



Gilles San Martin

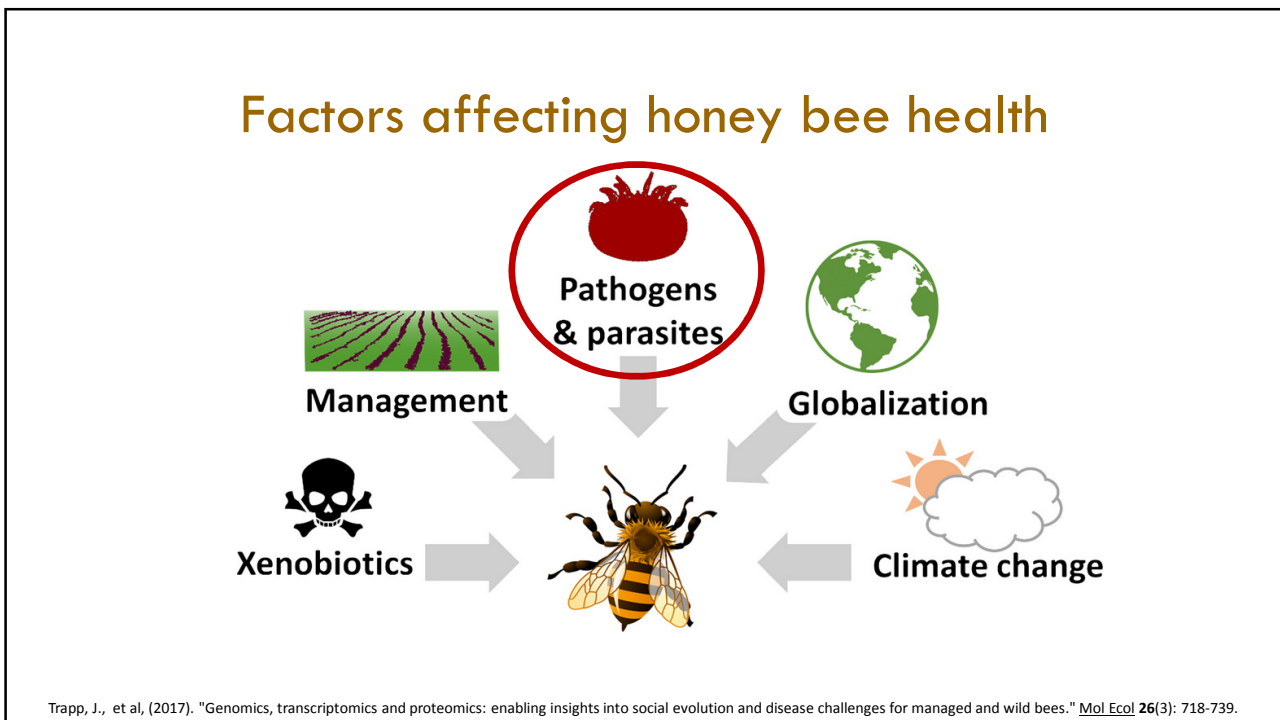
Can we immunise honey bees against virulent viruses?

Emily Remnant

*Behaviour and Genetics of Social Insects Laboratory
School of Life and Environmental Sciences*

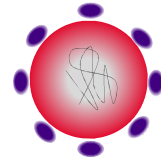
<https://www.facebook.com/sydneybeelab/>
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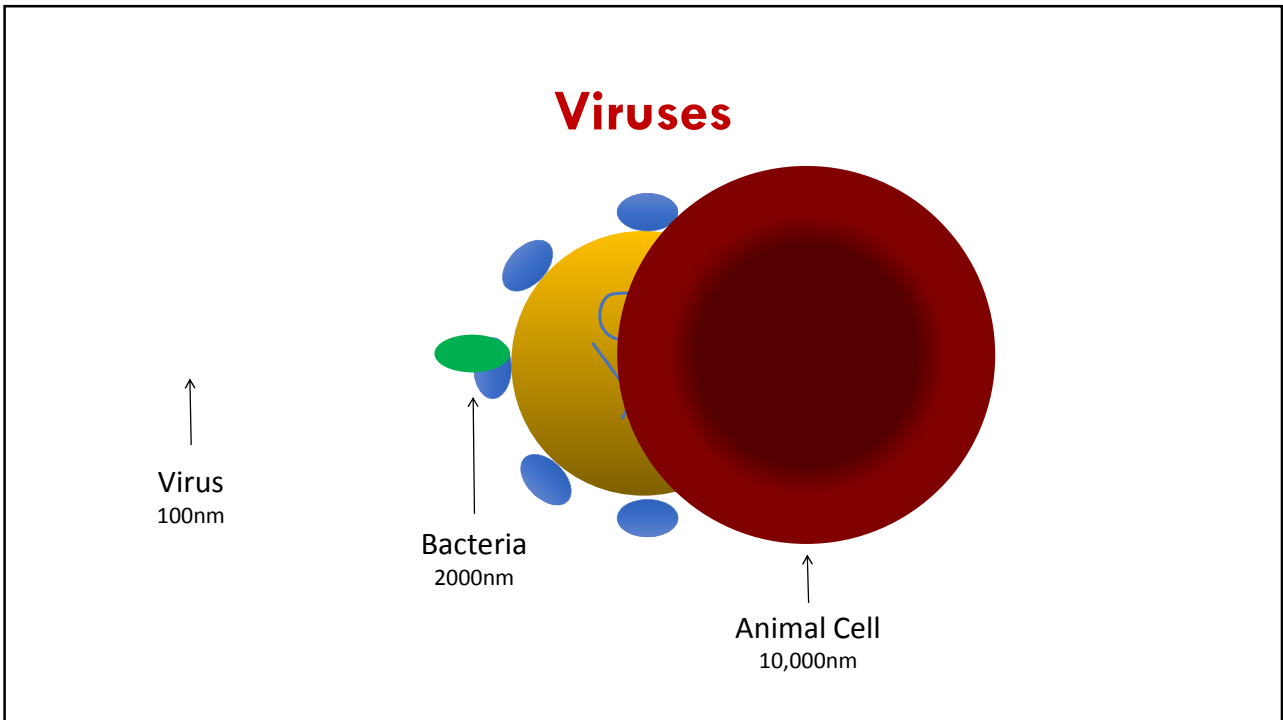
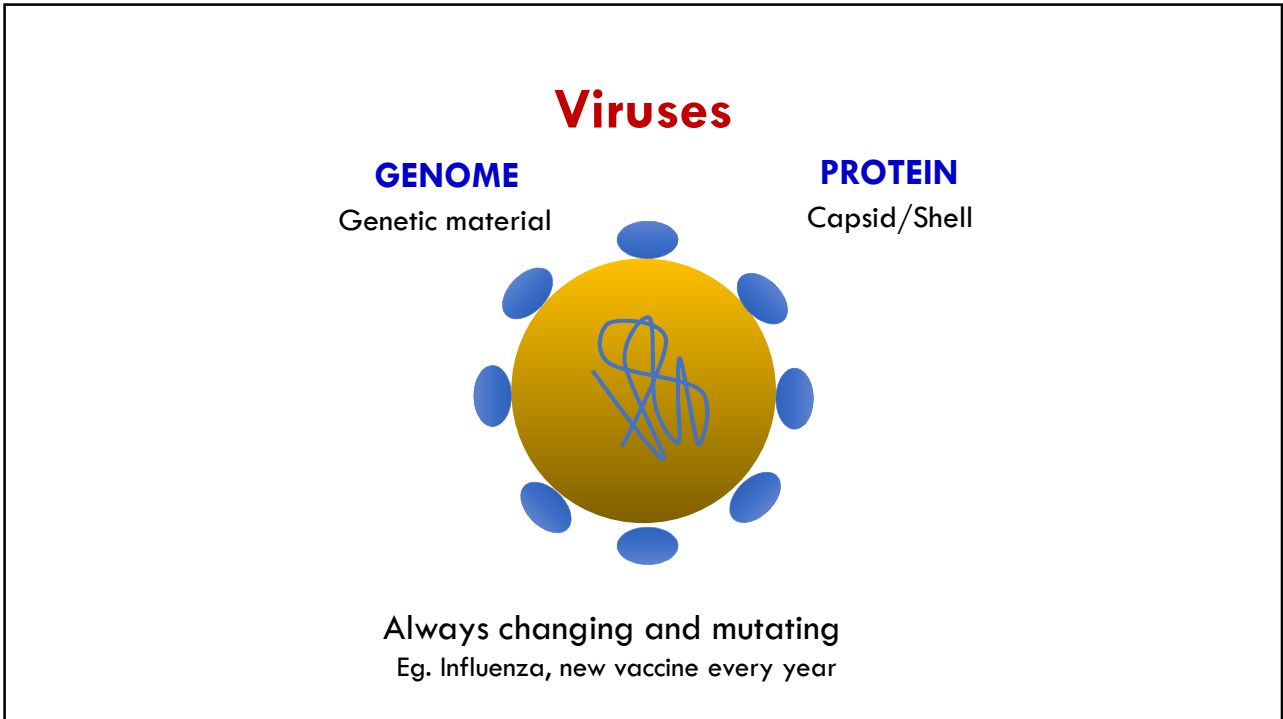
What can we do about bee disease?

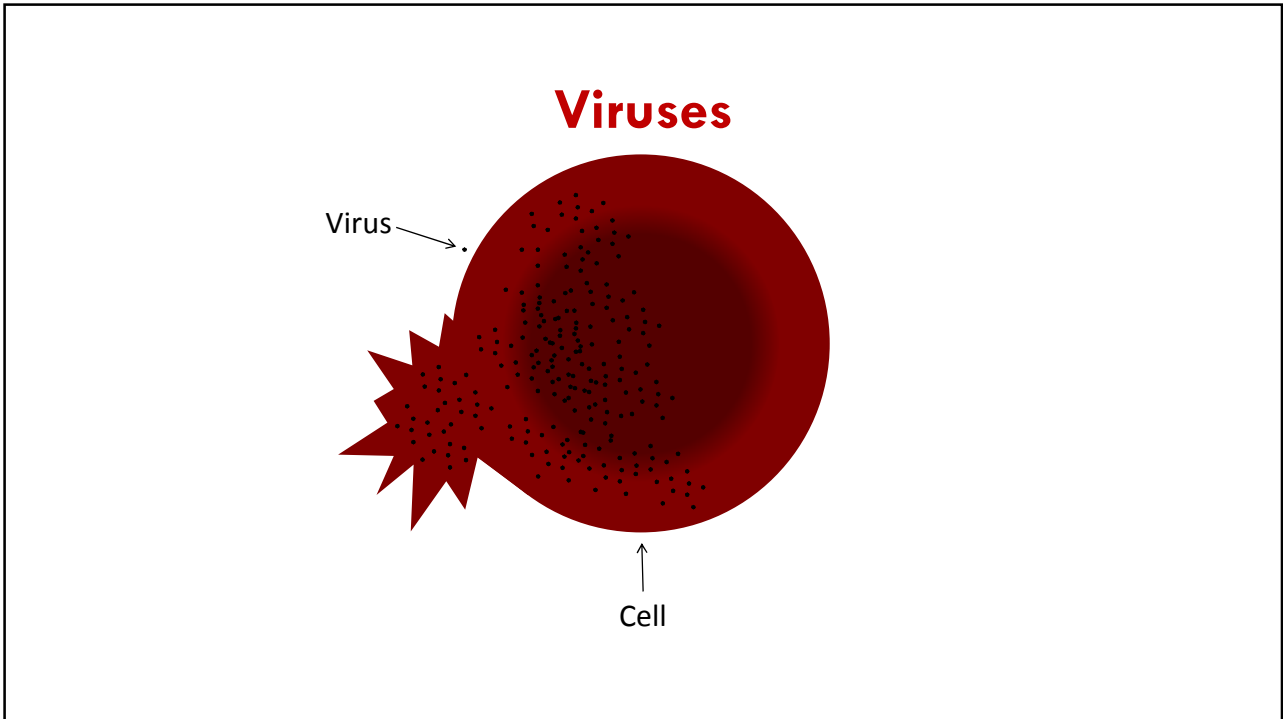
Parasites and pathogens are a major cause of colony loss
and suboptimal honey bee health



Are there genetic mechanisms that could reduce the impact of viruses
in honey bees?

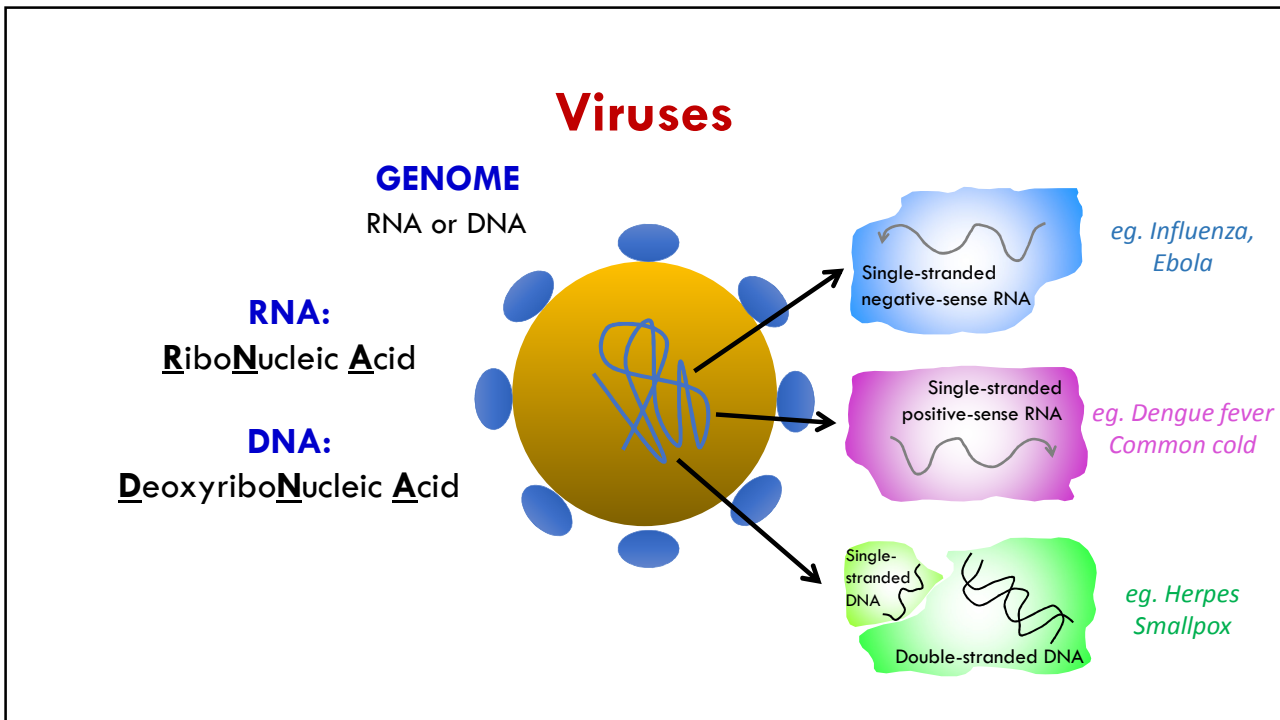
Honey bee viruses *and how to stop them*





...like unwanted house-guests...





Honey bee viruses

- Historically: around **24 viruses**
- **Australia** has 5 common viruses:
 - Black queen cell
 - Sacbrood
 - Lake Sinai I and II
 - Israeli Acute paralysis
- **New viruses** discovered all the time

Black queen cell virus

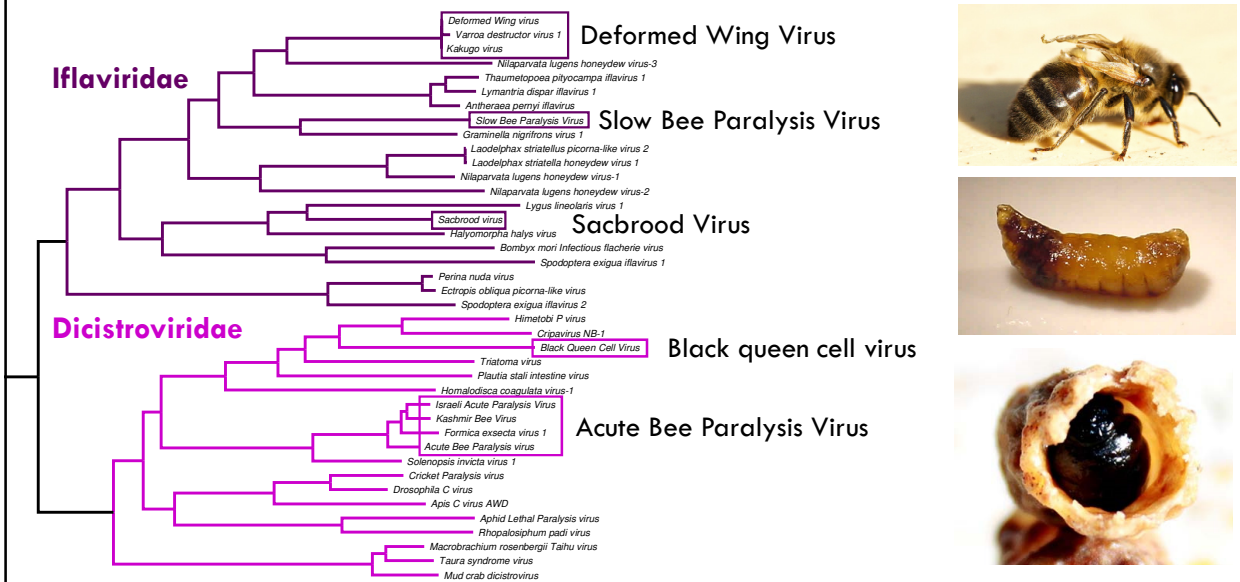


Sacbrood virus



Dr John Roberts, *CSIRO*
Session 8B (later today)

Honey bee viruses: *Picornavirales*



Virus Classification



Genome type

Honey bees

Insects

Single-stranded negative-sense RNA



Deformed wing virus

Single-stranded positive-sense RNA



Single-stranded DNA
Double-stranded DNA



Who's really in control?



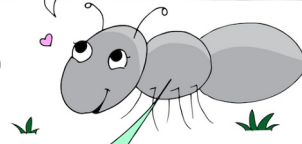
Us?

Parasites

Bacteria

Viruses

Ah, here I am, just living my life as an ant...



Oh, don't mind me. hehe
I'm just inside you, hehe
controlling your behavior.



Yessss, that's right.
Do my bidding.



All these fools
think they're
in charge. Heh.



Beatrice the
Biologist

Concept by Dr. Mark Martin, University of Puget Sound

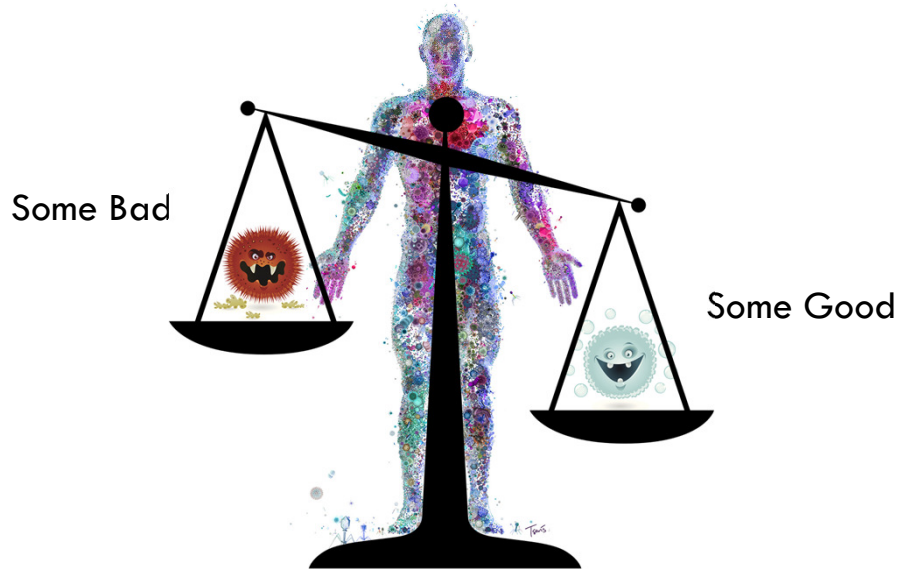
The Microbiome

We are FULL of
microorganisms



that impact how our
body works

The Microbiome



What's living inside our bees?

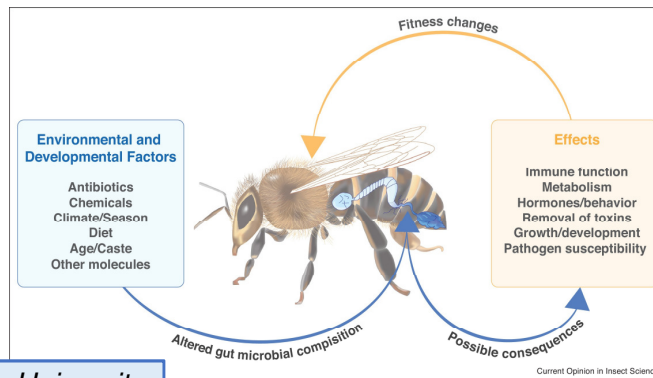
The good, the bad and the ugly



The bee gut



- 8-10 core species of bacteria in the gut, with key roles in health and development



Dr Julia Jones, *Uppsala University*
Session 3A (yesterday)



Honey bee diseases

VIRUSES

Deformed wing
Kakugo
Black Queen Cell
Sacbrood
Cloudy wing
Israeli Acute Paralysis
Kashmir Bee
Acute Paralysis
Chronic Paralysis
Slow paralysis
Lake Sinai 1 & 2
Apis Rhabdo 1 & 2
etc.....



FUNGI

Nosema
Chalkbrood

BACTERIA

American foulbrood
European foulbrood



Arrival of a new pest

Varroa destructor: Parasitic mite



Jumped from *Apis cerana* → *Apis mellifera*

P. Zborowski

Apis cerana
Asian honey bee



Apis mellifera
European honey bee



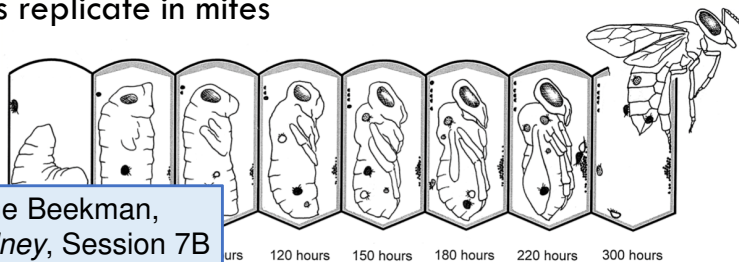
Prof Madeleine Beekman,
University of Sydney, Session 5A



National Geographic Japan; https://www.youtube.com/watch?v=IMtFYt7ko_o

Impact of *Varroa*

- Wounding and weakening
 - Damage to the cuticle
 - Feeds on haemolymph
- Vector for viruses
 - Spreads viruses
 - Viruses replicate in mites



Prof Madeleine Beekman,
University of Sydney, Session 7B

Bees brought to their knees

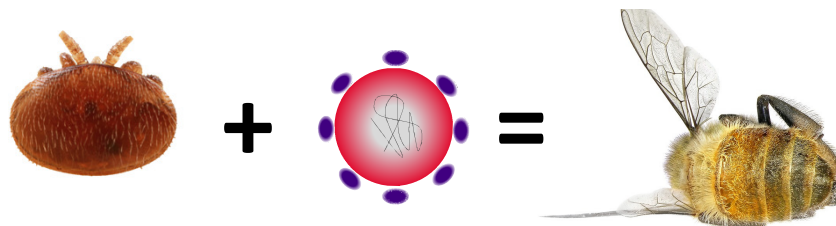
Viruses have changed since the arrival of *Varroa*

The main culprit: **Deformed wing virus (DWV)**

- *Varroa* arrives: DWV levels rapidly increase
- Left untreated for mites, hives die in 2-3 years



Deformed wing virus: Global pandemic



Global Honey Bee Viral Landscape Altered by a Parasitic Mite

Stephen J. Martin,^{1*} Andrea C. Highfield,² Laura Brettell,¹ Ethel M. Villalobos,³ Giles E. Budge,⁴ Michelle Powell,⁴ Scott Nikaido,³ Declan C. Schroeder^{2*}

8 JUNE 2012 VOL 336 SCIENCE

RESEARCH | REPORTS

HONEYBEE DISEASE

Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites

5 FEBRUARY 2016 • VOL 351 ISSUE 6273 sciencemag.org SCIENCE

So how do we stop viruses?

...get rid of mites...?

Ways to get rid of mites

- Chemical treatments
 - In hive residues bad for bees
 - Mites can become resistant

Dr Jody Wu-Smart, *University of Nebraska-Lincoln*, Session 4A

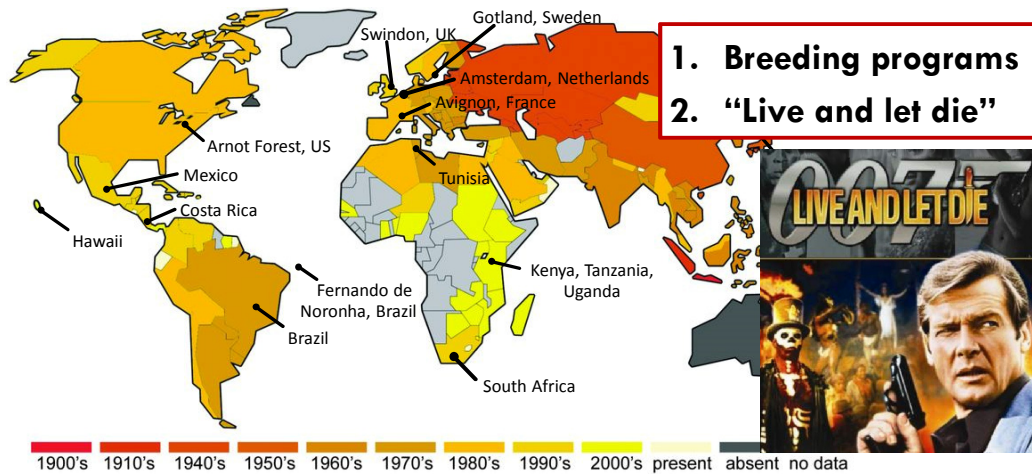
Dr Medhat Nasr, *Ministry of Alberta Agriculture and Forestry*, Session 5A

- Natural selection of Varroa-tolerance or resistance
 - Over time, bees learn to live with, or get rid of, mites

Prof Madeleine Beekman, *University of Sydney*, Session 5A

Worldwide evolution of *Varroa*-resistance/tolerance

Colonies survive mite infestations without chemical intervention

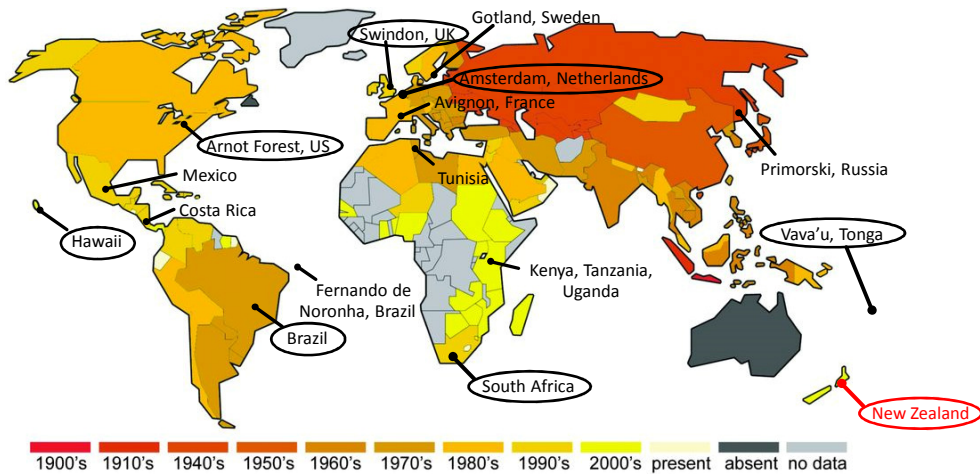


Wilfert *et. al.* (2016) Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. *Science* 351(6273), pp. 594-7
Adapted from: Locke, B., 2015 *Natural Varroa mite-surviving Apis mellifera honeybee populations*. *Apidologie*: 47: 467-482.

**But... are there viruses in
Varroa-resistant bees?**

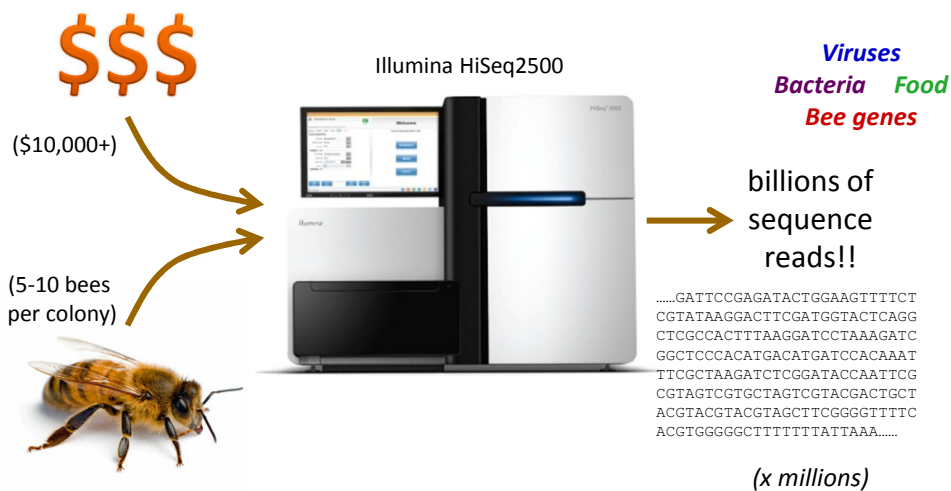
Worldwide evolution of *Varroa*-resistance/tolerance

Colonies survive mite infestations without chemical intervention

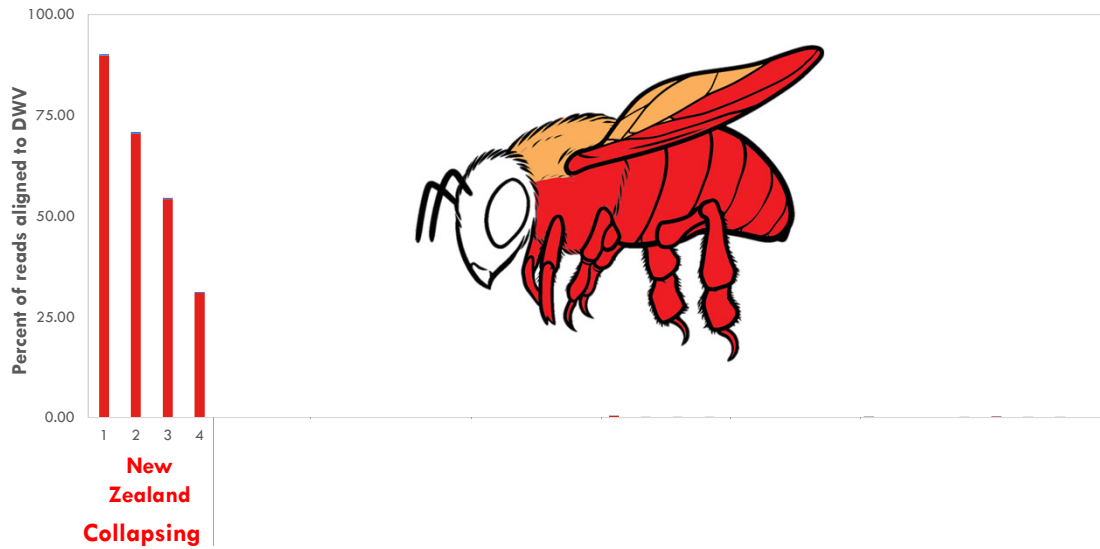


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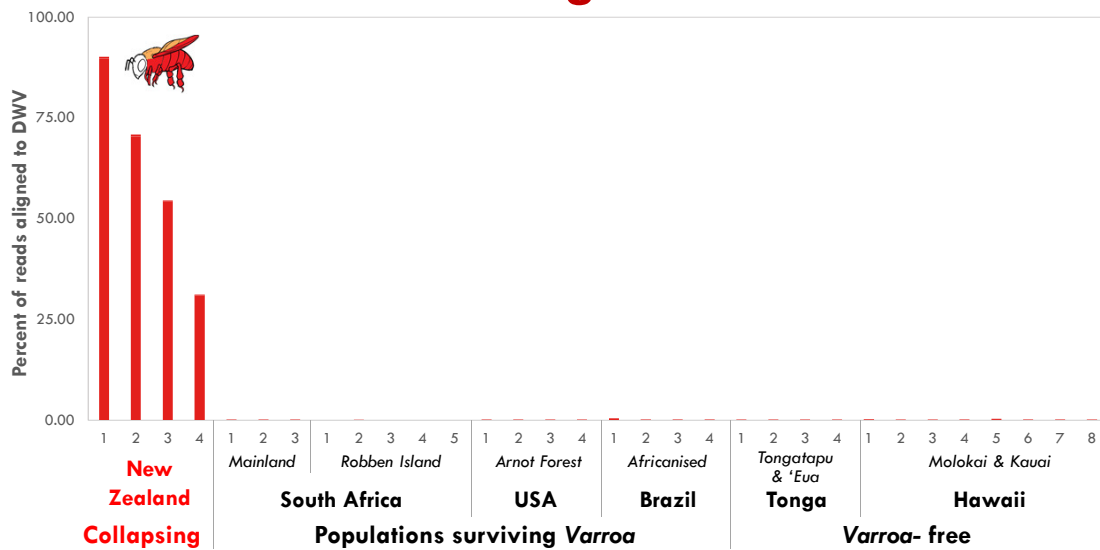
Sequence all the genetic material inside a bee



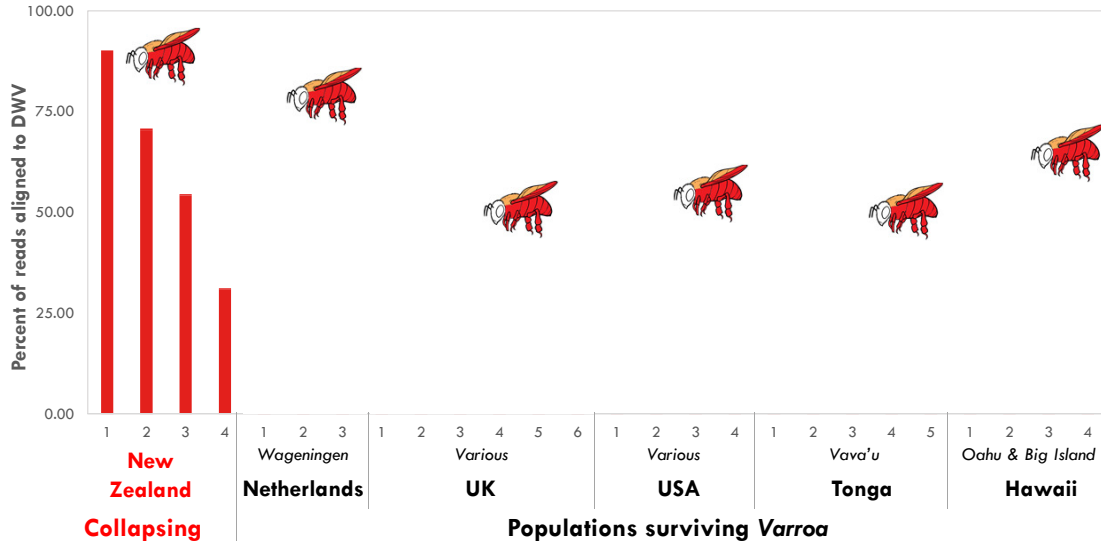
Virus levels in bees can be pretty high!



DWV is absent in some populations surviving Varroa

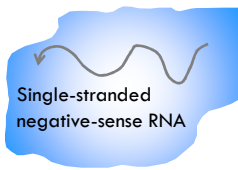



High levels of DWV in other populations surviving Varroa

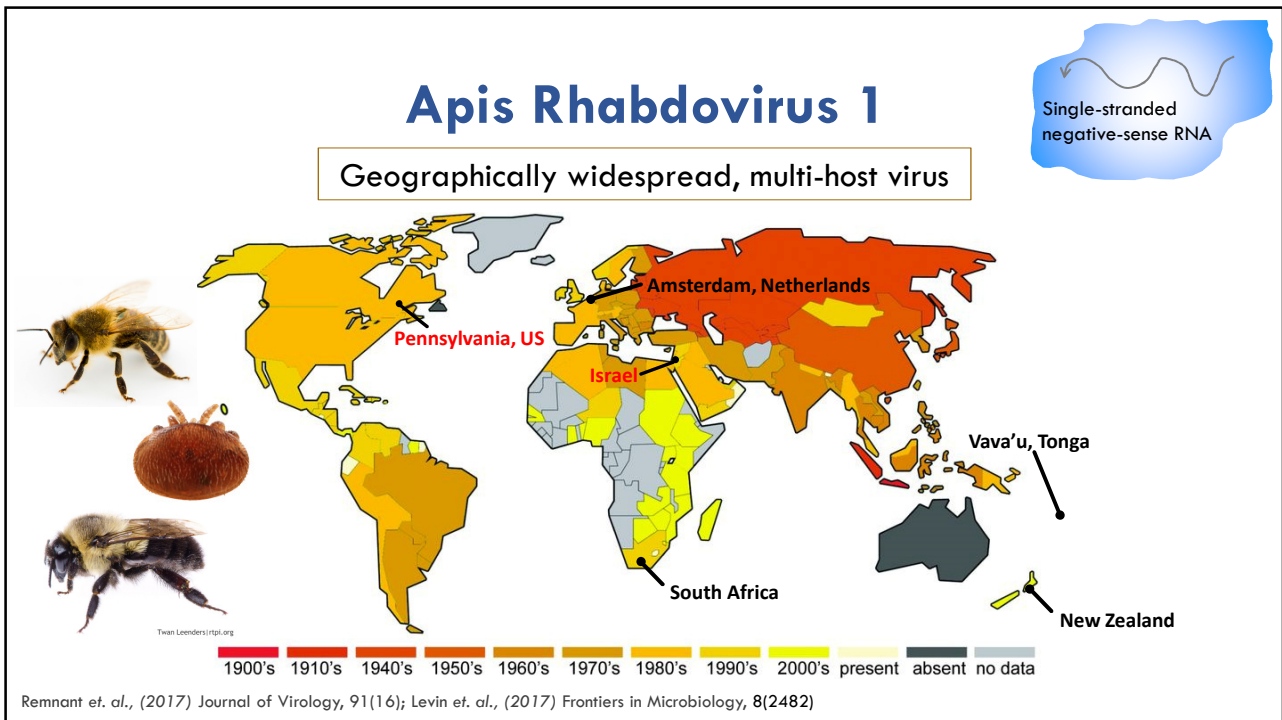


7 new RNA viruses

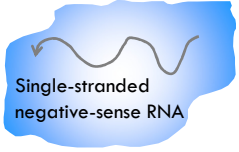

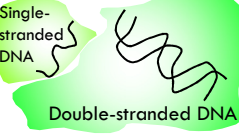

First negative sense RNA viruses in bees

| | Netherlands | South Africa | Tonga | New Zealand |
|---------------------------------------------------------------------------------------------------------------------------|--------------------|--------------|-------|-------------|
|  Single-stranded negative-sense RNA | Apis Rhabdovirus 1 | ✓ | ✓ | ✓ |
| | Apis Rhabdovirus 2 | ✓ | ✓ | ✓ |
| | Apis Bunyavirus 1 | | ✓ | |
| | Apis Bunyavirus 2 | | ✓ | |
|  Single-stranded positive-sense RNA | Apis C virus | ✓ | | |
| | Apis Flavivirus | | ✓ | |
| | Apis Nora virus | | ✓ | |

Remnant et. al., (2017) J Virol



Virus Classification

| Genome type | Honey bees | Insects |
|---------------------------------------------------------------------------------------------------------------------------|------------|---------|
|  Single-stranded negative-sense RNA | 4 | ✓ |
|  Single-stranded positive-sense RNA | 25 | ✓ |
|  Single-stranded DNA | 2 | ✓ |
|  Double-stranded DNA | | |

We don't know what the new viruses do

What can we do about it?

- *Varroa* has increased virus levels in bees
 - Long term effects on colony survival
- Impact of viruses remains high in most places
 - *Varroa*-resistance is not a complete solution
 - Can we reduce virus levels in bees by other mechanisms?

The Insect Immune System

- Insect immunity- less sophisticated than humans
 - No antibodies
 - Can't give them the bee version of a flu shot
- Bees rely on general immune pathways
 - At the colony level: **Hygienic behaviour**
 - Immune genes and pathways

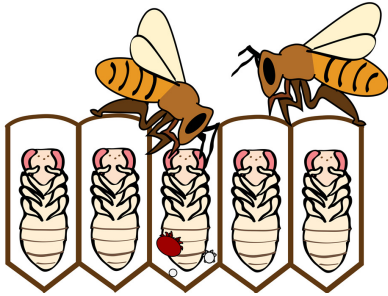


Dr Brock Harpur, *University of Toronto*, Session 3B

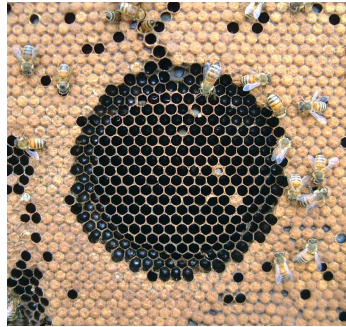
Social immunity

- Hygienic behaviour

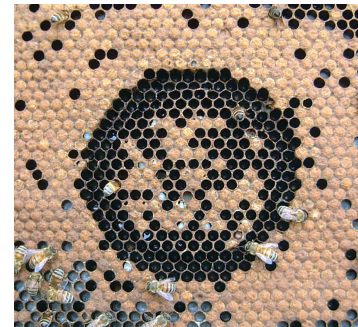
- Worker bees detect sick or dying brood and remove them from the hive
- Worker bees groom mites from other adult worker bees



A. McAfee



Good hygiene



Bad hygiene

<https://beeinformed.org/2011/07/25/hygienic-behavior/>

The Insect Immune System

- Insect immunity- less sophisticated than humans
 - No antibodies
 - Can't give them the bee version of a flu shot
- Bees rely on general immune pathways
 - At the colony level: Hygienic behaviour
 - Immune genes and pathways
 - The **Microbiome** is important



For example, in other insects...

OPEN ACCESS Freely available online

PLOS BIOLOGY

The Bacterial Symbiont *Wolbachia* Induces Resistance to RNA Viral Infections in *Drosophila melanogaster*

Luís Teixeira*, Álvaro Ferreira, Michael Ashburner

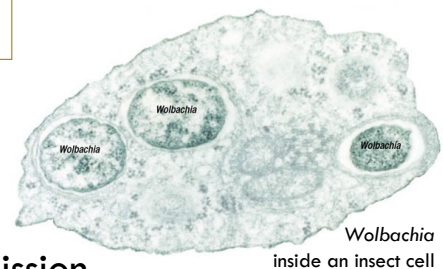
Department of Genetics, University of Cambridge, Cambridge, United Kingdom



What is Wolbachia?

Wolbachia pipientis

- Bacteria that lives inside cells
- Infects about half of all insect species
- Manipulates insect hosts to increase transmission
- Provides **resistance to viruses** in flies and mosquitoes



ELIMINATE
DENGUE
OUR CHALLENGE

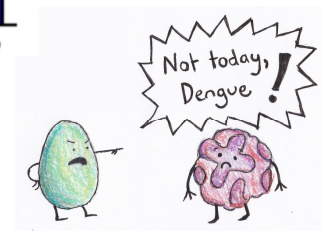
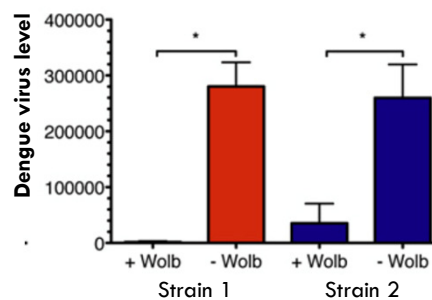
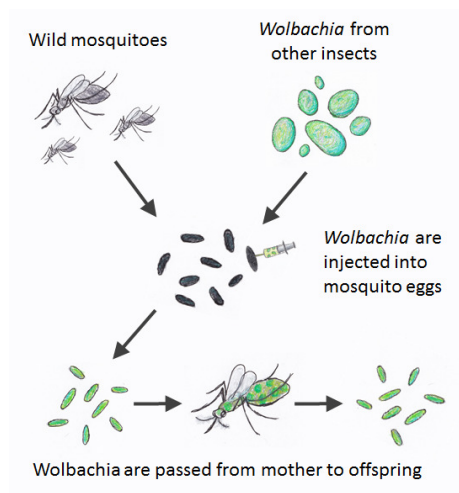
'Eliminate Dengue'

- Dengue fever: WHO #1 mosquito-borne disease
 - 30-fold increase in past 10 years
- *Aedes aegypti* mosquito:
 - vector of Dengue, Chikungunya, Zika virus
- Global team spearheaded by Australian labs
- Use a natural mechanism to prevent spread of Dengue fever: infect mosquitoes with *Wolbachia*

ELIMINATE
DENGUE
OUR CHALLENGE



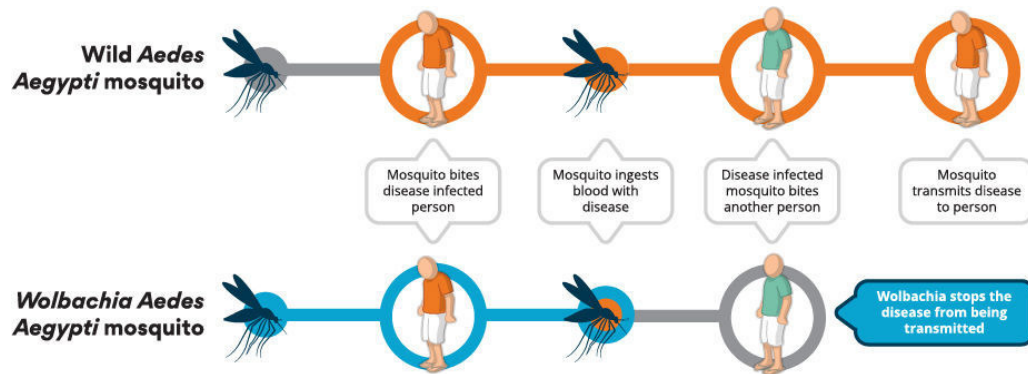
The Wolbachia method



Images: Perran Ross

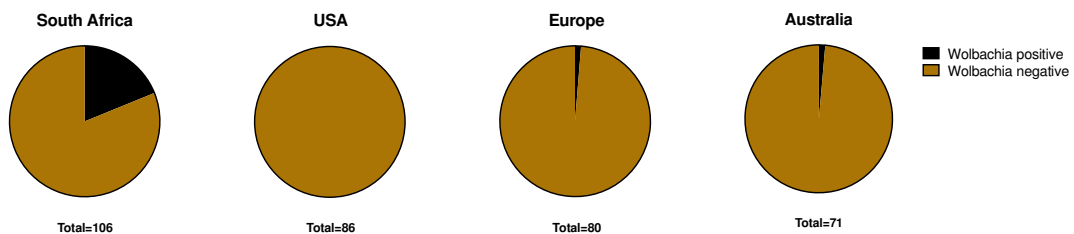
Moreira, L. A., et al, (2009). "A *Wolbachia* symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and *Plasmodium*." *Cell* 139(7): 1268-1278.

The Wolbachia method



Wolbachia in bees?

- Present in African honey bee subspecies
- Little evidence for *Wolbachia* elsewhere in *Apis*
 - Antibiotic treatments will remove *Wolbachia*



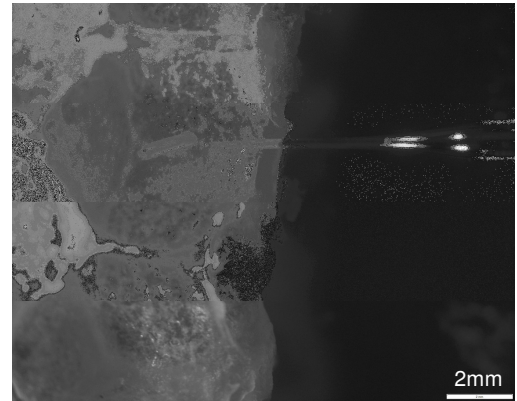
Can *Wolbachia* provide virus resistance to honey bees?

Can we immunise honey bees with bacteria?

Wolbachia transinfection:

- Contained in our quarantine lab
- Microinjection of honey bee eggs
- Injection into queen pupae → ovaries
- Next steps: test viruses in positive pupae

WATCH THIS SPACE!



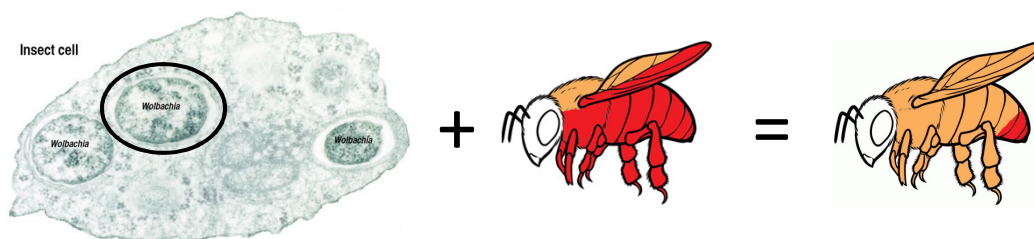
Prof Phil Lester, *Victoria University of Wellington*; Session 8B

ENDEAVOUR
Scholarships and Fellowships

Australian Government
Department of Agriculture
and Water Resources
**2017 Science
and Innovation Awards**
for Young People in Agriculture,
Fisheries and Forestry




Can we immunise honey bees with bacteria?




**ELIMINATE
DENGUE**
OUR CHALLENGE


Take home messages


1. Varroa + viruses = **Bad News**
2. We are in a unique position to prevent damage caused by viruses as seen elsewhere in the world, if *Varroa* mites arrive
3. Protective bacteria could provide a new way to reduce viruses




Acknowledgements

| | | |
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| <p><u>University of Sydney</u> Madeleine Beekman Ben Oldroyd Boris Yagound Tom Gillard Gabriele Buchmann Michael Holmes</p> <p><u>University of Salford</u> Stephen Martin Laura Brettel Jessica Kevill</p> | <p><u>Tonga</u> Bruce White Lamorna Osborne</p> <p><u>New Zealand</u> Phil Lester Jessica Russell James Baty Peter Dearden</p> <p><u>Wangeningen University</u> Tjeerd Blacquièrè</p> |  |
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