A Reductionist's Approach to Honey Bee Biology

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Every part of your body is made of cells. Different tissues in your body will have slightly different cells that are specialised to perform certain tasks. For example, neurons transmit electrical impulses around your body, red blood cells transport oxygen through your blood and white blood cells fight infection – even your fat cells are useful as they allow you to store energy for a time when nutrient availability is low.

Normally, your cells cannot survive unless they are a part of you, but scientists have been using a technology for over 50 years where they take cells from a living organism and cultivate them in a laboratory. This can work very well providing that the cells are fed with the nutrients that they would usually get from your body. The cells are stored in specially treated flasks and, when given the right environment, will start dividing and behaving in a similar way as if they were still part of the body tissue from which they came. In the laboratory, these cultivated cells are referred to as *cell lines* and are very useful for studying questions about how cells interact with their environment. They can also be used to determine the function of genes.

Cell lines have a benefit over using a live organism or superorganism as you can directly study certain cells such as neurons or blood cells while avoiding the complexity of the whole organism.

Cell lines can be made from almost any organism: humans, hamsters, beetles, plants, to name only a few. Cell lines avoid the ethical concern of experimenting on live animals (or humans), the cost is significantly reduced, experiments can be performed on a much larger scale and they allow you to tightly control the environment of your study system.

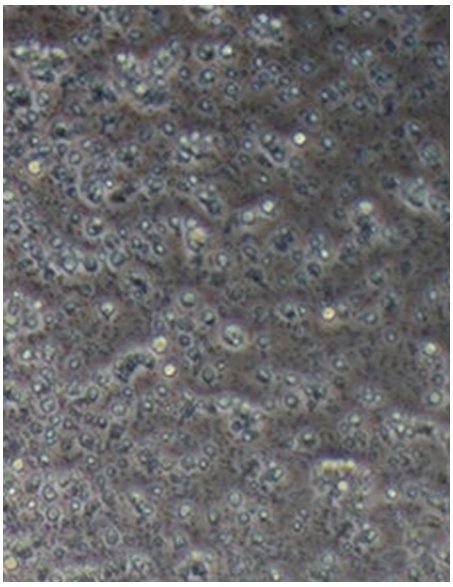
Honey Bee Cell Lines

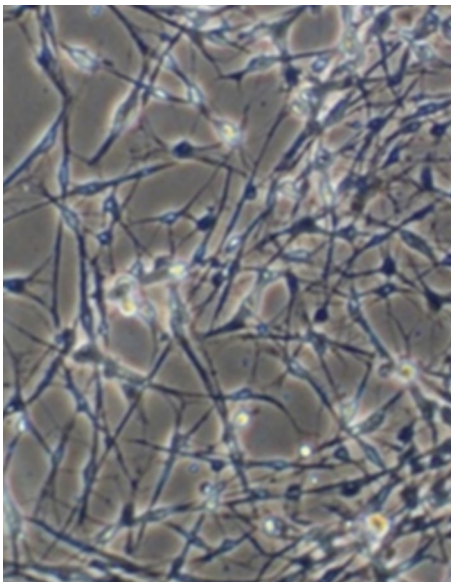
I had been interested in working with honey bee cell lines for many years. In my previous job, we used mosquito cell lines to study an insect bacterium called *Wolbachia*. We discovered that these bacteria interfere with the normal movement and processing of cholesterol in the insect cell, which then prevents some viruses from getting into the cell to replicate (Geoghegan, et al, 2017).

I thought it would be useful to have a honey bee cell line to gain a better

The University of Minnesota Bee Laboratory

Photography: Kirsty Stainton





Michael Goblirsch's honey bee cell lines seen through a microscope

understanding of how honey bee viruses interact with bee cells and how we could stop them in their tracks. So, I contacted Michael Goblirsch, a researcher in the United States of America (USA) who specialises in honey bee cell lines. In 2013, Michael created a cell line from cells collected from honey bee eggs (Goblirsch, et al, 2013). These cells could reproduce in the laboratory, allowing him to continue growing and studying the cells for many years.

Using his cell line, Michael was able to study the interactions between honey bee cells and different bee viruses. He infected the cell line with different viruses and showed that his cultured cells responded to the infection in the same way as the adult bees (Carrillo-Tripp, et al, 2016).

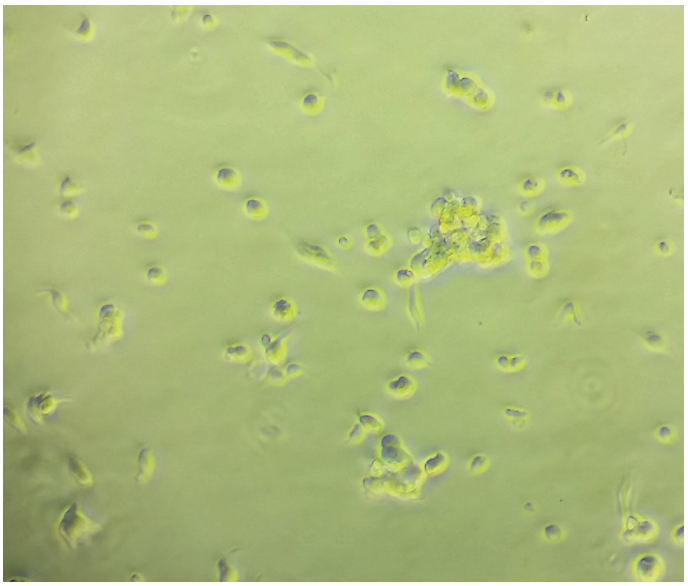
A cell line may not behave exactly as the cells in an organism. Some cells may need to interact with other cell types or factors in the host organism, so before using a cell line for study, you first need to make sure that the responses you are measuring will be similar in the host.

University of Minnesota Bee Laboratory

Michael wanted to impart his skills in creating a cell line for honey bees to fellow researchers, so that they could make lines for their own research projects.

In July 2017, I visited the University of Minnesota Bee Laboratory to learn this specialised technique alongside scientists from Australia, China and the United States. Michael showed us how to collect eggs from a comb, disinfect them and mash them up into a mixture which contains all the proteins, fatty acids and sugars needed for the cells to survive. This mixture or 'media' must be extremely clean; it is a rich and tempting treat for bacteria and fungi.

Infection with micro-organisms will result in a quick end to any cell line, so all work on cell lines must be conducted in a special unit called a biological safety cabinet. This helps prevent airborne invaders from getting in.



Cells extracted from honey bee eggs during the workshop

Once the eggs have been mashed up, the cells leak into the media and will attach to the bottom of the flasks. The living cells are 'sticky' as they usually stick to one another, but in the culture flask, they stick to the plastic and grow on it. Although this may sound strange, it is a useful feature as it allows you to remove the spent media and dead cells, and replace it with fresh clean media without losing the living cells.

The next step in making a cell line is patience. The cells are put into an incubator and grow very slowly to begin with. It will take months before the cells get used to the artificial environment and many cells will perish along the way. Michael found that he lost two-thirds of his cells in the first three months and it took him a year to obtain a cell line that grew quickly enough for research (Goblirsch, et al, 2013). Michael made 100 attempts before he got his cell line and it took him over a year. Although this may seem like an awful lot of time and effort, once you have made a honey bee cell line, you can use it in many research areas. For example, you could investigate how different bee pathogens act in bee cells. Understanding how they work on this level helps us to understand how we might combat them. Or, perhaps you could study how different diets affect the cells, or if certain compounds are helpful or harmful to cells. You can ask very simple questions on the cellular level which help you to refine your ideas for larger scale experiments at the whole bee or whole colony level.

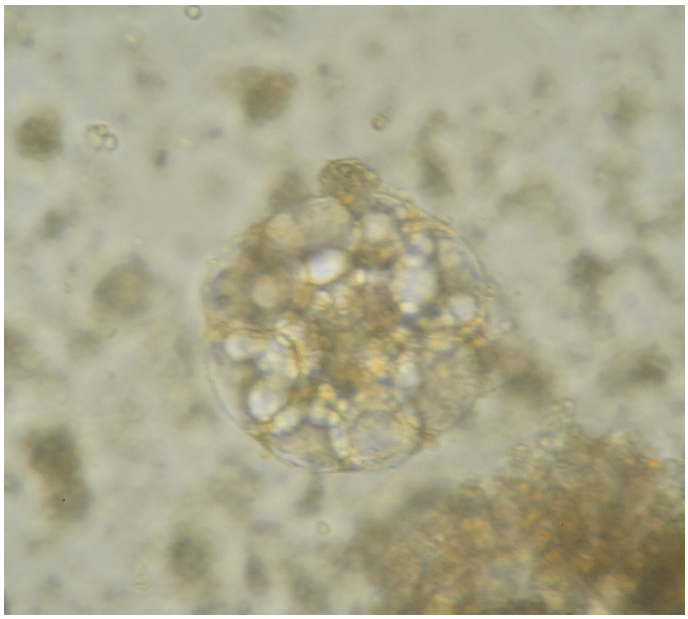
Cell Line Survival

The trick with cell lines is to try to get them to survive for as long as possible. Sometimes you can make a cell line that

divides continuously beyond the life span of the organism that the cells have come from. These cell lines are called 'immortalised' but are very hard to create.

Before I met Michael, I made three attempts to make a cell line. The first time I tried to make a cell line, I used pupae from the colony, but I didn't find any 'sticky' cells. On my second attempt, I got excellent growth of cells, especially from the brain, but the life span of those cells was short and they only lasted about six weeks. My third attempt was the most successful. I used eggs and pupae this time. I also tested different diets on the cells as I felt the diet I was using was not suitable. Two of these diets were very successful and my cells looked healthy for many weeks and seemed to be growing slowly. Unfortunately, the cells died out due to a technical problem and I decided that I would renew my efforts once I had visited the workshop in the USA.

Having returned from Michael's workshop, I feel renewed enthusiasm that I will be able to culture a honey bee cell line. There won't be any eggs in the apiary right now, but I think I can start testing my techniques on other tissues. Wish me luck … □



A surprise in my attempts to create a cell line from abdominal tissue – pollen!

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