DOES USING GM MOSQUITOES WORK?

The ultimate goal of producing and releasing genetically modified (GM) mosquitoes is to control the spread of human diseases by controlling the disease vectors. In this case, the disease vector is the *Aedes aegypti* mosquito.

To know whether the GM mosquito approach is effective in controlling this disease vector, one question you can ask is, “What effect, if any, has the GM mosquito program had on the local *Aedes aegypti* mosquito population?” That question leads to the following hypothesis: GM mosquitoes are an effective method for reducing the local *Aedes aegypti* mosquito population.

The Nature of Science

One way to test this hypothesis is to determine whether there is a change in the mosquito population before and after GM mosquitoes are released. A prediction you can make based on your hypothesis is that after the treatment period, areas treated with GM mosquitoes will have fewer wild mosquitoes than they did before the treatment. But if you do observe that the wild mosquito population decreases, how can you be confident that the decline was caused by the GM mosquitoes?

To increase your confidence in claiming that the GM mosquito program works, you need to include measurements from a control site. For example, the control site could be another area of similar size and environmental characteristics to the area in which the GM mosquitoes will be introduced. The control site is important because changes in the mosquito population could be caused by many factors, such as a change in weather patterns or a difference in mosquito predators. You want to be as sure as possible that any changes you observe are caused by the GM mosquito release.

Putting these ideas and considerations together helps guide a research design and is a critical characteristic of the nature of science.

Research Design

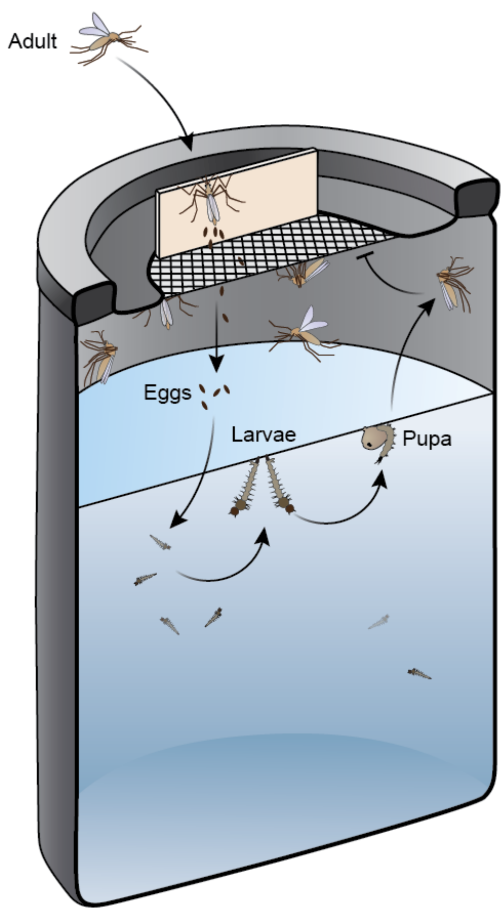
1. Identify the area in which GM mosquitoes will be introduced. Call this area the treated area.
2. Identify another area similar in size and environmental characteristics to serve as a control. Call this area the untreated area.
3. Measure the density of the mosquitoes in both places before the program begins and then several times after the program has started.
4. Remember that variables other than density must also be measured to make sure that they do not change or become different between treatments during the experimental period. These are called controlled variables.

Data to Collect

Your goal is to determine whether there is a change in the mosquito population before and after GM mosquitoes are released. To do that you will need to calculate some type of estimate of the population size.

In some studies, you may be interested in estimating the total number of individuals in a population. For example, you might want to estimate the number of elephants that exist to determine whether the species is endangered. But for an animal like the mosquito, *population density* is actually a more informative statistic for determining the risk of spreading disease. That's because being in an area with a greater mosquito density increases the probability of being bitten by a mosquito and getting a mosquito-borne disease.

Density is the number of individuals in an area divided by the size of that area. Determining the number of individuals in an area can be relatively easy. For example, if you want to know the number of students in your class, you would simply count them. But determining the number of small insects, like mosquitoes, in an area is more challenging. Mosquitoes are difficult to see, and their numbers may be very large. Thus, biologists collect samples and use those samples to estimate density.



Samples are taken using traps, called ovitraps, that capture mosquito eggs (Figure 1). Each ovitrap consists of a water container with dark sides, which makes them an attractive site for females to lay their eggs. Partially submerged cardboard or wood provides the surface on which to lay eggs. *Aedes* mosquitoes prefer to lay their eggs on a substrate that is subject to intermittent flooding, such as plant material, soil on the edge of a pond, or the sides of human-made containers. All the ovitraps in a study should be identical to each other so that any differences in the data from the ovitraps will reflect differences in mosquito density among the sampled areas.

Biologists can use ovitrap data to produce a statistic called an ovitrap index. They then can compare the ovitrap index between different collection sites or, in the case of our GM mosquito experiment, between the treated area and the untreated (control) area.

Scientists take samples from multiple sites within a larger area to reduce their uncertainty and get more-reliable estimates. In studies like this one of mosquitoes, one common approach is to take at least 10 samples per square kilometer. Each trap has a unique code (Sample No.) matched to its location.

Calculations

After the traps have been set for a number of days, they are brought to the lab and left to dry. After the drying process, the eggs in the ovitrap are placed in water where the eggs hatch. Scientists then count the number of larvae that develop to estimate the number of eggs that were laid.

Calculation 1: Ovitrap Index (OI)

The ovitrap index is a simple calculation where the number of ovitraps that contain at least one egg is divided by the total number of traps placed in the area.

$$\text{ovitap index (OI)} = \frac{\text{no. of traps with 1 or more mosquito eggs } (L)}{\text{total number of traps } (T)}$$

Look at the data in Table 1. Ten ovitraps were set in a treated area and later collected. In this example, there were 4 traps with eggs out of 10 total traps. The OI for this group is 4/10 = 0.4. (Note: "Sample no." in Table 1 shows the actual labels used by the researchers in a GM mosquito study.)

Table 1. Number of Eggs from 10 Ovitraps in a Treated Area *Before* Treatment

Sample No.	No. of Eggs
PIR-CE-001	0
PIR-CE-002	1
PIR-CE-003	0
PIR-CE-004	0
PIR-CE-005	2
PIR-CE-006	0
PIR-CE-007	2
PIR-CE-008	0
PIR-CE-009	0
PIR-CE-010	1

Calculation 2: Average Density (AD)

Another population statistic that can be calculated is called average density. The average density calculated from the ovitrap data is not the actual average density of adult mosquitoes in an area, but the statistic can be used to compare data across collection sites. *Average density is the average number of eggs from each trap*.

$$\text{average density (AD)} = \frac{\text{number of eggs from ovitraps } (E)}{\text{total number of traps } (T)}$$

Looking at Table 1 again, there were 6 eggs in 10 traps in the treated area before treatment. Therefore, the average density before the release of GM mosquitoes, AD = 6/10 = 0.6 eggs/trap.

Calculation 3: Relative Change

Remember that in our GM mosquito experiment we wondered what effect, if any, the GM mosquito program had on the local mosquito population. We can use the average density statistics from the treated and untreated areas to answer this question by looking at the relative change between the two areas:

$$\text{relative change} = \frac{\left(\frac{T_a}{U_a}\right)}{\left(\frac{T_b}{U_b}\right)} - 1$$

In this equation,

- T_b represents the average density (AD) in the treated area before treatment,
- U_b represents the AD in the untreated area before treatment,
- T_a represents the AD in the treated area after treatment, and
- U_a represents the AD in the untreated area after treatment.

(Note: Subtracting 1 from the ratio helps us see if there was a decrease or an increase in relative density.)

Using the data in Table 1, we calculated the average density of a treated area before treatment, which represents T_b . According to our calculations, $T_b = 0.60$. Now, suppose that the average density in the untreated area before treatment, U_b, equals 0.52. The ratio of the average densities for the two sites is $\left(\frac{0.60}{0.52}\right)$. The average density in the treated area at the end of the study, T_a, is 0.11, and the average density in the untreated area at the end of the study, U_a, is 0.49. The ratio of the average densities for the two sites at the end of the study is $\left(\frac{0.11}{0.49}\right)$.

The relative change in average density for this study therefore equals $\frac{\left(\frac{0.11}{0.49}\right)}{\left(\frac{0.60}{0.52}\right)} - 1 = -0.81$.

We can easily convert the relative change statistic to a percentage: −0.81 × 100% = −81%.

In other words, the treated area saw a decrease in mosquito density of around 81%.

Calculation 4: Mating Fraction

Although there may be a difference in the average density of mosquitoes between the untreated and treated areas, there may be alternative explanations for the difference other than the GM mosquito program. For example, perhaps a localized weather event or a predator caused the difference.

To help eliminate these alternative explanations, scientists also collect data on the percentage of females that are mating with GM males. If the density of mosquitoes declines when a high fraction of females are mating with GM males, then the case for the GM mosquito program having an impact is stronger.

Scientists can determine the number of larvae that come from GM males because these larvae fluoresce (glow red) when a specific type of light is shined on them. When eggs collected from an ovitrap are placed in water, they will hatch into larvae. Larvae are then screened for fluorescence using a fluorescent microscope. The GM mating fraction is calculated as the fraction of fluorescent larvae to total larvae:

$$\text{mating fraction } (M) = \frac{\text{number of fluorescent larvae } (F)}{number\ of\ nonfluorescent\ larvae\ (N) + F}$$

$$M = \frac{F}{N + F}$$