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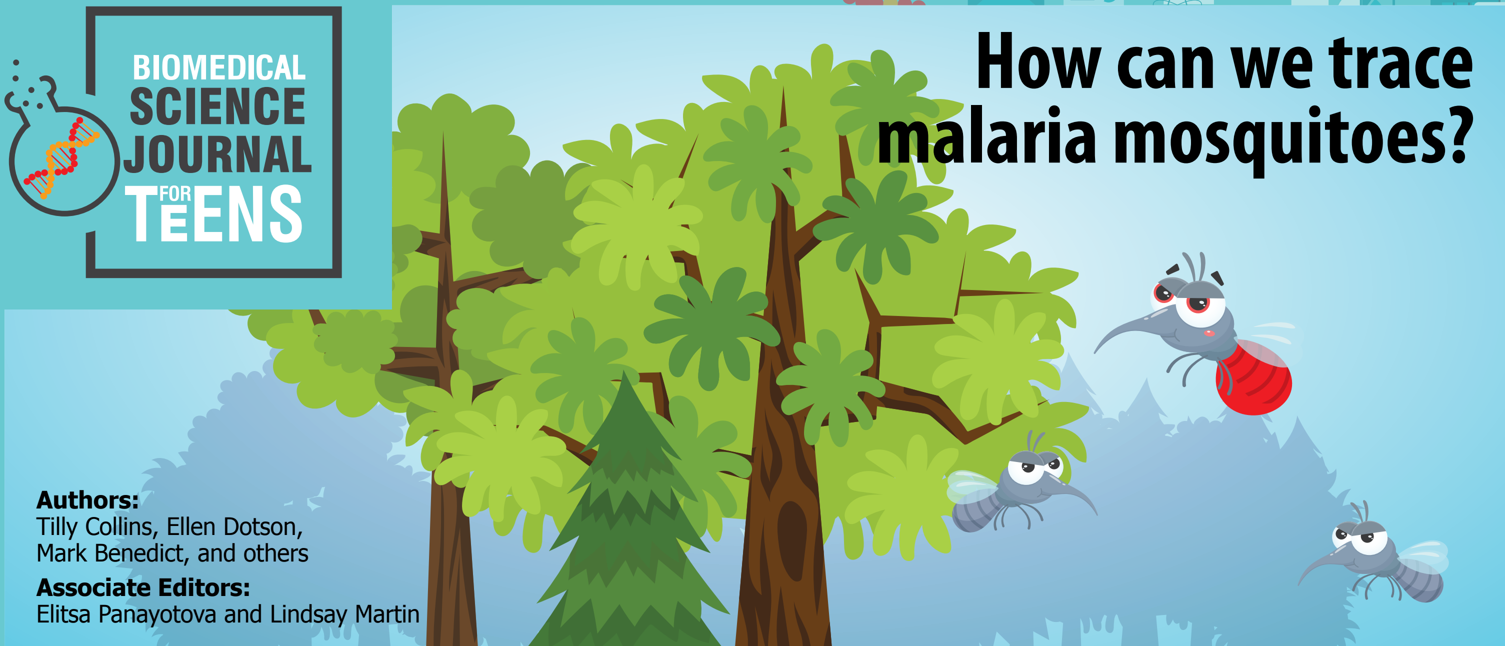
How can we trace malaria mosquitoes?



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Abstract

Do you find mosquitoes annoying? Not only can they be quite irritating, but they also carry many deadly diseases. One method to control these diseases is to control the mosquito populations. But we have to know more about them – how many there are, where they are, their mating behavior, etc. To do this, researchers most commonly use the mark-release-recapture technique. Current mosquito markers have some downsides because they can change the mosquitoes' behavior. This is why our research attempted to mark *Anopheles* mosquitoes with two dyes: rhodamine B and uranine. We found that both dyes are suitable for marking and tracking mosquitos.

Introduction

Mosquitoes can be quite a nuisance on a warm summer evening! They are also responsible for the deaths of millions of people around the world because they carry deadly diseases (such as malaria, dengue, yellow fever, and many others). So what can health authorities do to control these mosquito-borne diseases?

Vaccines are great for preventing diseases, but they often take a long time to develop. Also, there are diseases (like malaria) that are so complex that it's still difficult for scientists to develop effective vaccines. Most governments have methods for controlling mosquito populations. After all, the fewer mosquitoes there are, the fewer cases of mosquito-borne diseases there are. Using *insecticides* is popular, but *insecticide resistance* has spread among mosquitoes. Insecticides can also be bad for the environment.

In order for us to control mosquito populations, we must know where they are, how many there are, how far they fly, and their **mating behavior**. But how can we find this out? Mosquitoes are very tiny, so we can't put a tag or a radio device on them. Researchers most commonly use the *mark-release-recapture* technique. This means they capture mosquitoes, mark them, release them into the wild, and capture them again for observation. The proportion of marked mosquitoes in the recaptures made in different places helps us to understand many things about their behavior and survival.

There are different types of markers for mosquitoes, like different dyes and *isotopes*. But the most common among mosquito researchers is *fluorescent dust*. It can affect the insects' behavior, though. This is why we looked for better alternatives. Previous researchers successfully marked *Aedes aegypti* (the primary *vector* of dengue and yellow fever) with rhodamine B. But what about the primary vector of malaria, *Anopheles gambiae*? Would rhodamine B or uranine, an additional dye tested here, be suitable for marking?

Why is it important to understand **mating behavior**? Since some mosquitos are insecticide resistant, research has started to develop gentic tools to control mosquito populations. For example, the release of mosquitoes that are unable to have offspring (babies). If you are curious, here is more information:
https://sciencejournalforkids.org/articles/how-can-bacteria-stop-dengue/

Methods

We *cultured Anopheles gambiae* mosquitoes in the lab. For three nights we fed different groups of mosquitoes sugar water containing different concentrations of the markers.

We wanted to determine a concentration that was detectable, but not toxic for the insects.

- For each of the markers (rhodamine B and uranine), we prepared 0.1, 0.2, and 0.8% concentrations and fed each to a different group of mosquitoes.
- We then counted how many had survived after the third night.

We also wanted to see if feeding on the markers influenced *longevity*. To do this, we fed 90 marked and 90 unmarked mosquitoes with sugar water and recorded how many of them died each day (after the initial 3 days of marking).

To determine how long the markers remained detectable, we looked for the dyes every week for three weeks using a suitable microscope (Fig. 1).

What about mating behavior? Males transfer fluids to females when mating, so it was possible that the dyes would be transferred in the process. We allowed around 100 marked male mosquitoes and 30 unmarked females to mate for 4-7 nights. We then looked for the dyes inside the females.

Finally, we wanted to make sure that marked males had the same chance of mating with females as unmarked males. So, we combined 20 marked males and 20 unmarked males with 40 females. Then we counted how many of each group had mated.

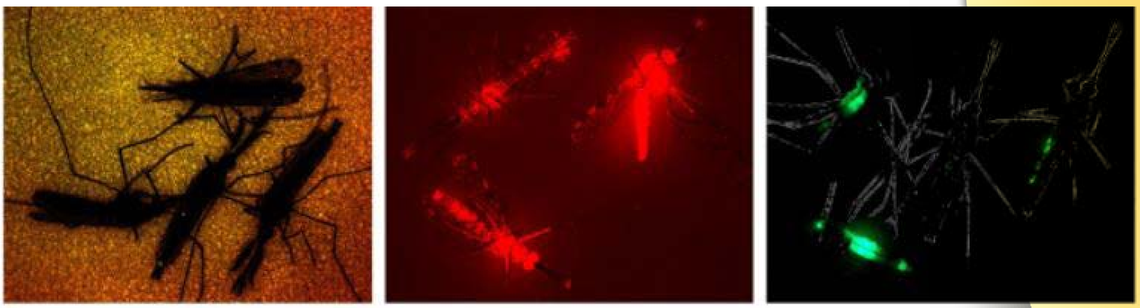


Figure 1: How mosquitoes look under a microscope: unmarked (left), marked with rhodamine (middle) and marked with uranine (right).

Results

We found that 0.8% of rhodamine was too toxic. Uranine, on the other hand, was not toxic at any of the examined concentrations.

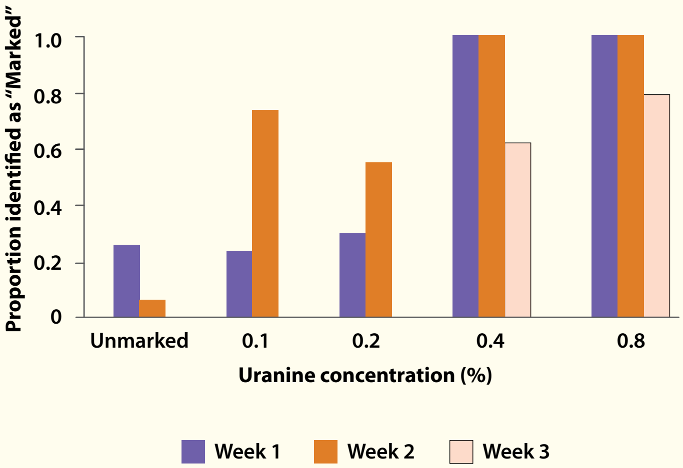
As for the duration of marking, we detected rhodamine each week for 3 weeks after marking. Uranine did not last as long, though. The color it produced was very similar to natural *autofluorescence*, so we had problems distinguishing it at the lower concentrations (Fig. 2).

We could detect the marker in almost all females after mating with a marked male, which means the dyes are suitable to track matings because they are transferred along with the mosquito sperm.

There is no reason to believe that markers made it harder for male mosquitoes to mate. However, marked males actually had a slightly better chance at mating with females than unmarked males. The markers didn't have a significant effect on longevity either.

Figure 2: Durability of uranine marker

Which concentrations of uranine are still detectable at Weeks 2 and 3?



Discussion

Our results show that both dyes can be useful for mosquito marking. Rhodamine B performed a little bit better. It turned out to be more permanent than uranine. Plus, rhodamine B can't be confused with autofluorescence as that appears red in color. Sometimes a uranine dye produces a yellow-green color, similar to autofluorescence.

The major disadvantage of our method is that researchers must feed the insects for 3 days before being used so that all the mosquitoes get the marker. Fluorescent dust is usually in the form of sprays, making it much easier and faster to use. However, dust can sometimes change the mosquitoes' behavior and longevity, and gradually comes off when they are released. And dust can't track matings among the mosquitoes like the two dyes we tested.

Conclusion

Health authorities take many measures to control mosquito-borne diseases trying to make you safe. But there are also things you can do to control mosquito populations:

- Remove standing water from your home (this is where females lay their eggs). Standing water is usually found in ponds, planters and pots, bird baths, or any type of containers that can hold at least 1 inch/2.5 cm of water.
- Use mosquito nets (with insecticides).
- Use window screens.
- Use approved insecticides to kill mosquitoes when appropriate.

And remember, avoiding mosquito bites is the best prevention! Use *repellents* and wear long sleeves and trousers.

Glossary of Key Terms

Autofluorescence – the natural emission of light from biological substances when illuminated with a UV light source.

Culture – to grow living material (like a mosquito) on, or in, a special substance.

Fluorescent dust/powder – tiny particles of fluorescent dye that researchers use to mark insects and other arthropods (insects, ticks, etc.) on the outside of the body. Usually fluorescent dust is visible to the naked eye, and sometimes a UV light is needed.

Insecticides – chemicals that kill insects.

Insecticide resistance – occurs when insect populations change as a result of the use of these chemicals. The insecticides can no longer kill the insects because over time a gene increases in the population that allows it to survive.

Isotopes – atoms with the same number of protons and electrons, but a different number of neutrons. (Picture three apples. One has five seeds, another has seven seeds, and the last has twelve seeds. Although they have a different number of seeds, they are all still apples.)

Longevity – length of lifespan.

Mark-release-recapture – A method used in ecology to estimate the size and characteristics of an animal population. For example, researchers capture a group of animals, mark them (with a tag or some other marker), then release them. Later, the researchers capture a group of wild animals and count the number of marked animals within the second group to estimate the overall size of the population.

Repellent – a chemical or natural substance that deters insects from approaching and biting.

Vaccine – an injection (or inhalation) made from very small amounts of weak or dead germs that can cause diseases — for example, viruses, bacteria, or toxins. It prepares your body to fight the disease faster and more effectively so you won't get sick.

Vector – an animal or insect that spreads diseases by carrying the pathogens that cause it. For instance, *Aedes aegypti* mosquitoes can carry dengue, Zika, and others. *Anopheles gambiae* can carry malaria.

Check your understanding

1. Why are insecticides becoming a less effective option for mosquito control?

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2. What are some of the methods for mosquito-borne disease **prevention** that were discussed? Can you think of any others?

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3. What are some other methods used to control the mosquito population?

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4. Why do we think rhodamine B is better than uranine for marking mosquitos?

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5. What are some advantages of the two dyes over fluorescent dust, the most commonly used marker for mosquitoes?

REFERENCES

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