Presenter: Associate Professor Beth McGraw, Monash University - *2015*

Title: Studying tiny things that matter: a story of viruses, mosquito spit and perseverance (11:56)

Time	*Dialogue*
00:15 00:57	Thanks. So it's Monday morning and we have to feed 5000 finicky girls food they don't like, convince them to fall in love with a boy not of their choosing and then spend the rest of their lives in a tiny little apartment raising their families with a pesky uninteresting husband. Thankfully these girls are not humans but mosquitos. My name is Beth McGraw and I study mosquitos. Particularly I study mosquito spit. Now the reason we put mosquitos in this cruel life is all in the name of science and in particular trying to understand the study of diseases transmission. So mosquitos are responsible for some of the most deadly diseases around the world, in particular Malaria that we all know about, but also Dengue Fever and my lab studies Dengue virus the virus that causes Dengue Fever. Upwards of 380 million people per year are at risk of Dengue Fever and as you can see it occurs primarily in the developing world.
01:13 01:46	When people get sick they have intense fevers that last for several weeks and sometimes they can die. There's no treatment, there's no vaccine so we are interested in finding ways to understand how this disease is transmitted. Now back to our sad hopeless girls in their apartments. Why do we do this to them? It's because the mosquitos play a really key role in that disease cycle in the field from an infected human into the mosquito and back to another human. Now what we see here is an image of inside a mosquito's body and we can track the process of the virus so when a mosquito bites and infected human and consumes blood that blood first has to infect the guts. And after it is in the guts it has to move out through a whole range of tissues and find its way to the salivary glands. Once it gets there it can be spat into the saliva. Up unto that point the mosquito is not capable of transmitting disease to another human.
02:08 02:37	Now we have a number of questions and the first is how long does it take for the virus to get from the gut to the spit? This is really key because if it gets there quickly that mosquito will have many more opportunities over its lifetime to infect humans. We also wanted to understand do mosquitos continuously secrete virus into their saliva and then lastly the one that is closest to my heart is how important are mosquito genes in this process and what mosquito physiologies are involved with the migration of the virus through the body. Now we have a number of predictions for our question. The first is we think it will take about 10 days for the virus to arrive and this comes from previous studies. We also think the mosquito will continue to secrete virus throughout her lifetime and that mosquito genes will play a small but significant role in dictating the nature of this process, but other things like the virus genome, or environmental temperatures or other variables might be important.
03:07	Now in order to answer these questions what we need to be able to do is get mosquito spit from a single mosquito, multiple times over her lifetime. This is challenging because mosquito saliva comes in very small quantities, 0.1 of a nanolitre each time they spit. That's 1/10,000 of a millilitre and inside that spit we need to be able to detect viruses. Viruses are only 50 nanometres in size and there might be only, 10 20, or 30 viruses when a mosquito spits. So this is a bit of a challenge and the way people have studied this in the past has been by this really destructive approach. You can see here a mosquito has been taken and her mouth parts have been jammed into a capillary tube and her wings and legs ripped off to stress her so she actually spits on command.

Time	Dialogue
03:54 04:26	Now our question is about asking the same female over and over again do you have virus in your saliva. So if we kill her this process is not going to work. So my lab wanted to come up with a way to get virus from female saliva over her lifetime and the people who took on this challenge are shown here, Henry Ye, a post doc in the group and Alison Carrasco a research assistant. Now these two thought they could handle this challenge and they first spent six months working in the laboratory every week trialling different approaches, different ways to try and solve this problem experimentally. Most of their approaches were based on the notion that the way that we keep mosquitos alive in the laboratory is by giving them sugar water to feed. Every time mosquitos drink in and out of that sugar water they are spitting and they are basically looking for Dengue virus backwash. It took many months and lots of problem solving.
04:49 05:17	They tried different approaches. They tried feeding mosquitos honey coated paper or sugar water in cottonwool but what we often found was these often led to problems with contamination and it was impossible to get the virus back out of those substrates. In the end they converged on this really beautiful design and it had to do with the give and take of ideas and both of their contributions. It's quite simple really. We put a mosquito which has been blood fed with Dengue virus inside one of these little cups, these are the little apartments. We give them the lid of a tube and we put into that their drinking liquid with the sugar. Every two days the mosquitos go in there and feed, and we can take away that cup and replace it with a fresh one. So the mosquito is unharmed and we've got the material to take into the molecular laboratory, extract and look for the presence of virus.
05:39 06:02	Now once we did this we weren't done with our challenge and we had to find a way to scale up these little assays for hundreds and hundreds of mosquitos because we needed a big strong data set. And more importantly because we are interested in mosquito genes we needed a way to connect this measure of how a virus moves through the body to a single mosquito's genome. We had to do this within the frame work of a family design so all of our mosquitos couldn't just come from one big population. We needed to know how they are relate to each other, so we spent three generations of breeding grandmother's, daughters, granddaughters, with each generation of egg to adult to egg, taking about a month. So three months just to get all the mosquitos ready for the feeding experiment.
06:27 06:53	In parallel we had to have virus grown and prepared and the way that we do that in the lab is we grow mosquito cells in liquid culture and infect it with virus. So we had to grow up huge quantities of virus. On the day of the experiment we needed to be able to take the virus and give it to the mosquitos in a blood meal, hope that they feed, as they often just don't. … and the next month would be the hard labour tracking the mosquitos and taking care of them and collecting their saliva day in and day out until they all died. Now on the day of the actual experiments Alison came into the laboratory shown here and that's what our mosquito cell culture looks like. She went to get the virus out and when she looked at the cells what she saw was they were contaminated with bacteria. So to her horror that one component of the experiment had failed in a way that we didn't predicted it would fail.
07:17 07:39	Henry and Alison had all sorts of contingency plans for the mosquitos because they are usually the tricky ones, but instead we had this contamination event that we hadn't experienced before. So all those grandmothers, daughters, granddaughters had all died for nothing and these staff in the lad had spent 8 months of their lives preparing for this one day and it all had come crashing down. The disappointment in the lab was really palpable. People were really quiet and supportive of each other. There was a lot of crying and beer drinking and cursing the career that they had chosen, you know but people went away from the lab and took a break and spent time with friends and family and tried not to think about mosquitos for a few weeks and then Henry and Alison came back and said we are the only people in the world that can do this experiment. We spent a year getting ready and we can do it and that contamination thing, that's nothing.

Time	*Dialogue*
08:13 08:38	We'll be ready for it next time. So they reran the experiments. This time they did it with a lot of help and they had much better logistical planning. They had learned from that first failed run. They did all their mosquito breeding in half the time. They bought hands in from the lab to help label apartments and tubes and so the whole thing went really well. The mosquitos fed on the day. The virus was fine and the real work began and that was sitting in a small room where we do our Dengue work because it's safer there. Small teams of two people doing day in and day out really monotonous work moving around spit. Right. You have to be careful because if you make a mistake those mosquitos could bite you or your partner and kill one of you. So really boring work but really dangerous. Henry kept everyone motivated by playing what he thought was the theme song for this experiment and that was … Daft Punk's, 'Get Lucky' and he played it a lot and nobody minded.
09:09 09:29	So as the weeks progressed there was this sort of quiet growing optimism that they might have pulled it off. And after 4 weeks of growing mosquitos Henry and Alison had 2000saliva samples jammed in the freezer. They began going through them and testing them for Dengue and then the entire team sort of staggered off to the physio to get treatment for repetitive strain injuries. But out of this we got some great data. Some really exciting findings. The first was that the virus turned up in only 4 days after the mosquitos had taken their blood meal. This is unheard of but it really helped to explain how when the virus had caused a massive outbreak in Queensland that it had moved so quickly though the human population because that intervening time in the mosquito was tiny.
09:54 10:22	The other thing that we found out was that the mosquitos stopped spitting virus after about 10 days of age, so what that tells us is only middle aged mosquitos are responsible for disease transmission and it changes how we think about who the culprits are out in the field. Lastly found that the mosquito genome was contributing a whopping 40% to the ability to transmit virus and setting us up on a path of being able to identify those genes in the future. Now this work has been presented at conferences and we have published a few papers. We've been really excited about how we can better inform about disease transmission and have accurate measures of these very important things happening in the mosquito helping us understand disease epidemiology. It's also a technique that we can take on to further our understanding of the genetic contribution of the mosquitos of this process as well of other people working with other disease vectors including Malaria.
10:46 11:12	Now soon after we finished the experiments another post doc in the lab got married and during the reception … Daft Punk – 'Get Lucky' came on and the whole lab got up and danced with Henry and Alison. It was a really emotional outpouring of support and respect for these two as everyone understood what they had pulled off and what the last year had been like for them. I'm surprised everyday how when I come to the lab my job is like watching my favourite genre of movie, which is the caper film – think the 'Italian Job' or 'Ocean's Eleven' and you know I really love watching a group of really talented people with diverse skills come together and really pull off the ultimate job. And that is what exactly happened here. Also knowing the information that we gleaned from these experiments will help us understand how human disease transmission is taking place in the field. All that is what gets me out of bed every day. Thanks very much.