breed with wild, non-GM mosquitoes, their offspring inherit the lethality gene. Without tetracycline in the environment to protect them, the offspring die.

In one study, Oxitec scientists released GM mosquitoes into a neighborhood in Brazil. Sustained release over the course of a year led to a reduction of the local *Aedes aegypti* population by 80% to 95% according to different measures (Carvalho *et al*., 2015). The scientists chose densely populated neighborhoods for their study because mosquito-borne diseases can spread most easily in areas where lots of humans and mosquitoes are present. They hypothesized that if they could reduce both the population size of the *A. aegypti* mosquitoes and the mosquito population density, they would reduce the probability that a person becomes infected with a pathogen spread by these mosquitoes. (An activity that shows how scientists measure mosquito density, based on data from Oxitec scientists, is available on the BioInteractive website as "Tracking Genetically Modified Mosquitoes.")

DISCUSSION QUESTIONS

PART 1: Mosquitoes and Zika

1. Do all mosquito species carry viruses or other pathogens?

Approximately 3,500 species of mosquitoes have been identified, with 176 species recognized in the U.S. Most of these mosquito species do not spread disease; only certain species do. They include species belonging to the Aedes, Culex, *and* Anopheles genera*.*

2. Why are health professionals concerned about the Zika virus?

The Zika virus was first identified in 1947 in Uganda, Africa, and was only known to cause mild symptoms, such as fever, rash, and headaches. In 2015, it came prominently to the public's attention when a widespread outbreak occurred in Brazil and then spread throughout the Americas. The outbreak was associated with cases of microcephaly.

3. What is microcephaly?

Microcephaly is a rare condition in which the circumference of a baby's brain is smaller than expected. Microcephaly can occur because a baby's brain did not develop properly during pregnancy and can be caused by genetic and environmental factors. In the absence of Zika infection, a typical rate of microcephaly is about 2 to 12 in 10,000 births in the U.S.

Children with severe microcephaly can have substantial cognitive delays, learning disabilities, and other neurological problems. Other consequences of microcephaly include difficulties with coordination and balance, decreased ability to learn and function in daily life, seizures, decreased hearing, and vision problems. Severe microcephaly can also cause death.

4. How does the Zika virus spread?

Zika virus has been detected in different monkey species and in humans, and these animals may act as reservoirs for the virus. A reservoir is an organism (or environment) in which the virus multiplies and develops and on which it is dependent for its survival in nature. Mosquitoes are the primary vector for transmitting the virus from one individual to another, although a few cases of sexual transmission from individual to individual have also occurred.

5. Which mosquito species carry Zika virus? What other diseases are carried by these mosquitoes? What is their geographical range?

Zika is primarily carried by the mosquito species Aedes aegypti *and* A. albopictus. *Both mosquito species can also spread the viruses that cause dengue fever, chikungunya, and yellow fever, and can carry filarial nematodes like* Dirofilaria immitis *that cause heartworm in dogs*.

A. aegypti *live year-round in tropical and subtropical climates, and in some temperate regions such as the southern United States. About 63% of the world human population lives in regions where* A. aegypti *also lives*. A. albopictus *is native to the tropical and subtropical regions of Southeast Asia, including Eastern Asia, India, Japan, and several islands in the Pacific. The species is now also in Italy and other regions in the Mediterranean basin, parts of Africa, Madagascar, Brazil, Central America, the Caribbean, and most of the United States (specifically the East Coast and the Midwest).* A. albopictus *can live in broader temperature ranges than* A. aegypti.

6. What are some of the methods used to reduce the spread of mosquito-borne disease and what are the pros and cons of each one?

Table 1. Common methods for reducing mosquito populations or preventing mosquitoes from biting people.

Method	Advantages	Disadvantages
Mosquito nets	Inexpensive, easy to install, efficient	Only provide protection if used correctly; nets can easily get torn
Window screens	Easy to install and inexpensive	Easily damaged; screens can get torn
Closing doors and windows and using air conditioning	Easy to use	Not available everywhere, moderately expensive
Removing standing water around homes to eliminate breeding sites to limit breeding areas	Effective and inexpensive	Needs to be checked regularly, needs large proportion of the community to participate, and is difficult to implement in areas with high rainfall
Keep grass and shrubs short to reduce places for mosquitoes to rest	Many communities promote short grass and shrubs already	Requires upkeep and needs large proportion of the community to participate
Wear pesticide-treated clothing or light-colored, loose-fitting clothing that fully covers arms and legs	Easy to implement	May be uncomfortable and moderately expensive

Method	Advantages	Disadvantages
Use chemical repellants on skin, such as DEET, picaridin, oil of lemon eucalyptus (OLE), or para-menthane-diol (PMD)	Inexpensive and easy to apply	Must be used constantly and reapplied; may irritate the skin and may be toxic to some people and animals in large amounts
Broadly used chemical sprays, including pyrethroid insecticides and other pesticides	Relatively easy to apply	Must be reapplied; have to be spread over a large area to be effective; toxic to many other insect species
Insecticide mist dispersed around buildings	Quickly kills mosquitoes	Expensive; has to be reapplied; toxic to many other insect species
Chemical methoprene to kill larvae in standing water and ditches	Easy to apply, effective, and safe for pets and birds	Must be reapplied; impossible to treat all breeding sites
Attract mosquitoes by mimicking human chemistry and trap in a vacuum	Effective	Expensive and not permanent

PART 2: GM Technology

7. What is a genetically modified organism (GMO)?

A genetically modified organism (GMO) is any organism whose genome has been modified by scientists through the use of a genetic engineering method (for example, by introducing mutations in the DNA or by inserting new genetic material).

Producing GMOs typically involves taking a gene (or genes) from the DNA of an unrelated species and inserting the gene (or genes) into a target organism's genome so that it's present in all the organism's cells. For example, in the case of the OX513A mosquito line, the foreign genes were introduced into mosquito eggs so that they are present in all the adult mosquitoes' cells. The mosquitoes in the OX513A line are referred to as GMOs, transgenic organisms, or just transgenics.

8. What are some examples of GMOs?

Genetic modification has been used to produce medicines and crops. For example, starting in 1982, E. coli *bacteria have been modified to carry the human insulin gene and produce human insulin, which is then given to patients with diabetes. Today, drug companies use transgenic* E. coli *and yeast to produce over 17,000 kg/year of insulin, which keeps tens of millions of people with diabetes around the world alive. In 1996, farmers were able to grow a transgenic corn variety that resists damage from insects. Called* Bt *corn, this transgenic plant produces a protein insecticide from the soil bacterium* Bacillus thuringiensis*. Bt corn has resulted in a 35% reduction in the use of insecticides worldwide and saves farmers over $1 billion annually in crop losses and insecticide costs.*

9. How does the lethality gene in GM mosquitoes work?

The lethality gene is not a naturally occurring gene. It was artificially synthesized by combining the DNA sequence of the tetracycline receptor gene from the bacterium E. coli *with the DNA sequence of a transcriptional activator from the herpes simplex virus called VP16. The lethality gene is more accurately called* tetracycline transcriptional activator variant*, or* tTAV *gene for short, and results in tetracycline-repressible dominant lethality in cells. So, how does it work?*

The tTAV *gene codes for the tTAV protein. In the absence of tetracycline, the tTAV protein binds to the* tTAV *gene promoter to activate its transcription and produce more tTAV protein in a positive feedback loop. High expression of* tTAV *is toxic because part of the protein (encoded by the* VP16 *gene from* herpes simplex *virus) binds to key transcription factors in mosquito cells, inhibiting gene expression, and killing the cells and eventually the whole organism. The tTAV protein also has an area (encoded by the tetracycline receptor gene from* E. coli*) to which tetracycline can bind. When tetracycline binds to the tTAV protein, the protein can no longer bind to the* tTAV *promoter and* tTAV *gene expression shuts down. Tetracycline is therefore an antidote to* tTAV *toxicity.*

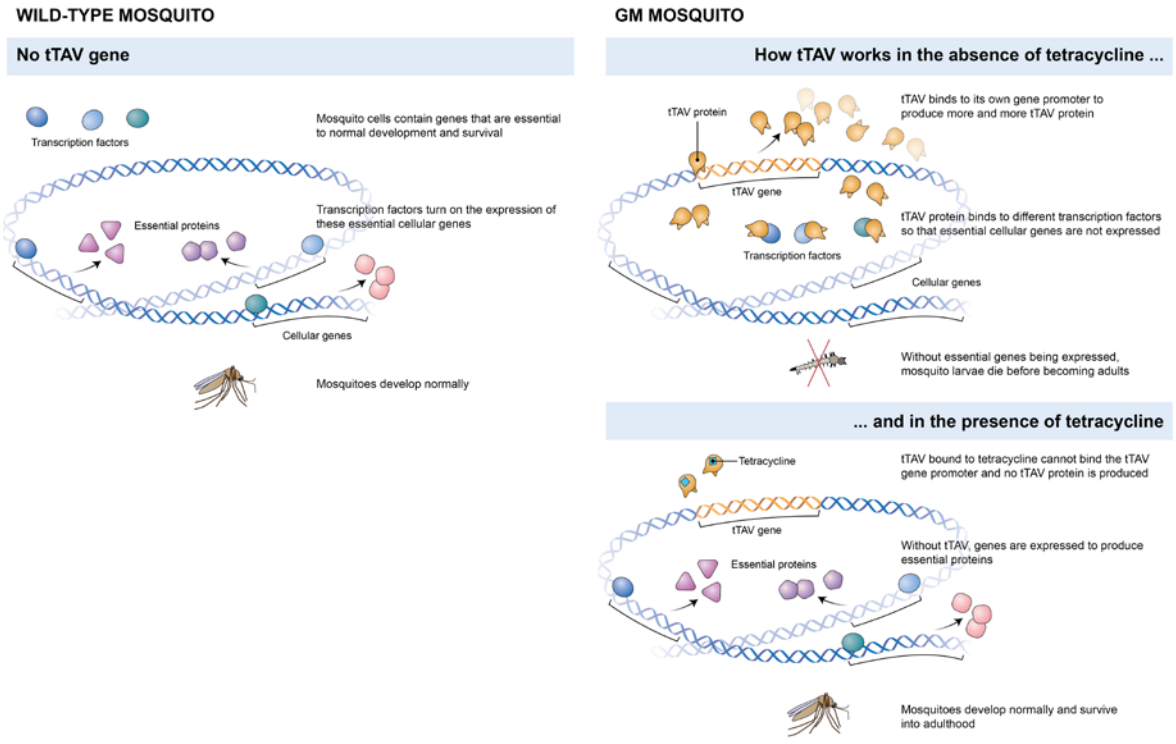

Figure 1.
A summary of how the *tTAV* gene works in the absence and presence of tetracycline.

Table 2. Summary of how tTAV and tetracycline affect typical and GM mosquitoes.

Mosquito	Tetracycline present?	Amount of active tTAV protein in cells	Result
Wild-type *Aedes aegypti*	Not in appreciable amounts in the wild	None, because the *tTAV* gene is not present	Normal level of transcription, healthy cells and mosquitoes
GM *Aedes aegypti* (OX513A line) grown in the lab	Yes, if provided in the lab	Very low because tetracycline binds to the tTAV protein and disables it	Normal level of transcription, healthy cells and mosquitoes
GM *Aedes aegypti* (OX513A line) released in the wild	Not in appreciable amounts in the wild	High (toxic levels)	Repression of transcription, death of mosquitoes

10. How does the red fluorescence gene work?

*The red fluorescence gene (*DsRed2*) comes from a colored marine coral species in the genus* Discosoma *that codes for a red fluorescent protein. When a special laser that emits light at a certain wavelength is shone on the red fluorescent protein, the protein becomes excited and emits red light. In the transgenic mosquitoes, the fluorescent protein indicates to scientists that a mosquito has been genetically modified.*

11. How were these two genes inserted into the genome of mosquitoes? Are they inserted randomly in the genome?

Scientists created small circular DNA segments called plasmids. One plasmid contained both the lethality and red fluorescence genes that scientists wanted to integrate into the mosquito DNA. That plasmid also had some segments of DNA from the fruit fly Drosophila melanogaster *required for proper gene expression, such as promoter, enhancer, and terminator sequences. A second plasmid has a gene that codes for an enzyme that helps integrate the first plasmid into the mosquito genome.*

Small concentrations of these two DNA plasmids were microinjected into mosquito eggs. In some of the eggs, the lethality and fluorescence genes were integrated into the DNA of the eggs. Scientists used DNA sequencing to identify the locations of the insertion. Those studies show that the genes are inserted as one unit at random locations throughout the genome.

When eggs develop into adults and the mosquitoes breed, the parents pass on the integrated genes to their offspring. Several studies show that the sequences are stable in their position in the genome over many generations.

PART 3: The OX513A Mosquito Line

12. How did researchers establish a colony of GM mosquitoes homozygous for the lethality and fluorescence genes?

Scientists injected the two plasmids into mosquito eggs and grew the eggs in the presence of tetracycline. Once the eggs grew into adults, the adult mosquitoes were mated with wild-type mosquitoes and their offspring were screened for fluorescence at all developmental stages. Offspring positive for the fluorescence, which should be heterozygous for the lethality and fluorescence genes, were mated back with their parents multiple times to eventually create a line of homozygous GM mosquitoes (OX513A). These mosquitoes carry two copies of the lethality and fluorescence genes.

13. How are male GM mosquitoes selected?

GM mosquitoes are reared to pupae and then the pupae are mechanically sorted to remove females. Male pupae are smaller than female pupae and can therefore be separated according to size. The mechanical sorting typically leads to less than 1% female contamination.

14. Why are only male GM mosquitoes released in the wild?

Only GM males are released because the males do not bite humans or any other animals, so they do not contribute to the spread of the Zika virus or other pathogens.

15. How many male GM mosquitoes are released?

In one trial in Brazil, Oxitec scientists released about 500,000 mosquitoes per week and in a second trial 1.5 million per week (Garziera et al.*, 2017). The median survival of the male GM mosquitoes released into the wild is 2 days compared to 60 days for wild-type male mosquitoes. Two days is long enough for the GM males to mate with wild-type females. However, the method needs constant release to ensure a large enough population of GM males.*

16. What happens when GM males mate with wild-type females?

The GM male mosquito is homozygous for the fluorescence and lethality genes. When the GM male mates with a wild-type female, the GM father passes on one copy of the genes to all of the offspring. Because the female does not have these two genes, all the offspring will have one copy of each of these genes. The lethality gene and the fluorescence genes act in a dominant pattern, so all the offspring will glow red under fluorescent light and need

tetracycline to survive. Because tetracycline does not occur in large quantities in the wild, the offspring die before becoming adults.

PART 4: Safety

17. What are some of the things scientists monitor to make sure GM mosquitoes are safe?

The GM mosquito technology is new and has only been tested in a few locations. However, scientists have not found any evidence of negative effects on human or animal health. For example, they have performed studies on GM mosquitoes to show that the inserted genes do not create any products that are known to act as toxins or to cause allergic reactions in humans. Scientists also studied animals that normally feed on mosquitoes in the wild. They found that there was no significant difference in the development, lifespan, size, or survival rates of these animals after feeding on GM versus wild mosquitoes. Scientists have also been monitoring the effects on local ecosystem in areas where GM mosquitoes have been released. When health officials release the GM mosquitoes, they ensure that the area of release does not overlap with the ranges of endangered animals.

Some people are concerned that GM mosquitoes may have increased ability to survive in a range of environments, meaning that they could spread beyond the area where that species of mosquitoes normally lives. Additional studies showed that GM mosquitoes respond in the same way to changes in the environment as do wild mosquitoes, which supports the claim that GM mosquitoes would be limited to the same environments as wild mosquitoes.

Another concern is that over time, mosquitoes could mutate and be able to survive even in the absence of tetracycline. This is a possibility with sustained release, and scientists need to continuously gather and sample mosquitoes in the wild to collect data. For example, scientists collect GM mosquito larvae to study the location of the inserted DNA in their genomes and sequence the DNA to see whether it has changed in any way.

18. What is the evidence that releasing GM mosquitoes in the wild is effective in reducing mosquito populations?

Trials conducted in Brazil, Panama, and the Cayman Islands showed that sustained release of male GM mosquitoes can dramatically reduce the population size of wild A. aegypti *mosquitoes. These different trials reported reductions in mosquito populations ranging from 80% to 99%. It is not yet clear what percentage reduction is necessary to significantly reduce the spread of diseases like dengue, malaria, and Zika because there are many variables that impact the spread of pathogens.*

Additional Point of Discussion

The ethics involved in making public health decisions, especially when a novel technology is involved, are complex and there are no easy answers. Students may be familiar with previous attempts to limit pest populations that have had unintended consequences, such as the extensive use of the chemical DDT or the introduction of mongooses to Hawaii to control rats. You may wish to emphasize to students that the information gleaned from scientific research may inform discussions, but making the decisions involves having reasoned conversations among many stakeholders and weighing different options. In many cases, communities are directly engaged to make their own decisions about the risks and benefits of introducing a new technology into their environment, such as with the GM mosquito release in the Florida Keys.

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