

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*



THE UNIVERSITY OF
SYDNEY

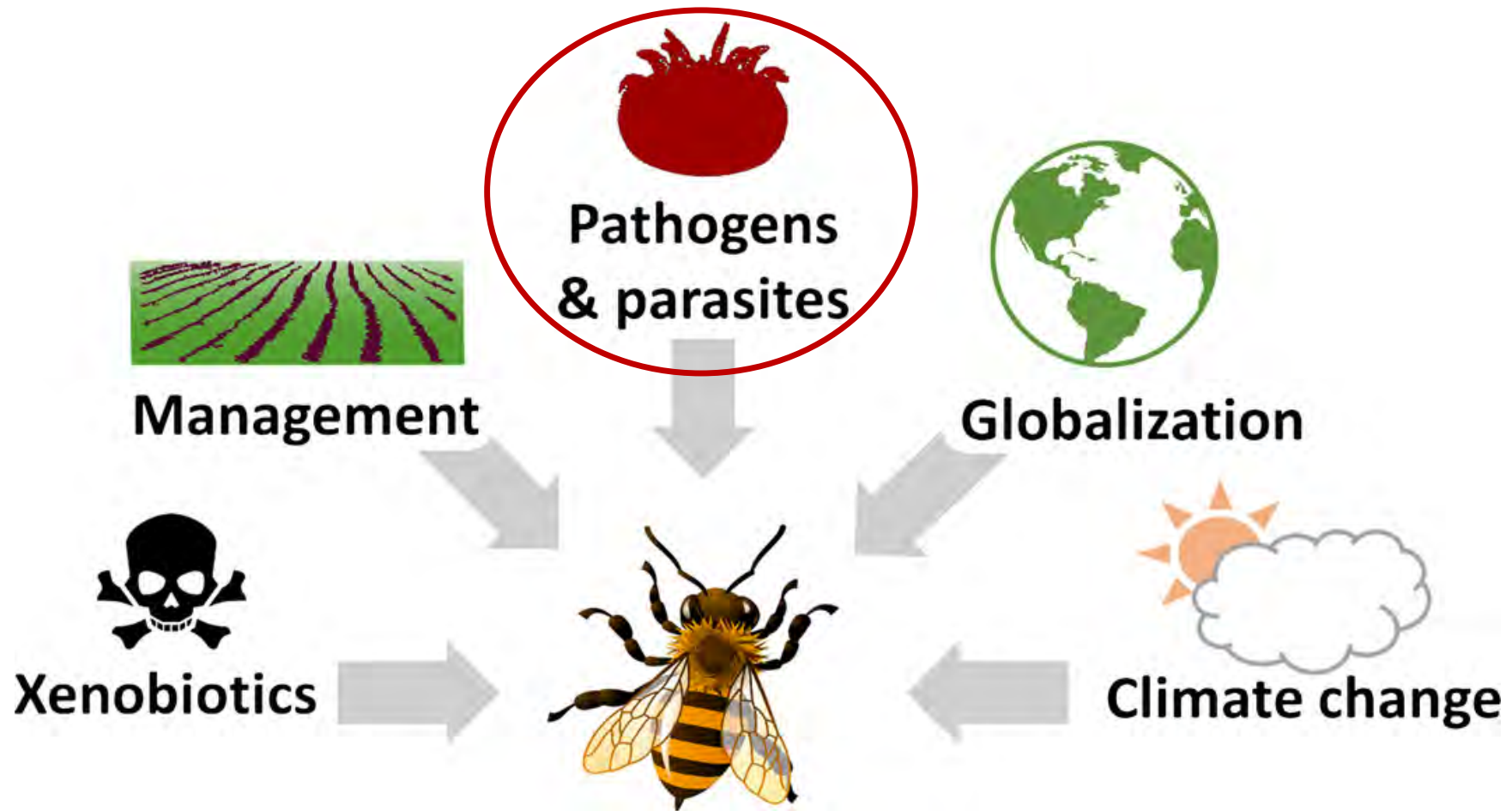
<https://www.facebook.com/sydneybeelab/>

emily.remnant@sydney.edu.au



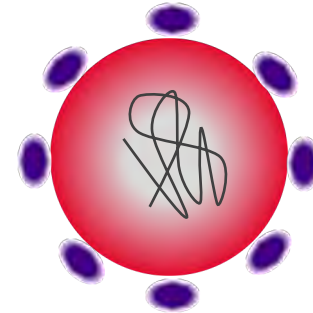
@EmsyRemsy

Factors affecting honey bee health



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

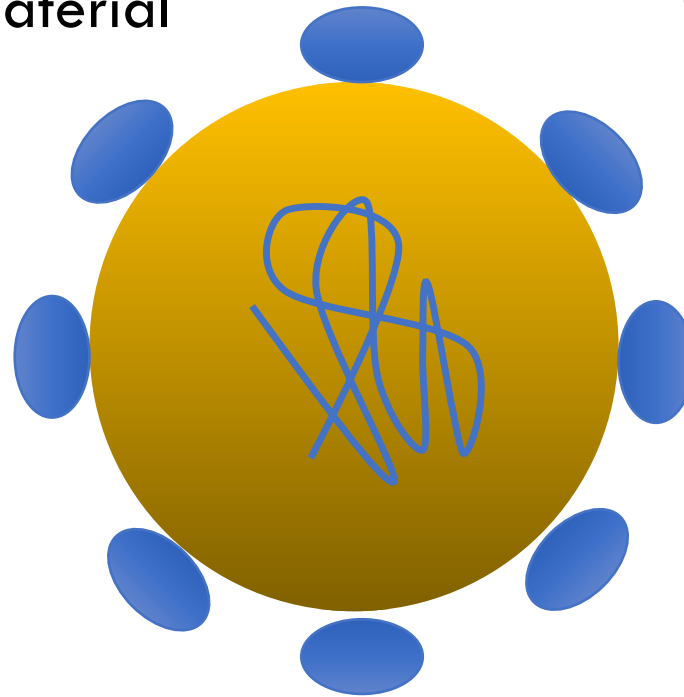
Viruses

GENOME

Genetic material

PROTEIN

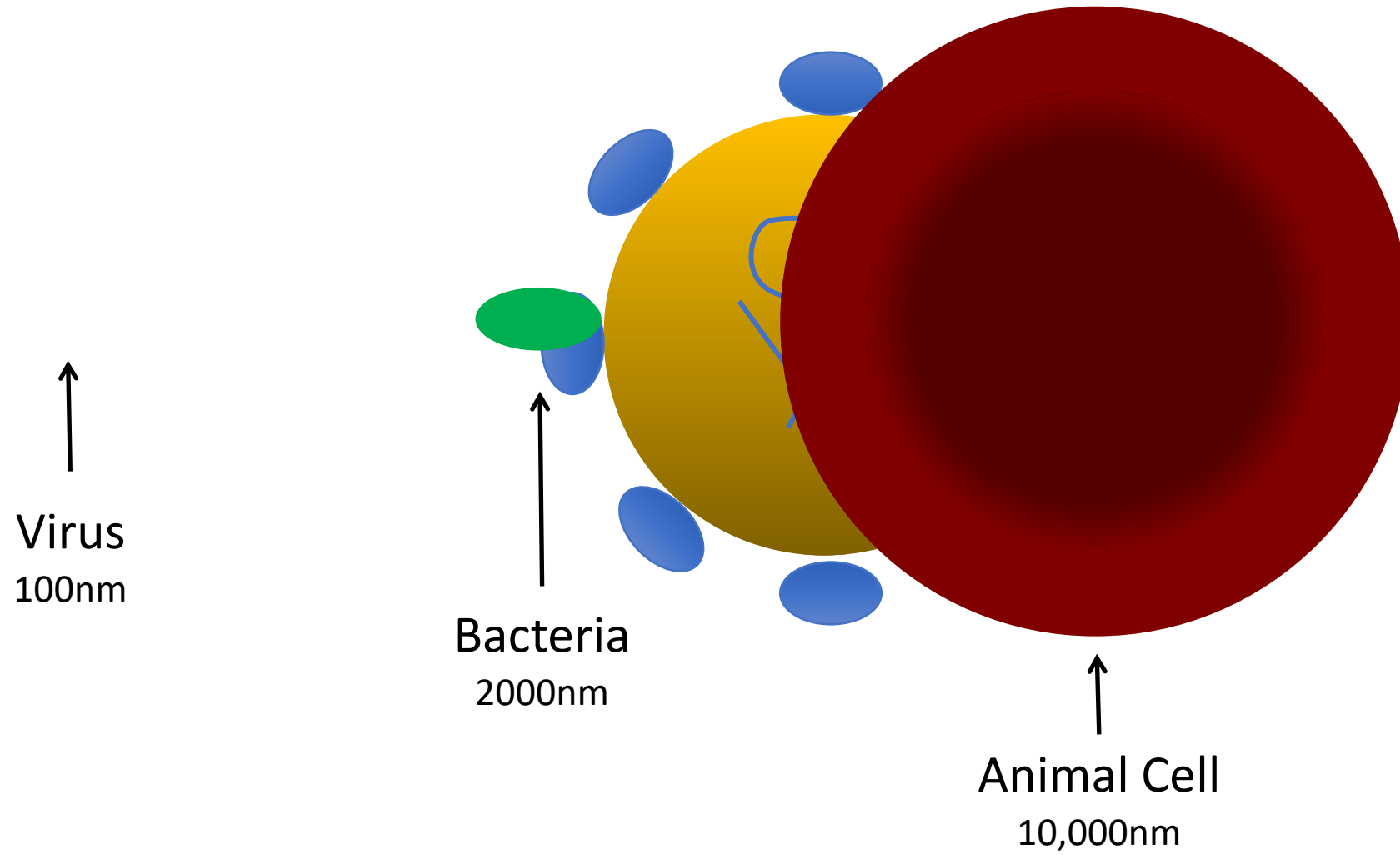
Capsid/Shell



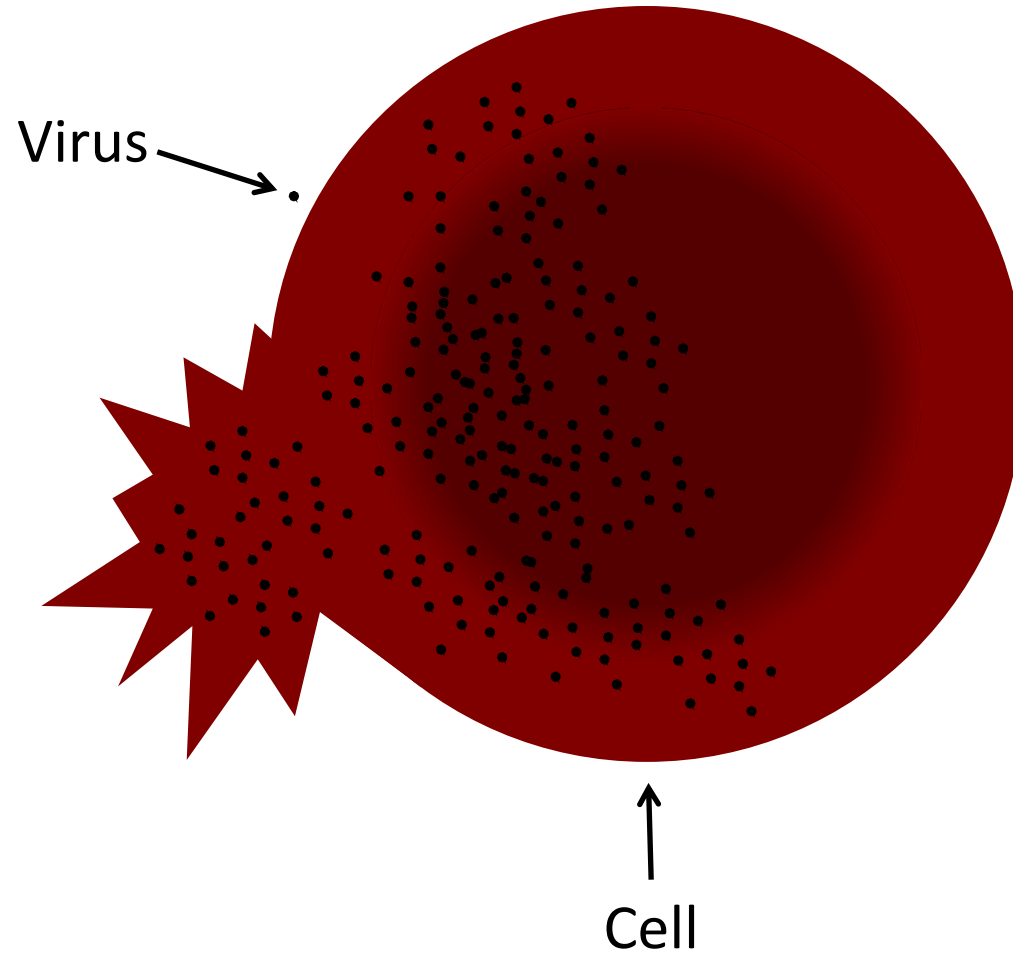
Always changing and mutating

Eg. Influenza, new vaccine every year

Viruses



Viruses



...like unwanted house-guests...



Viruses

GENOME

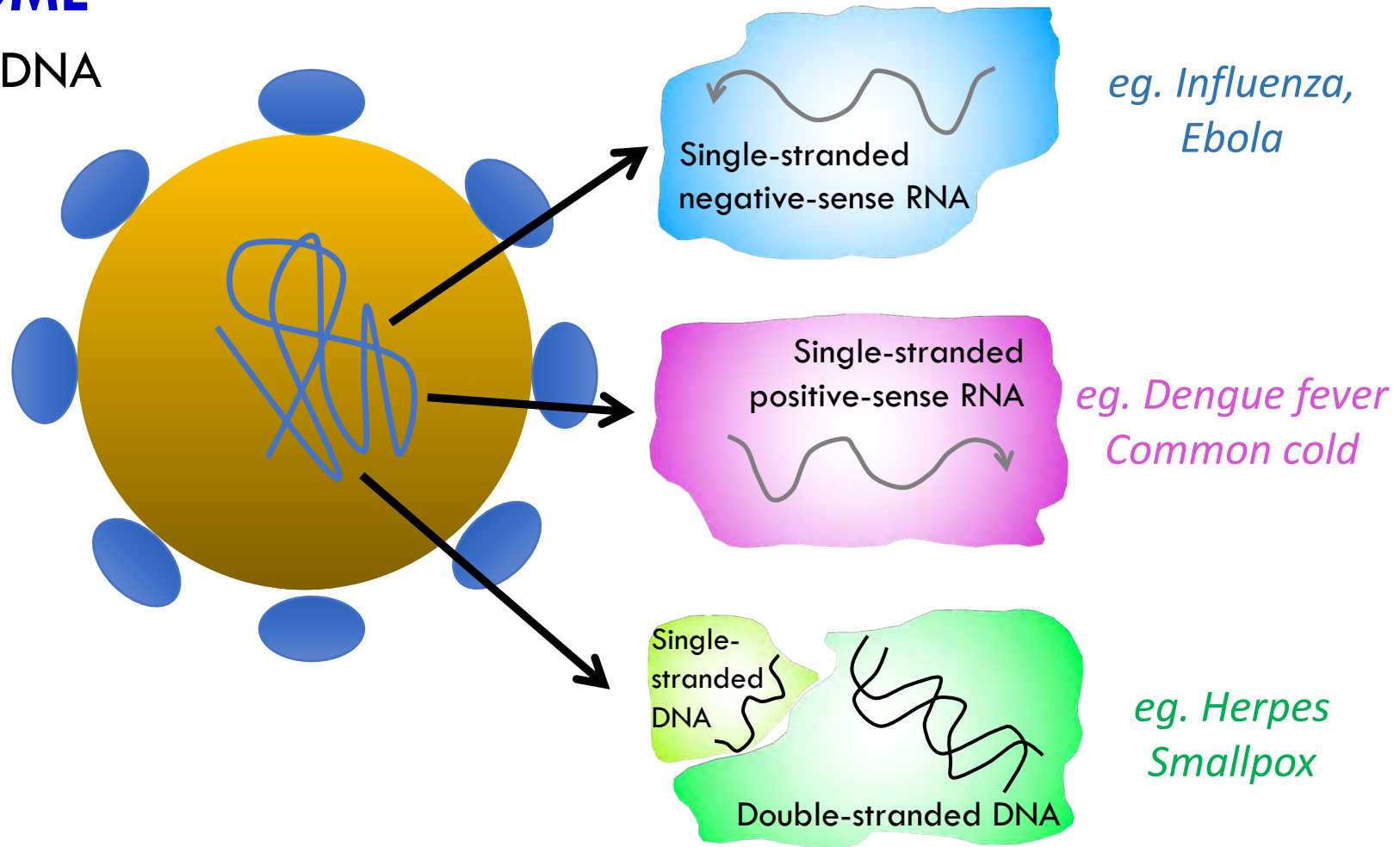
RNA or DNA

RNA:

RiboNucleic Acid

DNA:

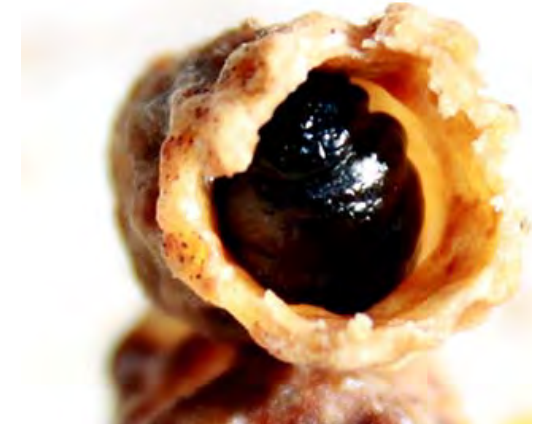
DeoxyriboNucleic Acid



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



Honey bee viruses: *Picornavirales*

Iflaviridae

Deformed Wing virus
Varroa destructor virus 1
Kakugo virus

Deformed Wing Virus

Nilaparvata lugens honeydew virus-3

Thaumetopoea pityocampa iflavirus 1

Lymantria dispar iflavirus 1

Antheraea pernyi iflavirus

Slow Bee Paralysis Virus

Slow Bee Paralysis Virus

Graminella nigrifrons virus 1

Laodelphax striatellus picorna-like virus 2

Laodelphax striatella honeydew virus 1

Nilaparvata lugens honeydew virus-1

Nilaparvata lugens honeydew virus-2

Lygus lineolaris virus 1

Sacbrood virus

Sacbrood Virus

Halyomorpha halys virus

Bombyx mori Infectious flacherie virus

Spodoptera exigua iflavirus 1

Perina nuda virus

Ectropis obliqua picorna-like virus

Spodoptera exigua iflavirus 2

Dicistroviridae

Himetobi P virus

Cripavirus NB-1

Black Queen Cell Virus

Black queen cell virus

Triatoma virus

Plautia stali intestine virus

Homalodisca coagulata virus-1

Israeli Acute Paralysis Virus

Kashmir Bee Virus

Formica exsecta virus 1

Acute Bee Paralysis virus

Acute Bee Paralysis Virus

Solenopsis invicta virus 1

Cricket Paralysis virus

Drosophila C virus

Apis C virus AWD

Aphid Lethal Paralysis virus

Rhopalosiphum padi virus

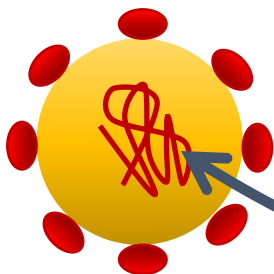
Macrobrachium rosenbergii Taihu virus

Taura syndrome virus

Mud crab dicistrovirus



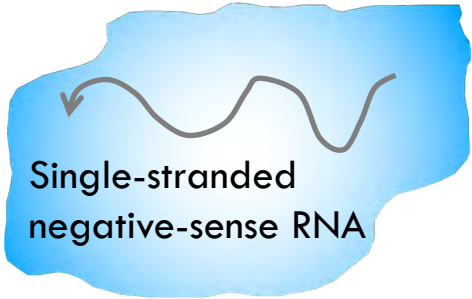
Virus Classification



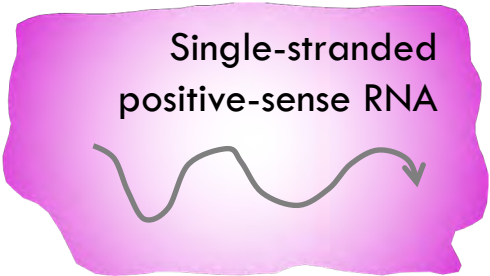
Genome type

Honey bees

Insects



Single-stranded
negative-sense RNA



Single-stranded
positive-sense RNA



Single-
stranded
DNA

Double-stranded DNA



Deformed wing virus

A collection of colorful, hand-painted ceramic dolls. The central figure is a large, rounded doll with a yellow face, large blue eyes, and a red mouth. It has a black body decorated with green leaves and red flowers. To its left are two smaller dolls, one with a yellow face and one with a brown face, both with large blue eyes and red mouths. To its right is another small doll with a yellow face and large blue eyes. All the dolls have a black body decorated with green leaves and red flowers. The dolls are arranged in a cluster, with the large one in the center and the smaller ones around it.

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

A cartoon illustration of a yellow nematode (roundworm) inside a green circle. The word "NEMATODE" is written in a curved path along the bottom of the circle. A speech bubble from the nematode says "Oh, I'm com".

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

Concept by Dr. Mark Martin, University of Puget Sound

The Microbiome

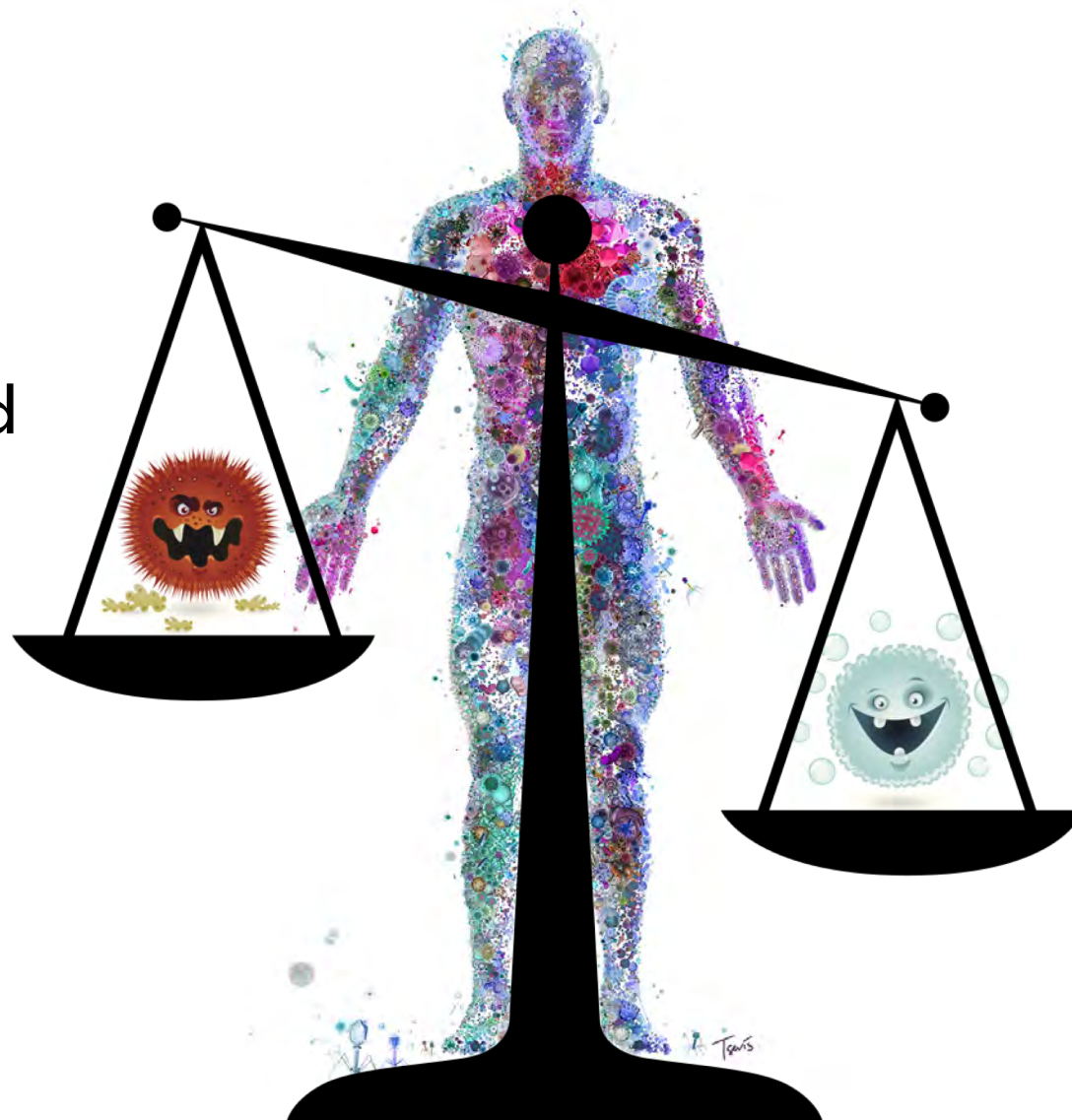
We are FULL of
microorganisms



that impact how our
body works

The Microbiome

Some Bad



Some Good

What's living inside our bees?

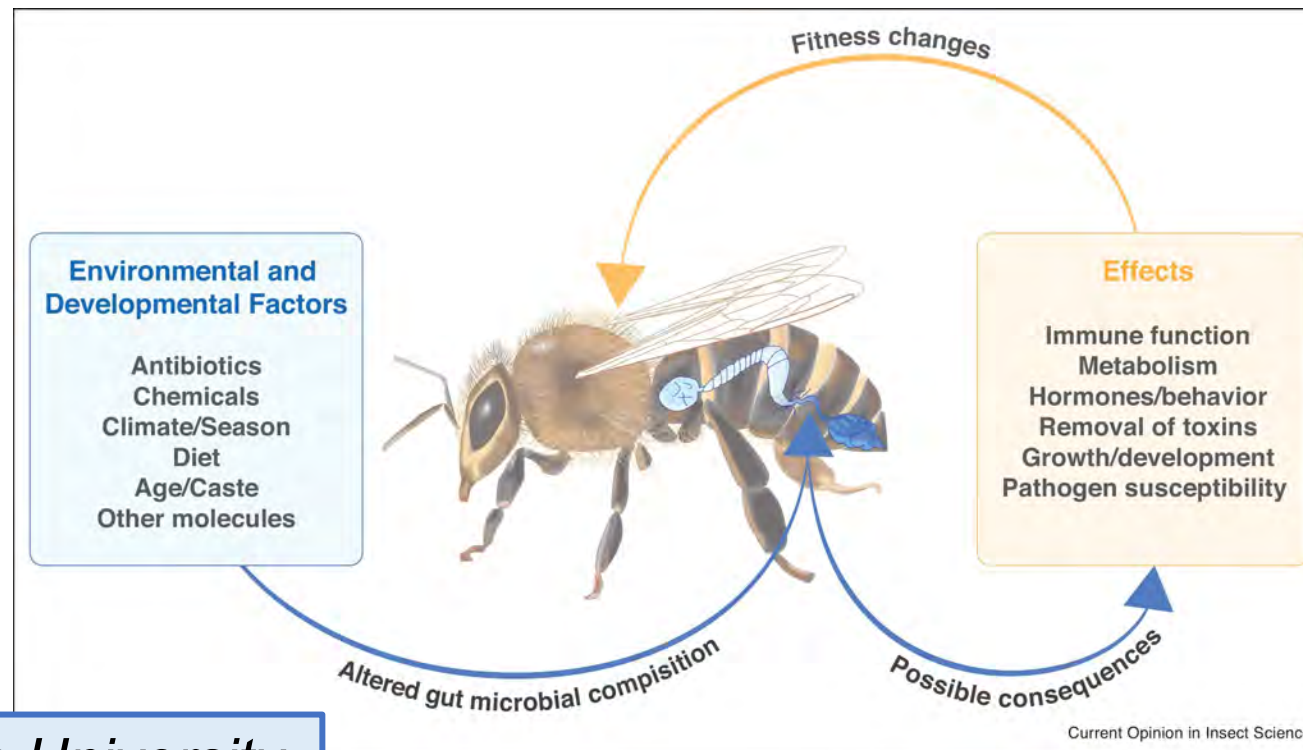
The good, the bad and the ugly



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



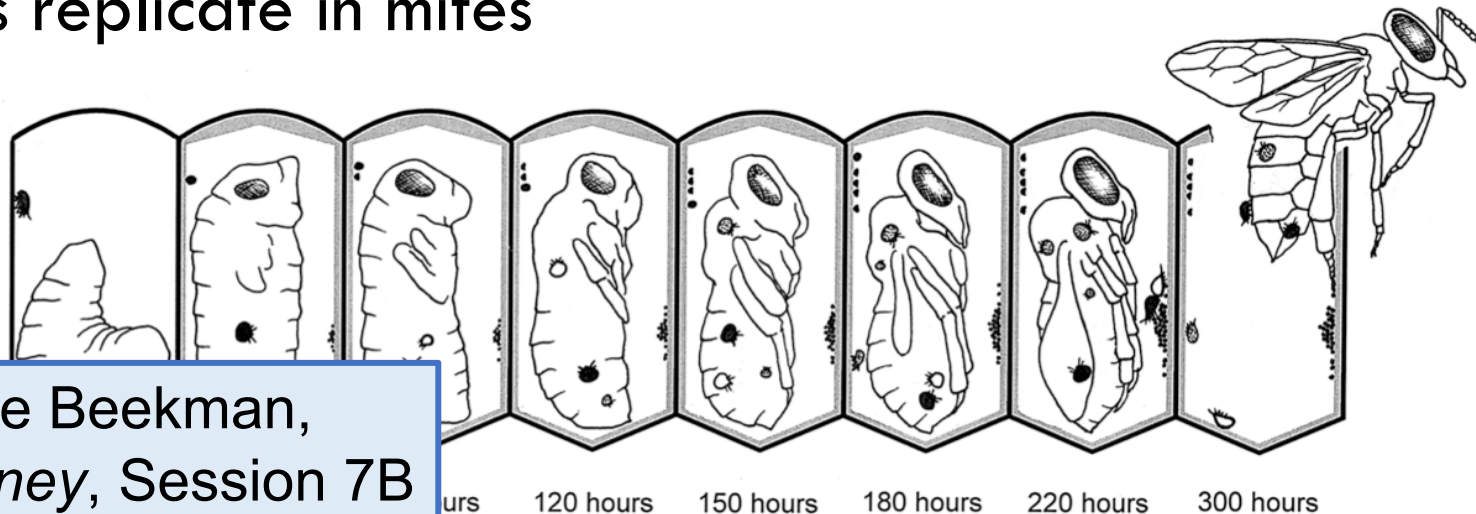
Prof Madeleine Beekman,
University of Sydney, Session 5A



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



Bees brought to their knees

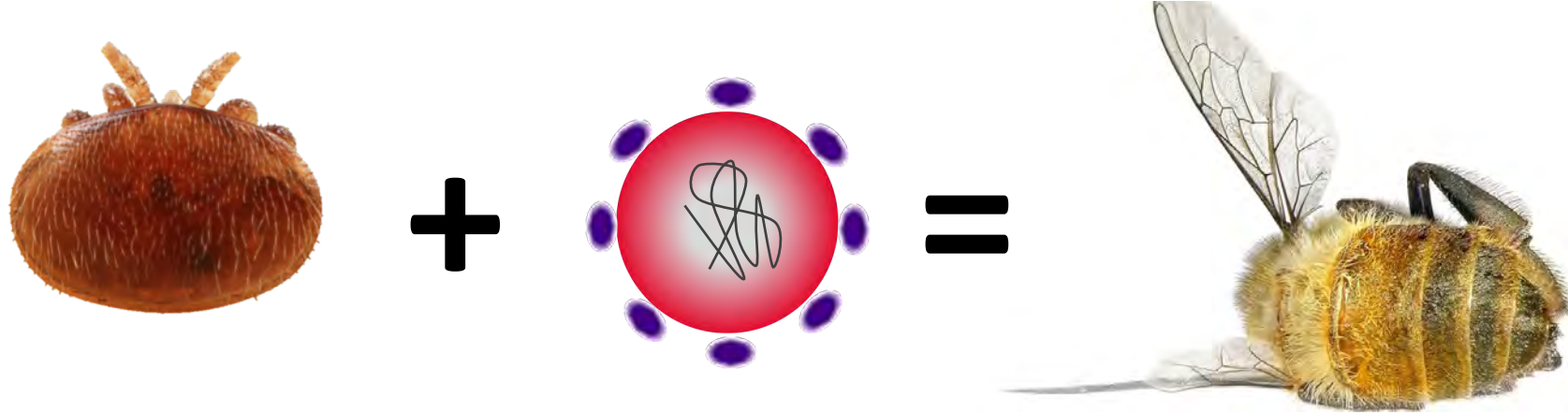
Viruses have changed since the arrival of *Varroa*

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

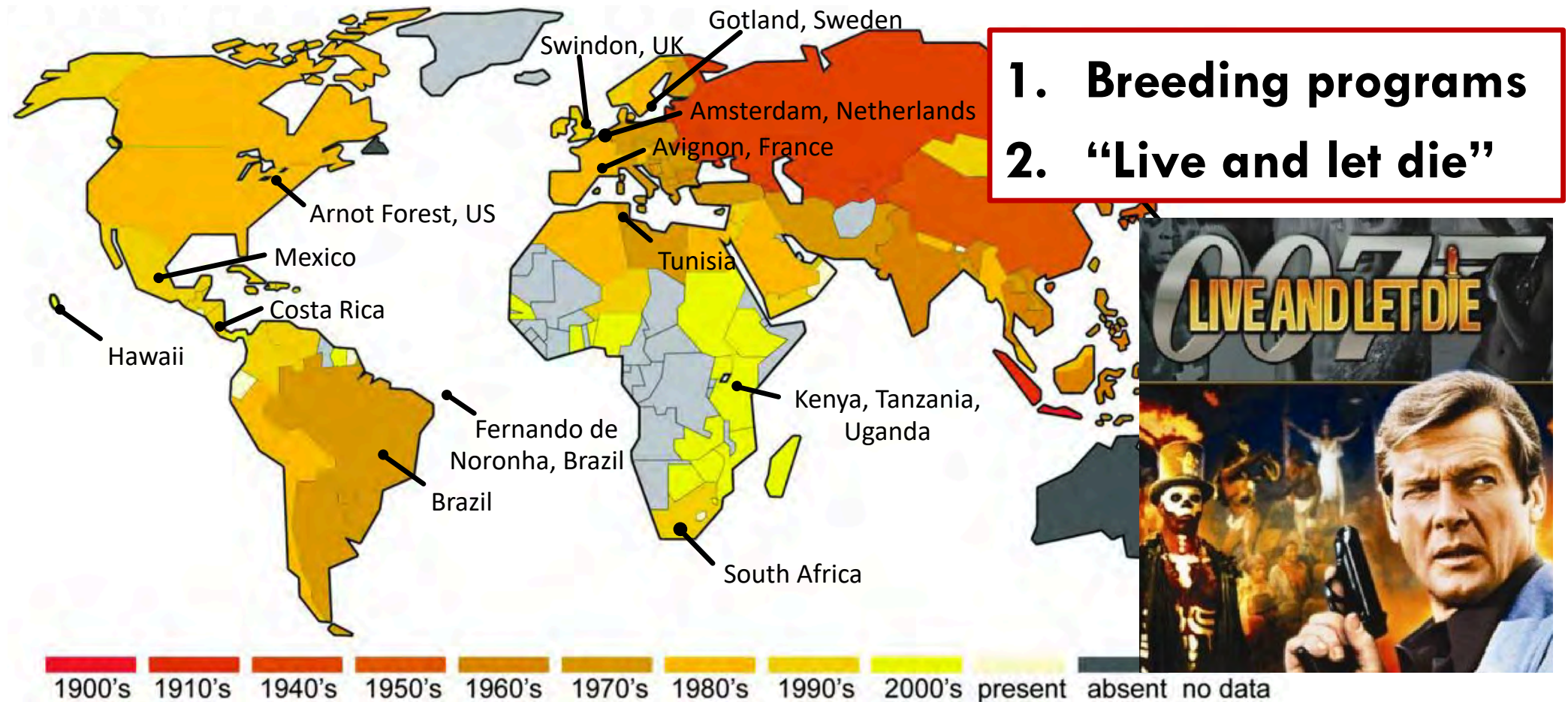
Dr Medhat Nasr, *Ministry of Alberta Agriculture and Forestry*, Session 5A

- 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



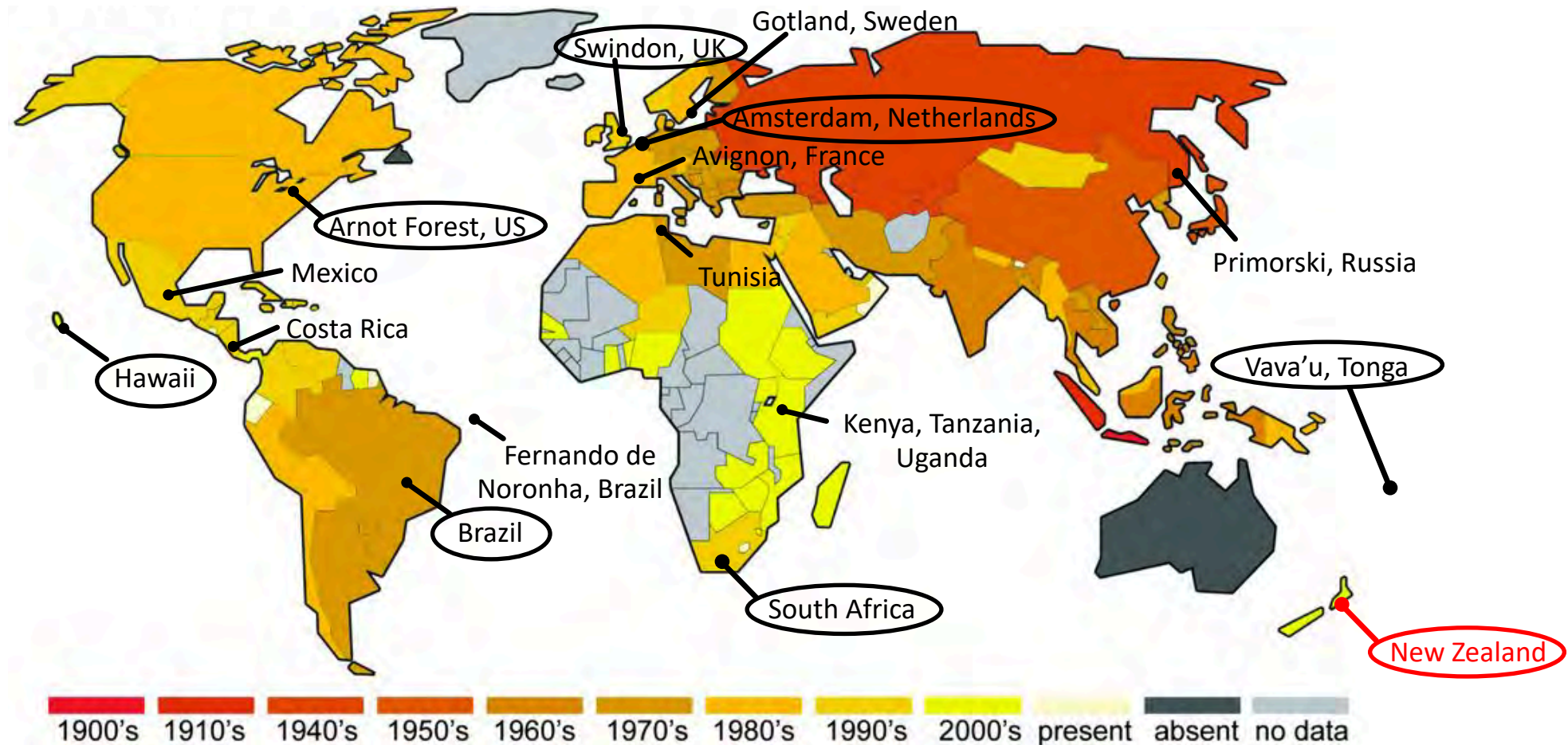
Wilfert *et. al*, (2016) Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. *Science* **351**(6273), pp. 594-7

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

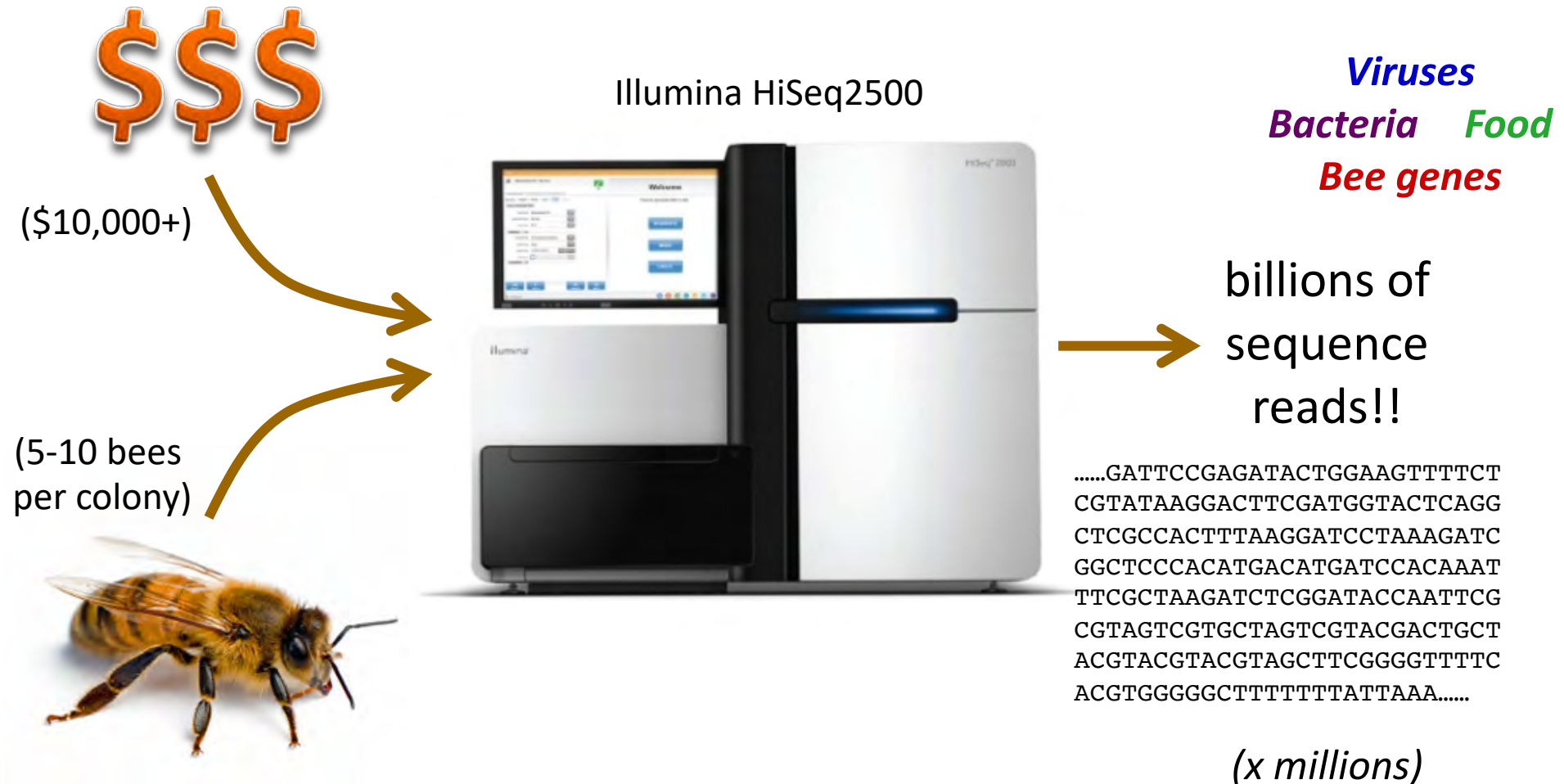
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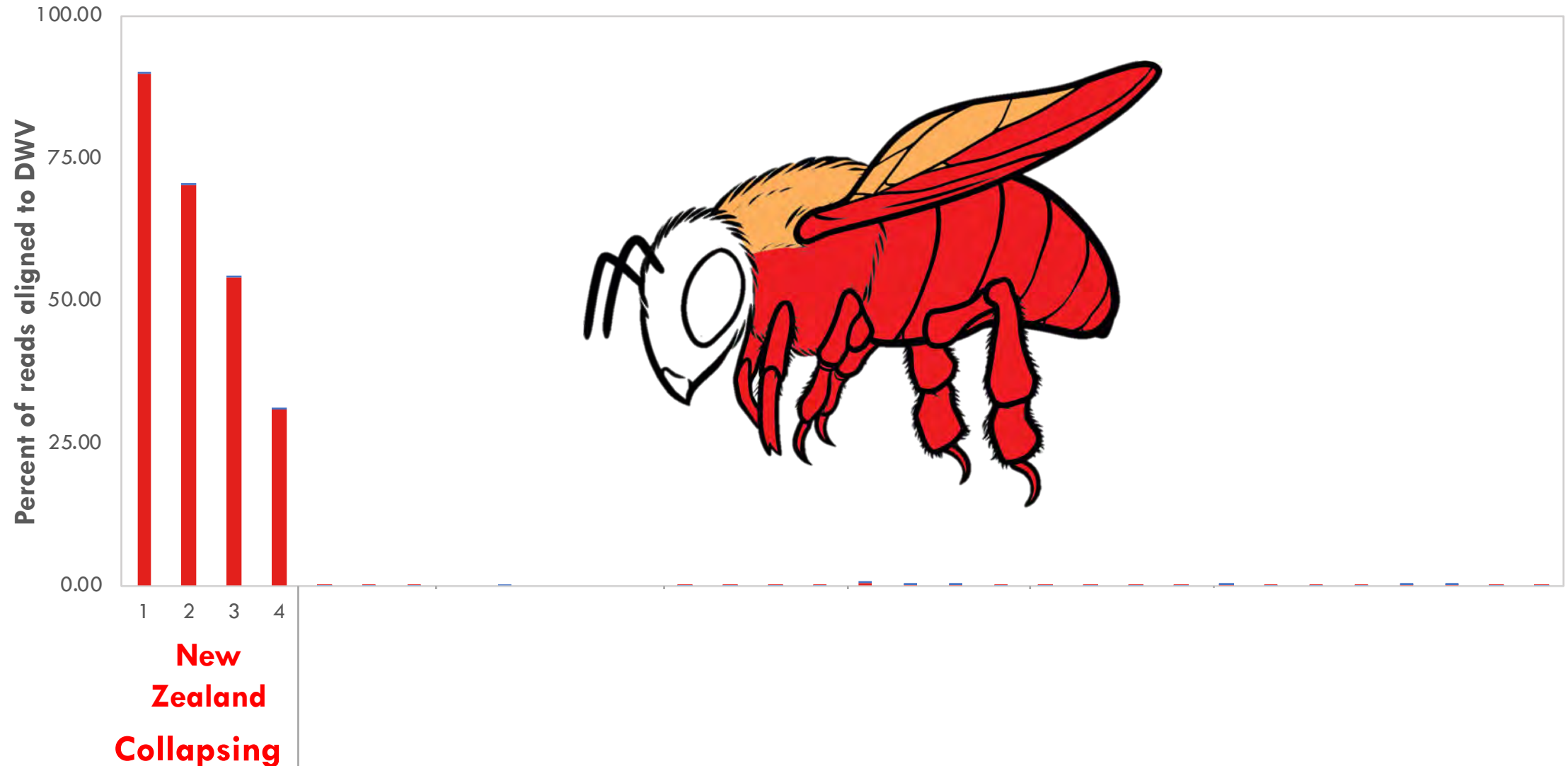
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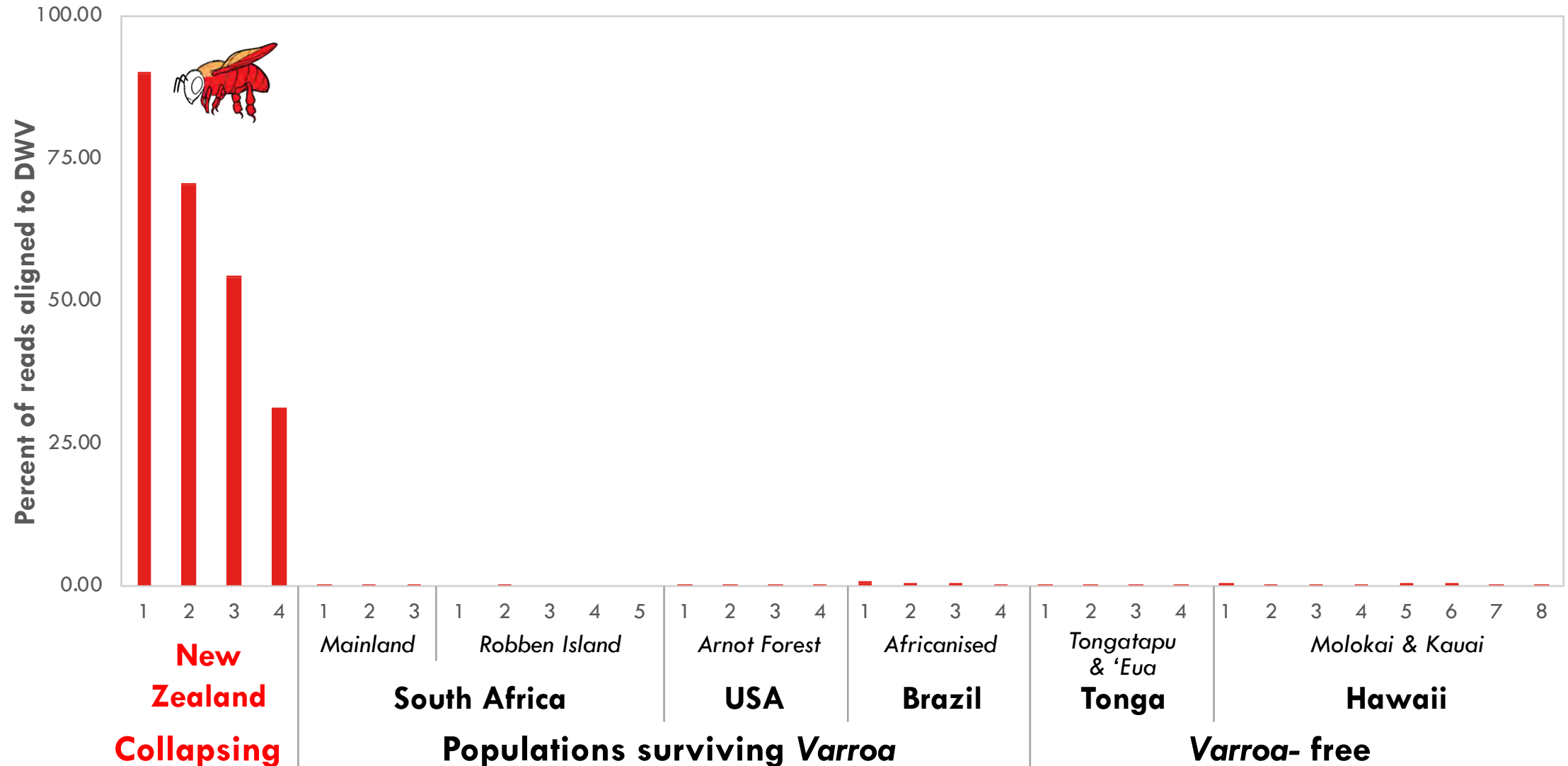
Sequence all the genetic material inside a bee



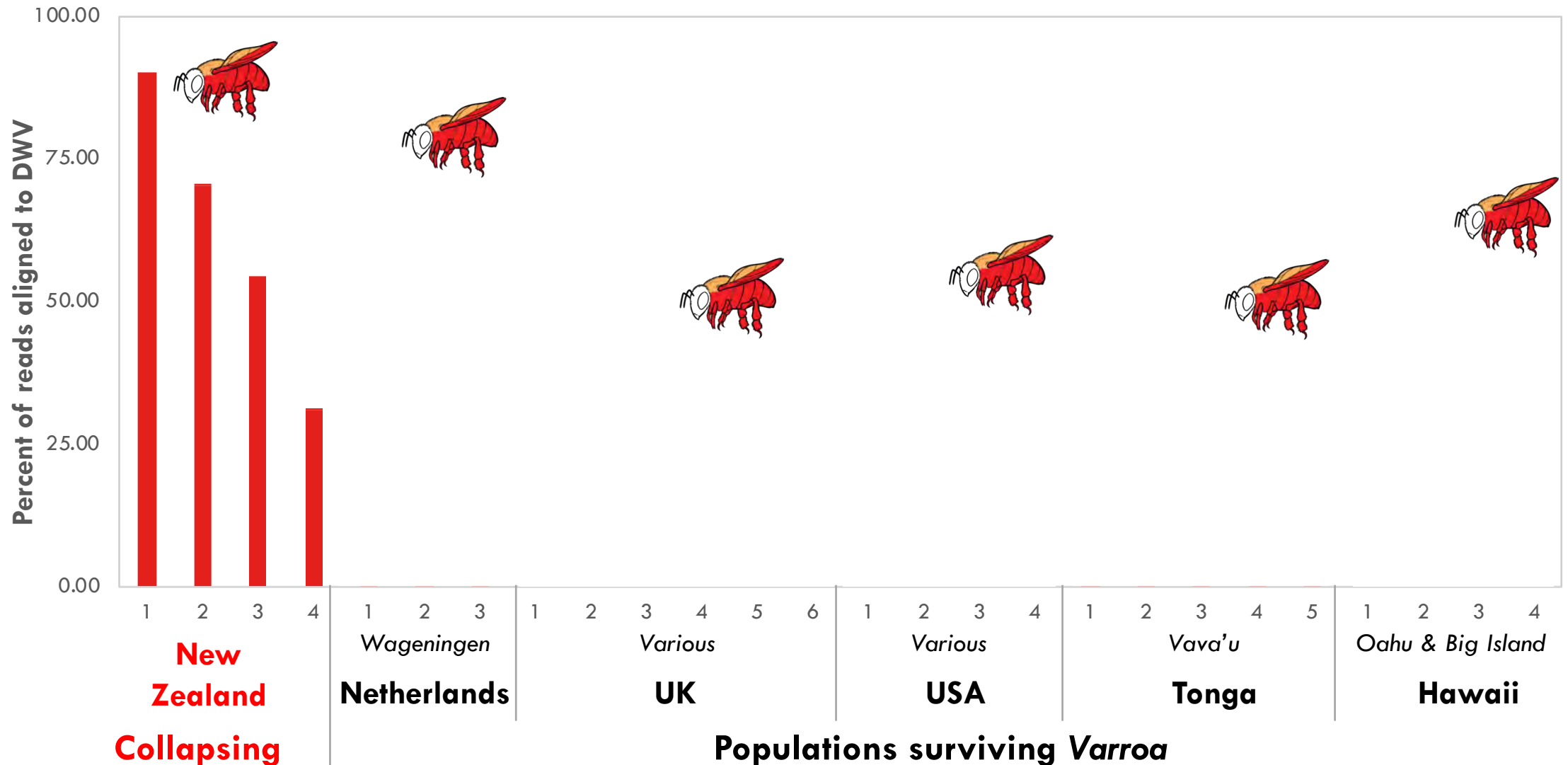
Virus levels in bees can be pretty high!



DWV is absent in some populations surviving *Varroa*

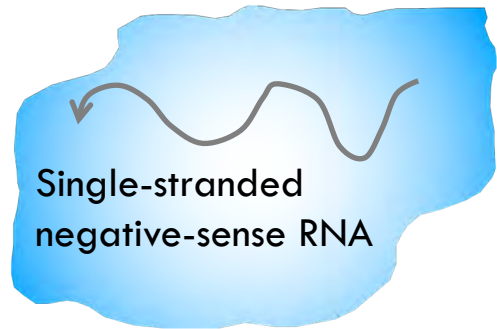


High levels of DWV in other populations surviving *Varroa*

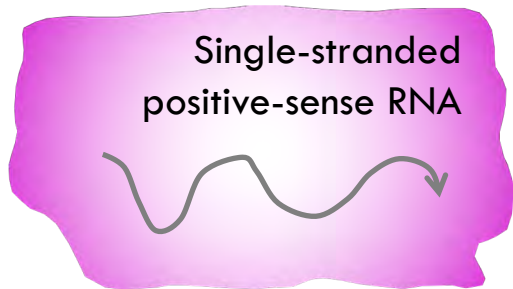


7 new RNA viruses

First negative sense RNA viruses in bees



	Netherlands	South Africa	Tonga	New Zealand
Apis Rhabdovirus 1	✓	✓	✓	✓
Apis Rhabdovirus 2	✓	✓	✓	✓
Apis Bunyavirus 1		✓		
Apis Bunyavirus 2		✓		

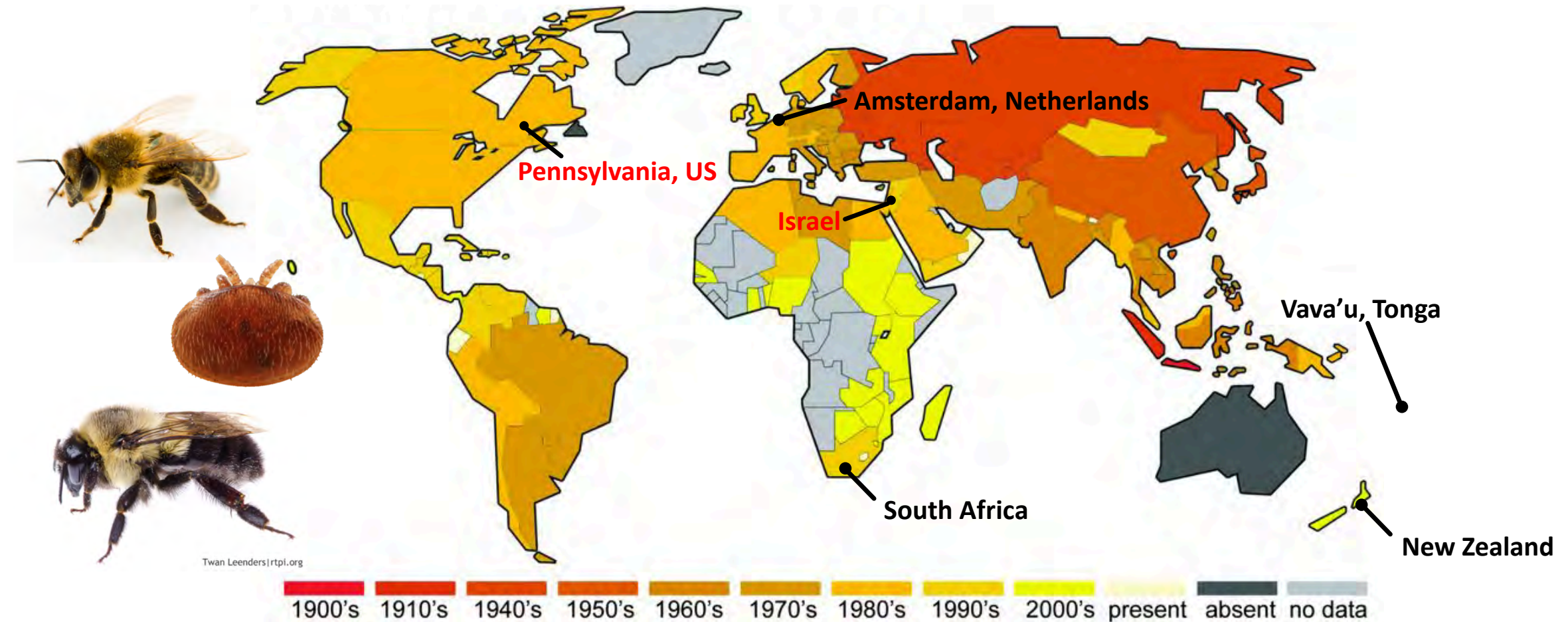


Apis C virus	✓			
Apis Flavivirus		✓		
Apis Nora virus		✓		

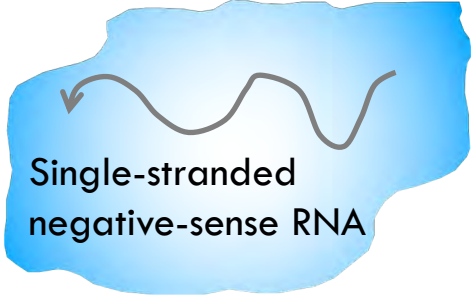
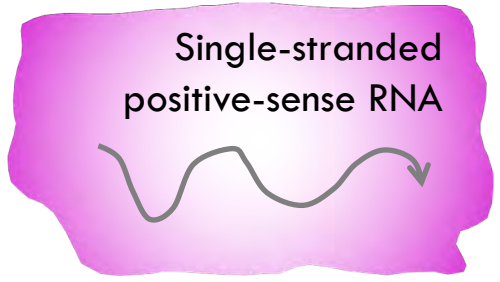

Apis Rhabdovirus 1

Geographically widespread, multi-host virus

Single-stranded
negative-sense RNA



Virus Classification

Genome type	Honey bees	Insects
 <p>Single-stranded negative-sense RNA</p>	4	✓
 <p>Single-stranded positive-sense RNA</p>	25	✓
 <p>Single-stranded DNA</p> <p>Double-stranded DNA</p>	2	✓

*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

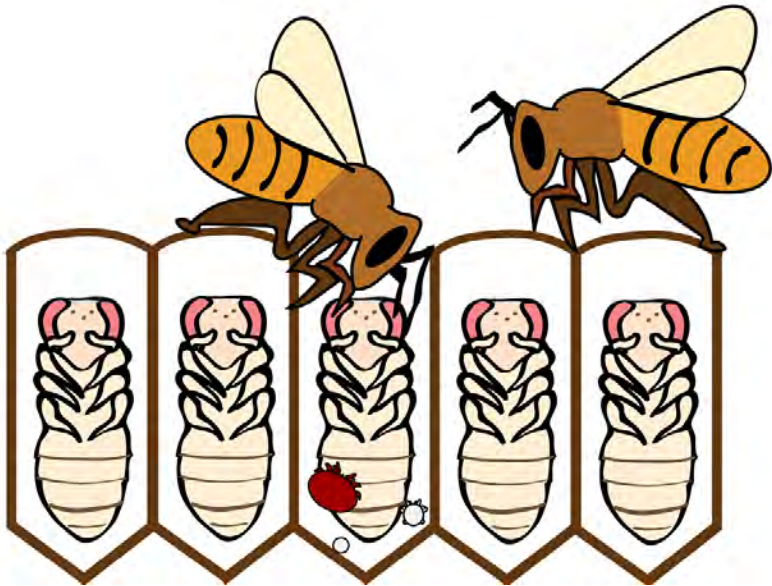
- Insect immunity- less sophisticated than humans
 - No antibodies
 - Can't give them the bee version of a flu shot
- Bees rely on general immune pathways
 - At the colony level: **Hygienic behaviour**
 - Immune genes and pathways



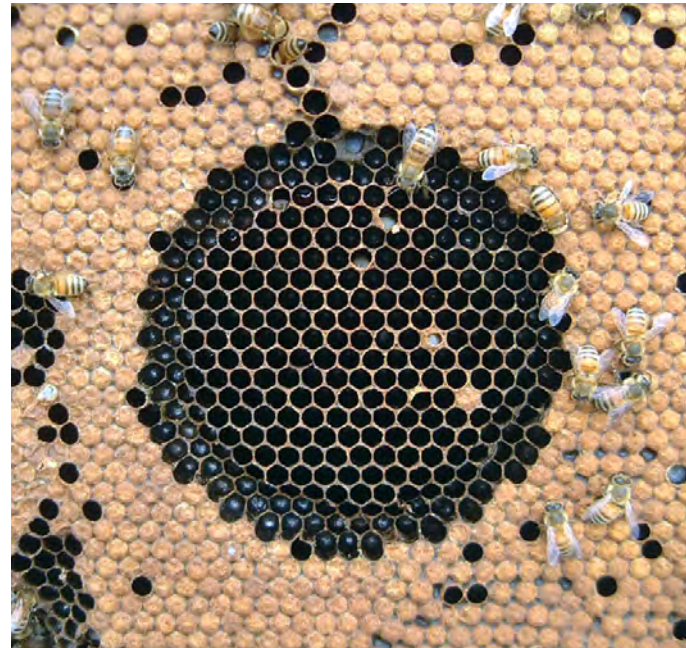
Dr Brock Harpur, *University of Toronto*, Session 3B

Social immunity

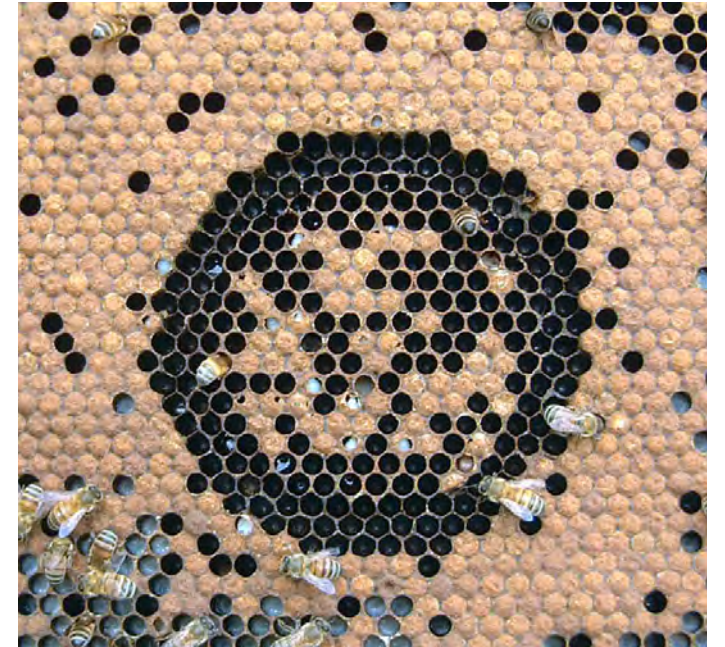
- Hygienic behaviour
 - Worker bees detect sick or dying brood and remove them from the hive
 - Worker bees groom mites from other adult worker bees



A. McAfee



Good hygiene



Bad hygiene

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

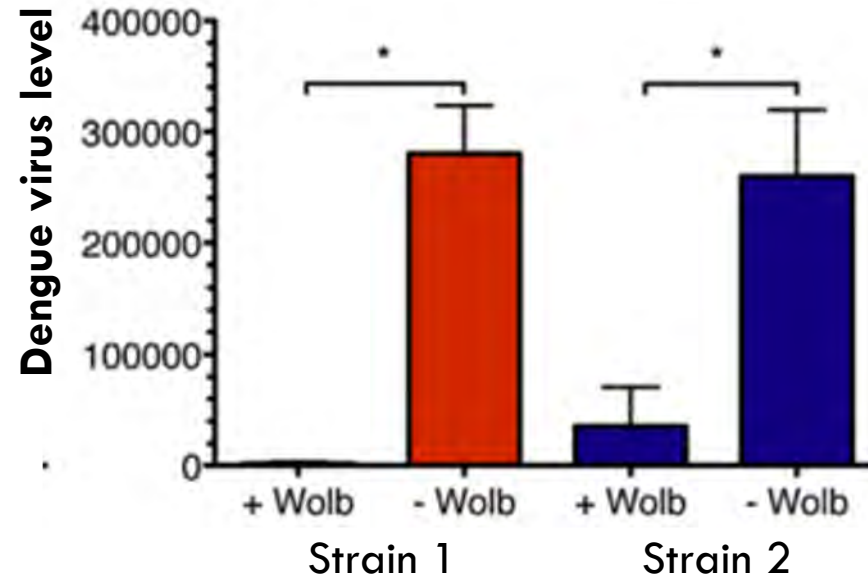
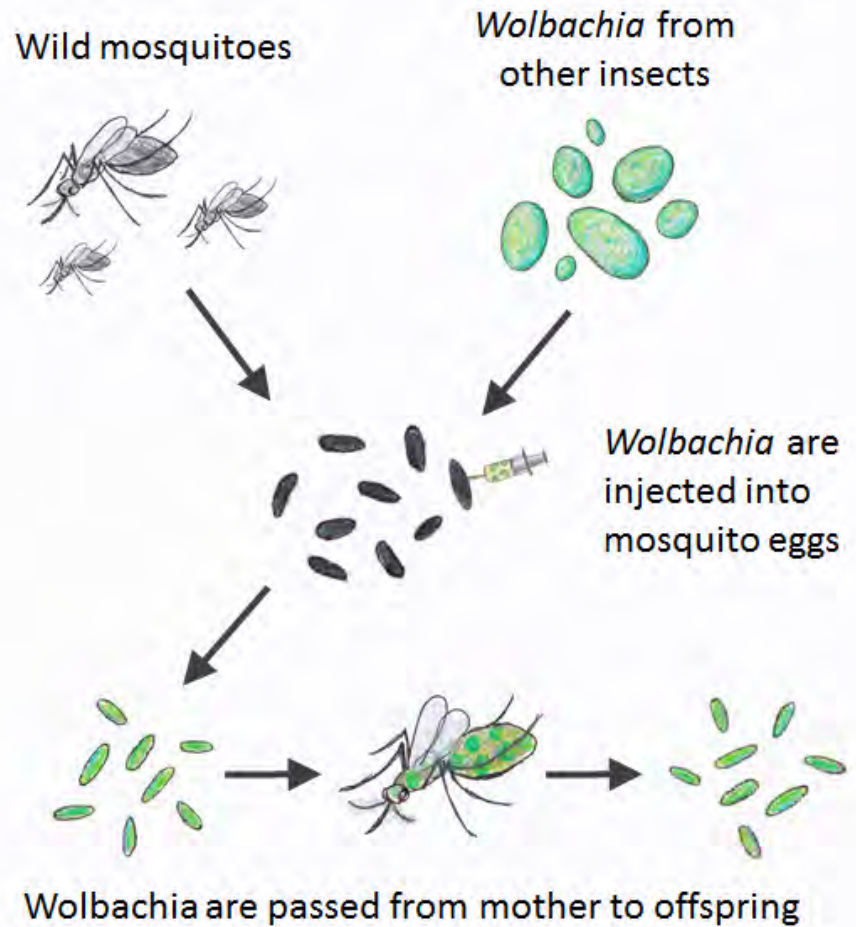


‘Eliminate Dengue’

- 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*



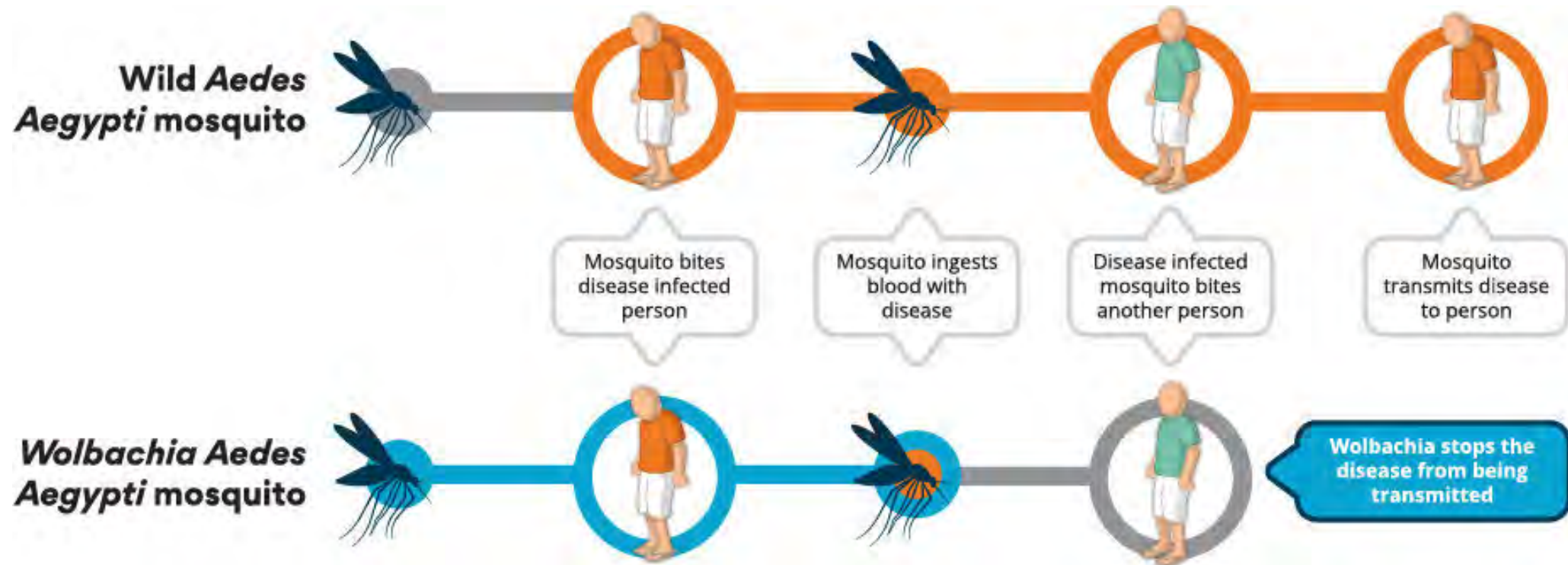
The Wolbachia method



Images: Perran Ross

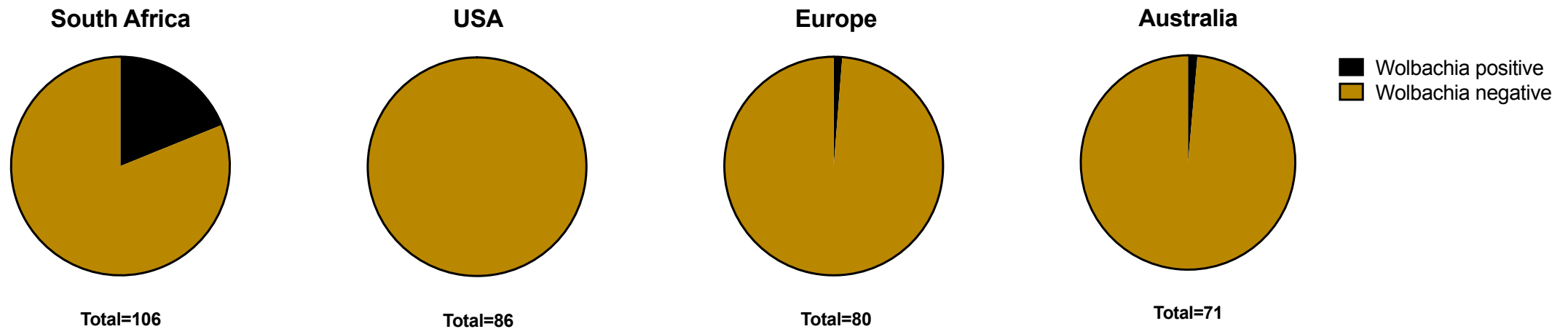
Moreira, L. A., et al, (2009). "A *Wolbachia* symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and *Plasmodium*." *Cell* **139**(7): 1268-1278.

The Wolbachia method



Wolbachia in bees?

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



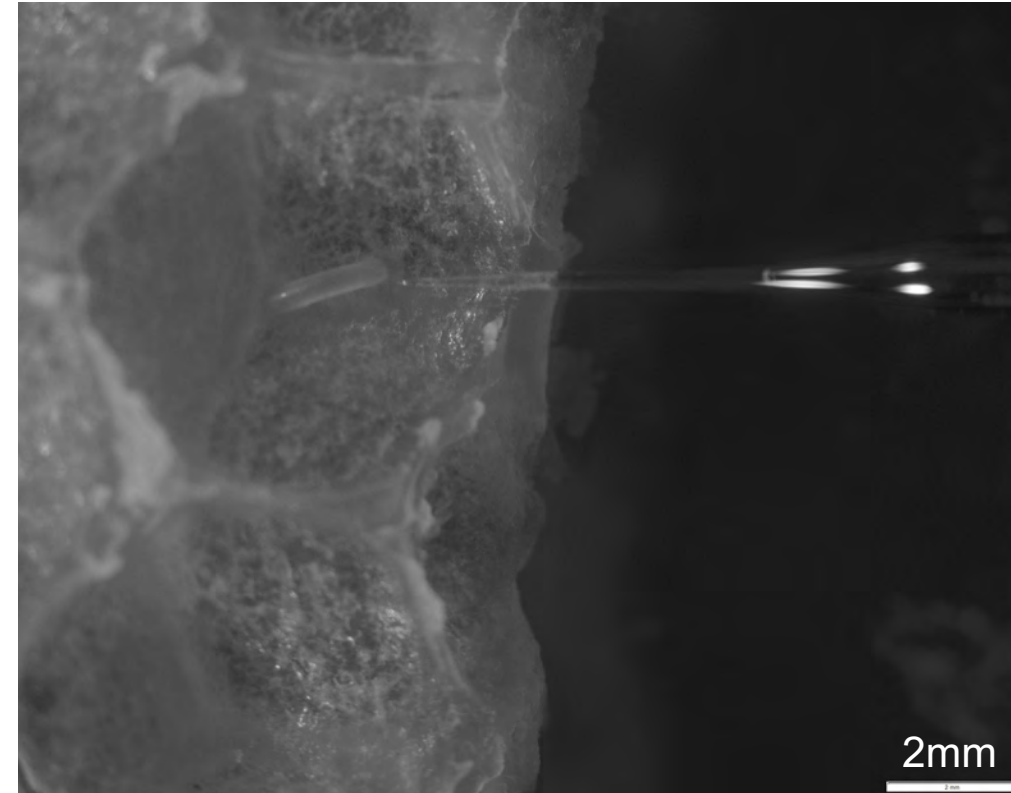
Can *Wolbachia* provide virus resistance to honey bees?

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!

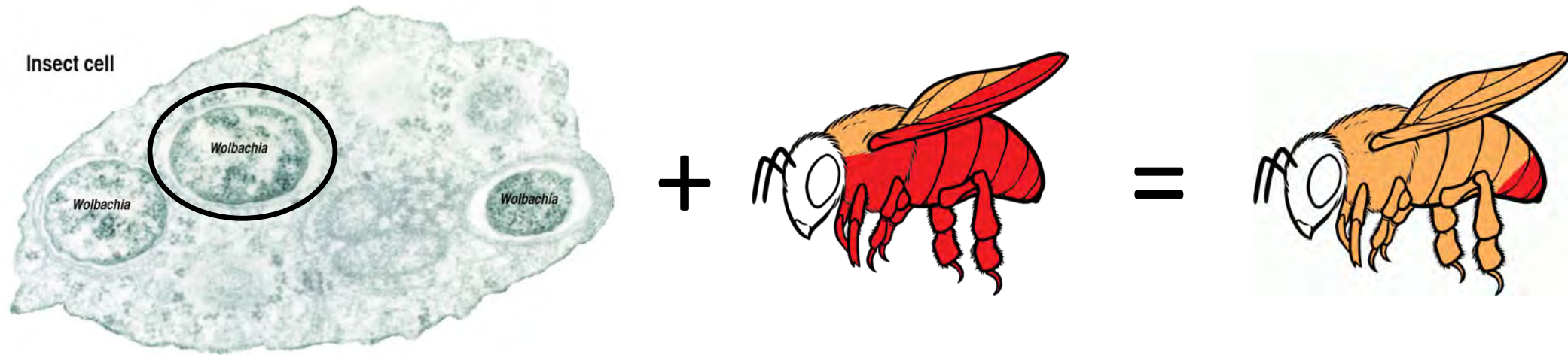


Prof Phil Lester, *Victoria University of Wellington*; Session 8B

ENDEAVOUR
Scholarships and Fellowships



Can we immunise honey bees with bacteria?



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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Phil Lester

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James Baty

Peter Dearden

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Tjeerd Blacquièrè



emily.remnant@sydney.edu.au



@EmsyRemsy

