PROFESSIONAL BUTTERFLY FARMING – PART I
The Livestock and Production Processes - By Nigel Venters
Monarchs and O.e. – A Scientist's View
(Contributing Author: Dr. Sonia Altizer)

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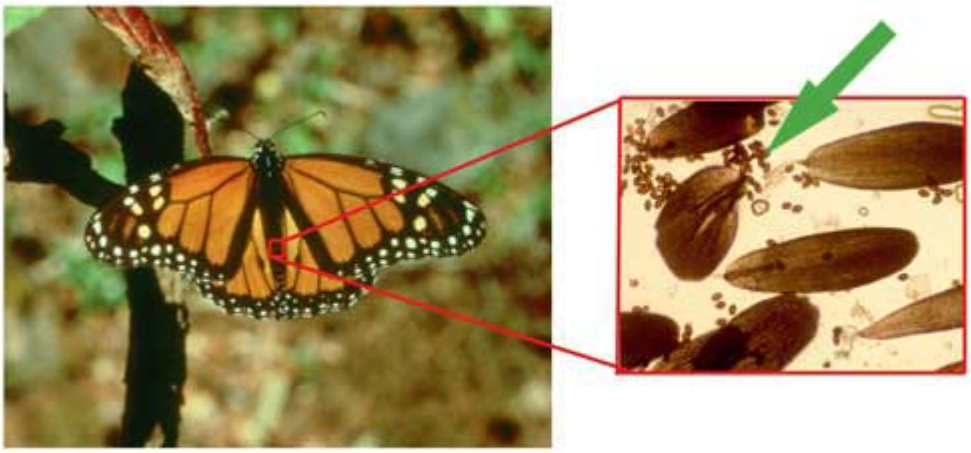
Monarch Health Program, University of Georgia
http://monarchparasites.uga.edu/

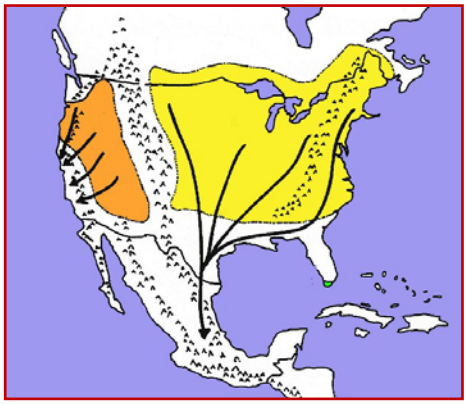
What is O.e.?



*Ophryocystis elektroscirrha (*OE*)* is an obligate, protozoan parasite (shown by the green arrow above) that infects monarch and queen butterflies. In simpler language, OE isn't an animal or a plant, but a protozoan. Protozoans are single-celled organisms, living things that have many of the same characteristics as animals. In fact, protozoans are often called animal-like protists.

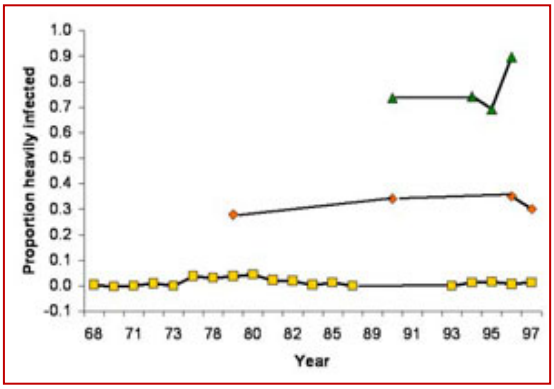
OE is considered an obligate parasite because it must live within a host to grow and multiply. Between infections OE survives as spores that are resistant to extreme environmental conditions. OE was first discovered infecting monarch and queen butterflies in Florida in the late 1960's. There are no other known hosts. It has since been found in all other monarch populations world-wide. Because of this world-wide range, all indications are that this parasite has co-evolved with monarchs.

How common is OE in North American Monarchs?



There are three major Monarch populations in North America. Most research has focused on the population that breeds east of the Rocky Mountains and migrates every year to wintering sites in the transvolcanic mountains of central Mexico (shown in **yellow** on the map). After mating in the spring they migrate north to their summer breeding grounds in the United States and Canada. A second monarch population west of the Rocky Mountains has a shorter, less dramatic migration to their roosting areas on the coast of California (shown in **orange** on the map). There are also non-migratory populations that breed year-round in southern Florida (shown in **green** on the map), coastal Texas, Hawai'i, the Caribbean Islands, and Central and South America. Since milkweed plants grow here all year round, the butterflies do not need to leave the area. These resident Monarchs reproduce throughout the entire year.

How common is OE in North America?

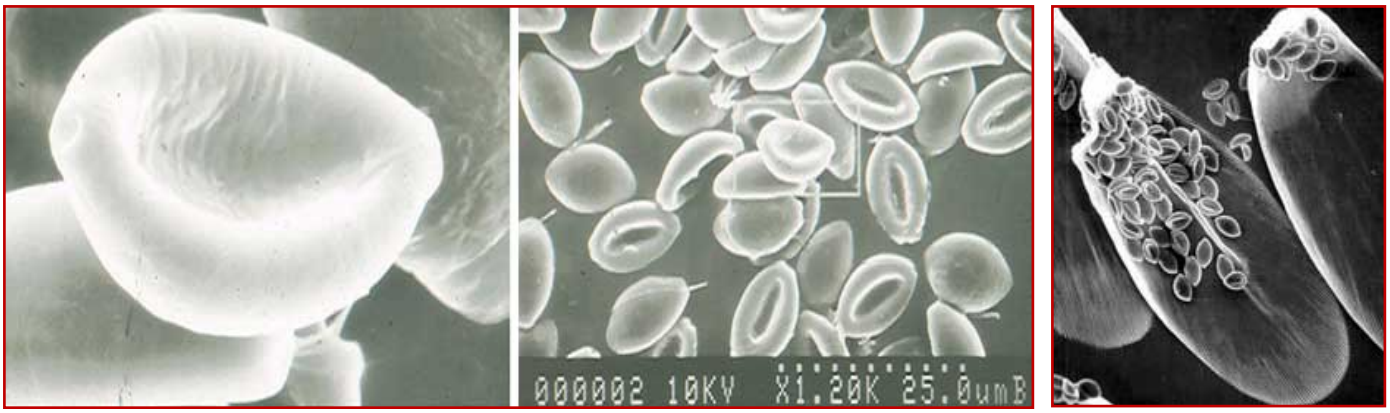


OE infects monarchs in all three North American populations. The **eastern** migratory monarchs have the lowest infection rate. Less than 8% of these butterflies are heavily infected with OE. More monarchs have OE west of the Rocky Mountains. About 30% of the **western** migratory population is heavily infected with OE. The highest rate of OE in North America occurs in the **non-migratory** monarchs of South Florida. More than 70% of these monarchs have OE infections. The infection rates for monarch populations in North America have been constant for many decades.

OE Spores

OE spores are dormant cells found on the outside of infected monarchs. These tiny spores are sandwiched in between the scales that cover a butterfly's body. The greatest concentration of spores is usually on the abdomen. Spores are much smaller than scales. In fact, a monarch scale is about 100 times larger than an OE spore. You must use a light microscope set at 40 to 100X to see a spore. Even at this magnification spores look like small, brown or black lemon-shaped objects. Here is a picture of OE spores at 400X:

The images below (taken by Chip Taylor) show scanning electron micrographs of parasite spores clustered on abdominal scales from a parasitized monarch.

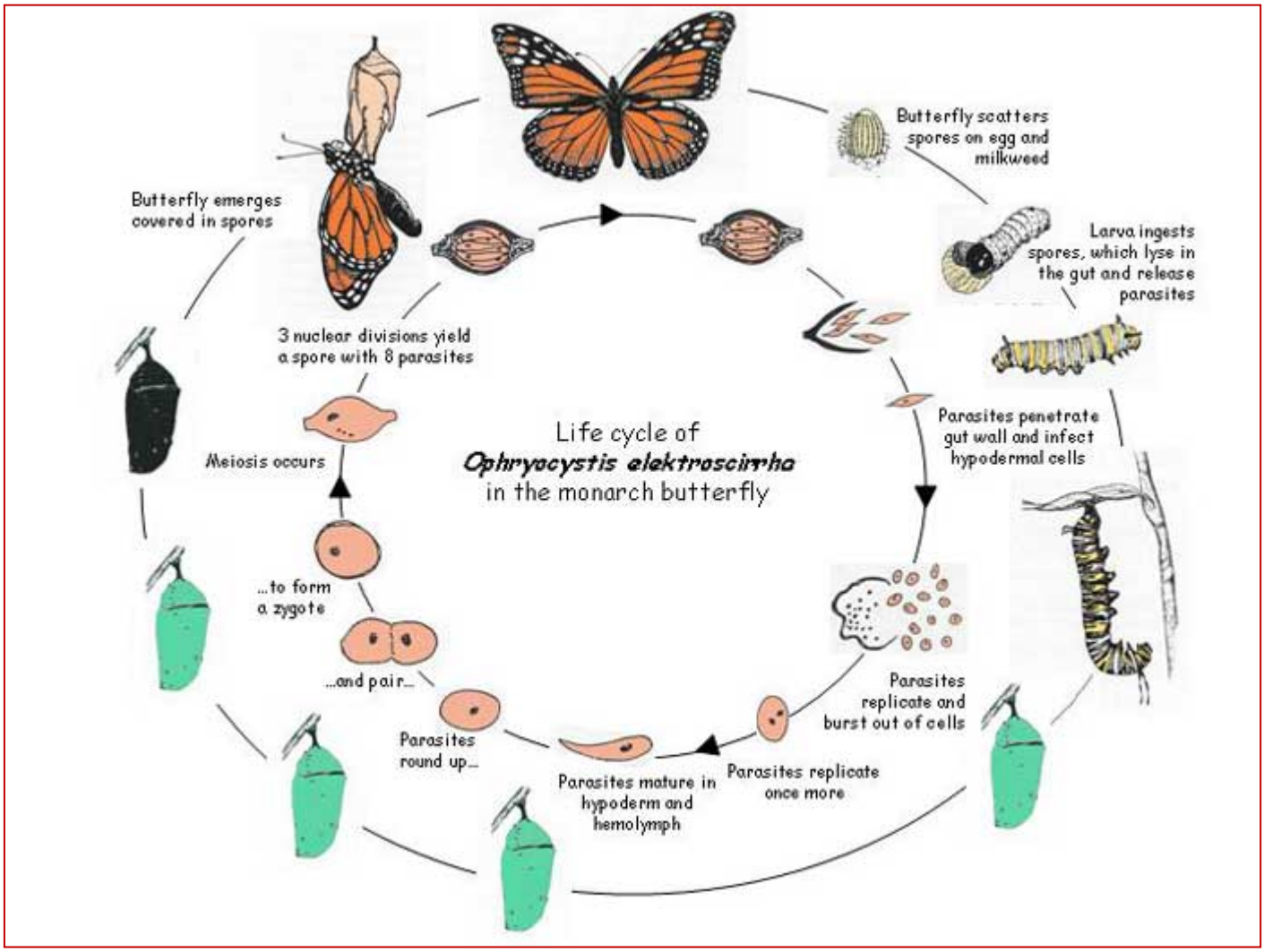


Life Cycle and Transmission

The life cycle of OE is very closely related to the life cycle of the monarch butterfly. OE can only reproduce inside the insect's body. Infected females pass on the parasite to their offspring when they lay eggs. Dormant spores on the outside of the female's abdomen are scattered on the eggs and milkweed leaves. When a caterpillar hatches, its first meal is the egg shell. The caterpillar will eat OE spores along with the shell and milkweed.

The dormant spores move through the larva to the midgut. Digestive chemicals break open the spores releasing the parasites. The parasites move into the intestinal wall to the hypoderm. Here OE reproduces asexually. Each OE parent cell divides many times, greatly increasing the number of parasites.

Most damage to the butterfly happens during the pupal stage. The OE parasite goes through sexual reproduction. Again the number of parasites in the monarch increases. About three days before the adult emerges from the pupa, OE spores will begin to form. Spores allow OE to survive outside of the monarch's body. The spores can be seen through the integument or outside layer of the pupa. Infected adults emerge covered with spores. *Once butterflies are infected, they do not recover.* By the time adults emerge with parasite spores, all physical damage by the OE parasites has been done –the parasites do not grow or reproduce on the adults. The spores are inactive or dormant until they are eaten by another caterpillar.


Butterfly scatters spores on egg and milkweed
Larva ingests spores, which lyse in the gut and release parasites
Parasites penetrate gut wall and infect hypodermal cells
Parasites replicate and burst out of cells
Parasites replicate once more
Parasites mature in hypoderm and hemolymph
Parasites round up...
...and pair...
...to form a zygote
Meiosis occurs
3 nuclear divisions yield a spore with 8 parasites
Butterfly emerges covered in spores
Life cycle of
Ophryocystis elektroscirrha
in the monarch butterfly

How To Tell If Butterflies Are Infected With OE

An infected pupa may develop dark spots or blotches two or three days before the butterfly emerges. These abnormal dark areas are parasite spores. Spores form on the eyes, antennae, wing veins, but mostly on the abdomen. You can see the spores through the outside layer of the pupa a day or two before pigments that color the butterfly normally darken the pupa. Before a butterfly emerges from the chrysalis, pigments are laid down coloring the scales that cover the butterfly. This normal change in the color of the pupa is symmetrical. The color change of an infected monarch happens earlier and does not create a balanced pattern on the pupa.

Adults that are heavily infected with OE are weak and often have difficulty emerging from the chrysalis. Some monarchs die before emerging. Others emerge, but are too weak to cling to the pupal case. They fall to the ground before fully expanding their wings. These severely deformed monarchs do not survive long. analysis.

Mild OE infections also harm butterflies. Infected adults are often smaller than healthy monarchs. They weigh less and have shorter forewing lengths than normal. Parasites also damage the cuticle or outside layer of the monarch's abdomen. This damage causes the butterfly to dry out and lose weight faster than normal. This is especially a problem if there is a shortage of nectar or water. Studies have shown that monarchs infected with OE can not fly as far or as long as healthy butterflies. Since infected males are weak, they are less likely to mate and produce offspring than uninfected males. Infection does not appear to harm the ability of females to reproduce.

While these may all be symptoms of OE infection, many infected monarchs look healthy. They emerge normally and are not deformed. The only way to really know if your monarch is infected is to check for spores.

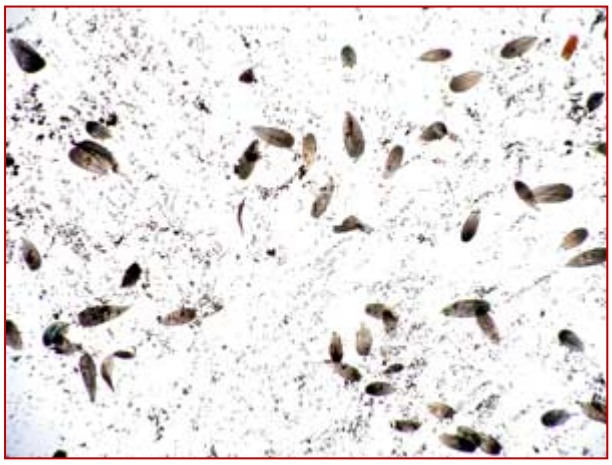
Sampling Monarchs for Parasites

Monarchs can be easily assessed for parasite loads by pressing a piece of ultraclear Scotch TM tape on their abdomens and counting the number of spores in a 1cm x 1cm area. This slide shows how spores appear relative to abdominal scales under the light microscope at 200x.

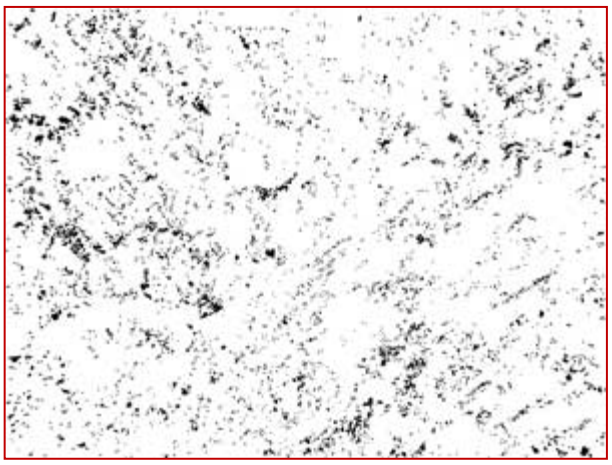
In our lab, we use this 'tape method' to categorize parasite loads on an approximate logarithmic scale of 0-5, with 5 being the most heavily infected class, and 0 being butterflies with no detectable spores.

This method allows for rapid classification of disease status and the severity infection – and is highly correlated with the log of total infection loads estimated using a destructive wash-and count method.

We also create tape samples from swabs sent in by our *MonarchHealth* participants. People in our lab have also developed more innovative methods for using digital image analyses to get more refined and continuous measures of spore densities (*below*).



Digital picture of scales and spores obtained from an infected monarch. OE spores look like dust particles in this picture.



Same picture after digitally removing scales. Computer then does the rest. There are approximately 3600 spores in this picture!

What do infected monarchs look like?

The answer to this question depends on how heavily infected the monarch is. Many OE infections, especially of eastern North American monarchs, are 'mild' cases, and the infected adult monarch will look nearly identical to a healthy adult. Mildly infected adults will also act normal, so it is usually impossible to know if they are infected without testing them using the methods described below. Unfortunately for those of us that rear monarchs in captivity, these mildly infected adults can spread their spores around their cages and rearing containers just as well as heavily infected ones, which is why it is essential to test all adult monarchs upon eclosion, and remove any infected ones immediately. Also, since there is no way to 'cure' adult monarchs once infected, they must be destroyed. Releasing them to the wild will only spread the parasites further and you will risk contaminating your favorite local milkweed patch.

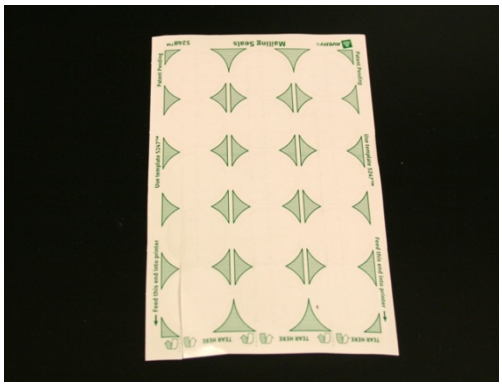
Below are some pictures of various OE infected monarchs. The monarch in the *bottom right* image is indeed infected, but appears otherwise normal.

How to Test for Parasites

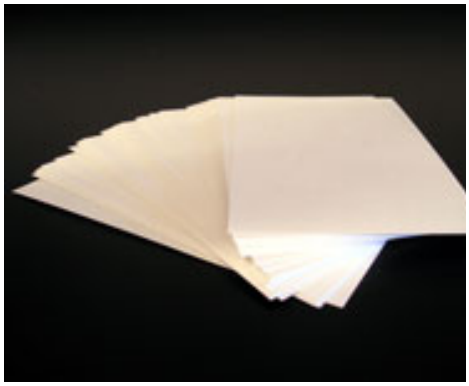
If you are interested in examining your own butterflies for the OE parasite, you can follow these steps using the supplies sent to you in your new kit. Most of our participants use the tape method below for project *MonarchHealth*.

Things you will need for testing using the tape method:

Disposable gloves



Clear tape stickers



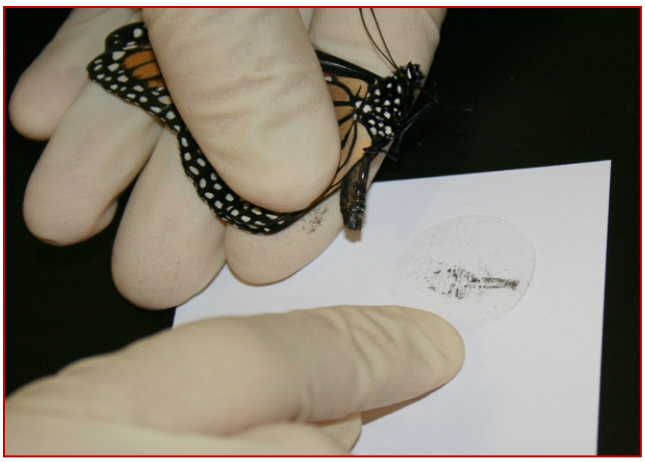
Blank index cards

Step 1. Put on your gloves!

Step 2. Hold the monarch firmly as shown in the picture below, using a gloved hand. Be sure not to use your other hand to touch the butterfly because that hand will be used to hold the tape sticker and sample for spores. It is critical that you keep your bare hand completely free of touching the butterfly throughout this process!

Step 3. Pick up a tape sticker using your other hand and gently place the sticky side of the piece of tape to the abdomen of the monarch. Press down so that it wraps around and sticks to the sides of the abdomen.

Step 4. Gently peel the tape sticker off and stick it to the index card. You will remove some scales in the process, but don't worry, it will not harm the monarch.



Finish by labeling the sticker sample on the spore card with the identity (we use a number) of the monarch. Continue these steps until you have sampled all of your monarchs. In the end, your index cards should look similar to this:

The numbers on the bottom of each sample refer to the number of the butterfly in our lab, and the numbers above refer to the parasite 'spore load' on each monarch (we use a 0-5 scaling system). Notice that one monarch from these samples was infected and was given a 5 score (heaviest infection category).

While you have the butterfly out, determine if it is a female (photo on left) or male (photo on right). The two sexes are easy to tell apart because males have a small raised black spot on a vein on each hind wing that is not present in females.

Step 5. Record data on data sheet for each butterfly: date collected, date sampled, gender, etc.

Step 6. Prepare the sample for mailing.

Once filled out completely, place the index card into the larger mailing envelope. Once you have sampled 10 or more butterflies, or at the end of the summer season, return the pre-addressed envelope to our lab at the University of Georgia. Samples should be mailed in the envelope provided to:

Project Monarch Health - c/o Sonia Altizer
Odum School of Ecology
University of Georgia
Athens, GA 30602-2202

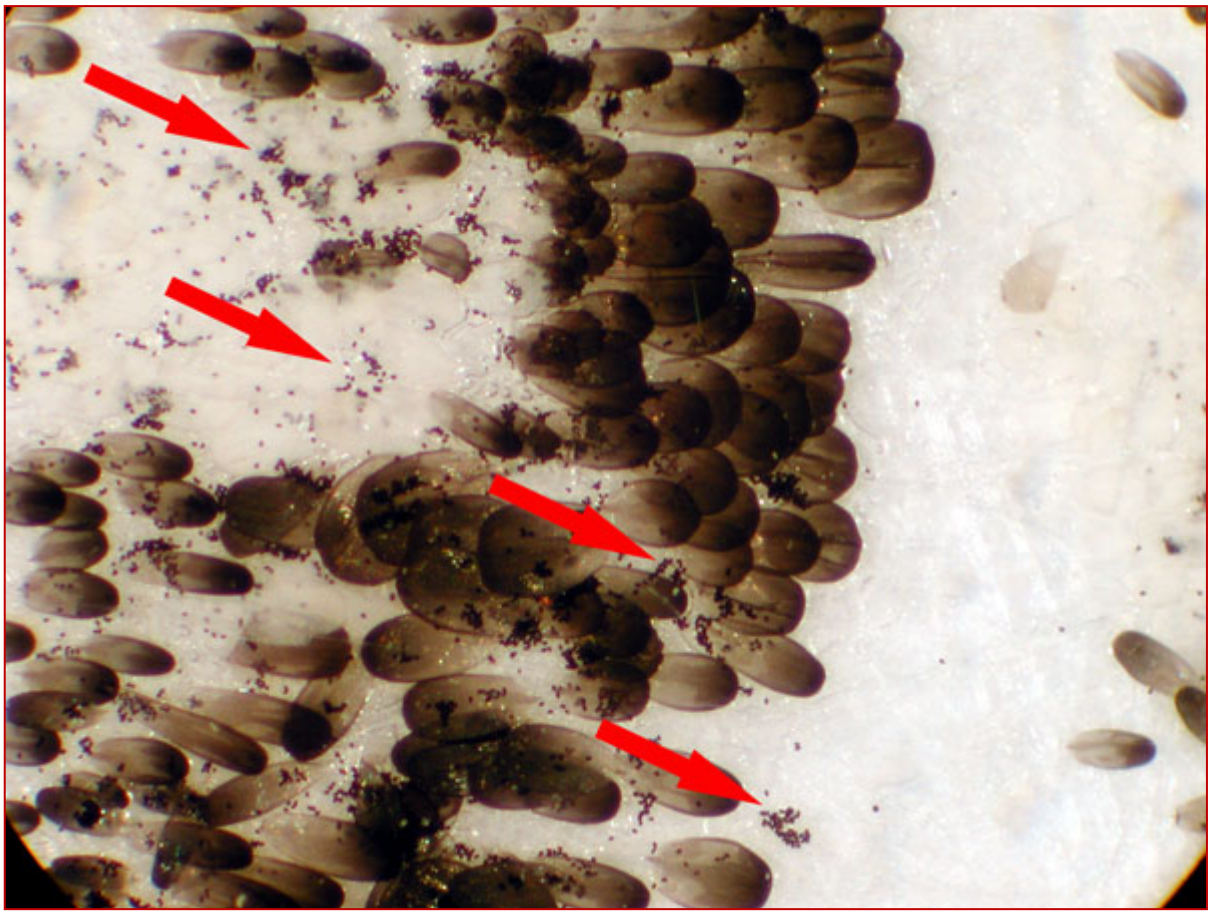
Step 7. Wipe down your work surface with the bleach wipes when you finish and dispose of your gloves before handling another monarch.

It's important to try and keep a clean work area when sampling the butterflies for parasites. One monarch may be infected, while another may not. Changing gloves and wiping areas with bleach wipes will prevent cross-contamination of different samples and help avoid touching a non-infected sample with infected spores from another.

Are the monarchs I sampled infected?

We will examine if your samples show if the monarchs are infected. Once we have the results, we will notify you the infection status. Results from all participants will be compiled and sent to all volunteers and updated on the website.

Here's what a sample looks like when we examine it under the microscope:



The red arrows indicate the parasite spores in this image. The big objects are the monarch scales. The spores look like tiny lemon-shaped objects, often clumped together. They often have a reddish tinge. This monarch would be considered heavily infected.