RNA viruses in Australian bees

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This thesis is dedicated to my father Wing Kock,

Who had the most beautiful soul and hearth….The greatest man I ever knew. He gave me the best he could and encourage me to dream big, set goals and work to achieve them. He is responsible for the person I am.

Although he is not here to celebrate with me, I know he is always by my side looking after me with his unique smile ☺

I MISS HIM everyday..!!

I LOVE HIM

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Abstract

Bees play an important role as pollinators of angiosperms in most terrestrial ecosystems and they are exposed to numerous threats. In many regions in the world, bee abundance and species richness are in decline due to the combined effects of habitat loss, pesticide use, and parasites and disease. Worldwide, diseases caused by RNA viruses are among the greatest threats to the health of the European honey bee (*Apis mellifera*) predominantly when the parasitic Varroa mite (*Varroa destructor*) functions as a vector and incubator of these viruses. While research on RNA viruses in bees has been intensifying around the world, in Australia, information about RNA viruses is limited to managed hives of *A. mellifera*, but no information is available for unmanaged, wild colonies of *A. mellifera*, introduced bumble bees (*Bombus terrestris*) or solitary bees.

While knowledge of the distribution of RNA viruses is important in the context of managing and understanding bee declines, it is also important to have baseline data of prevalence and distributions of RNA viruses prior to an incursion of the Varroa mite. The mite is known to influence the infectivity and virulence of different viruses, but so far, baseline data that allow proper monitoring of this process have been scant. Hence, a survey of the RNA viruses carried by Australian bees is timely and necessary.

For many decades, *A. mellifera* has been perceived as the original and only host of a range of RNA viruses. However, recently "honey bee" RNA viruses have been detected in different species of non-*Apis* bees. This raises questions regarding the original hosts and the direction of transmission of these RNA viruses. Our study confirms the association of some RNA viruses with native bees and show that the probability of South Australian native bees carrying *Black queen cell virus* (BQCV) and S*acbrood virus* (SBV) is higher in non-arid areas with abundant managed and feral *A. mellifera*. Furthermore, the results indicate that BQCV and SBV were introduced into Australia with *A. mellifera*.

Since the introduction of *B. terrestris* onto the Australian island of Tasmania in 1992 from New Zealand, no research has been undertaken to determine whether these bees had brought new viruses to the island. Australia is free of a number of RNA viruses including the epidemic *Deformed wing virus* (DWV), which is present in New Zealand. Using RT-PCR, we found that *Kashmir bee virus* (KBV) and SBV are present and shared between Tasmanian *B. terrestris* and *A. mellifera*, while BQCV was detected only in *A. mellifera*. Because we did not find DWV in either *A. mellifera* or *B. terrestris*, we conclude that introduction of the latter species did not coincide with introduction of this virus. While this is the first report of KBV in Tasmania, we believe it may have been previously detected but misclassified.

Recent studies have reported RNA interference (RNAi) as an immune response of *A. mellifera* to different RNA viruses. The RNAi pathway is activated by presence of double-stranded RNA and degrades the viral genome in 21-22 nucleotides-long small interfering RNAs (siRNAs). siRNAs matching different RNA viruses have been reported in *A. mellifera*, but generation of a complete viral genome using assembly of siRNAs has not been achieved. Our results show that *A. mellifera* larvae activate the RNA interference (RNAi) immune response in the presence of SBV. We generate three complete SBV genomes from three individual larvae from different hives in a single apiary, and demonstrated the presence of different SBV quasispecies within the country.

In summary, this study provides new insights into the epidemiology and ecology of bee RNA viruses. This information is important for understanding the impact of RNA viruses in bee health and for elaboration of mitigation or control strategies.

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Overall percentage (%)	70		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Abbreviations

(-)ssRNA	negative- and single-strand RNA
(+)ssRNA	positive- and single-strand RNA
%	Percentage
°C	Degree Celsius
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
BQCV	Black queen cell virus
CBPV	Chronic bee paralysis virus
CCD	Colony Collapse Disorder
cDNA	complementary DNA
CP	Conservation Park
CSBV	Chines sacbrood virus
CsCl	Caesium chloride
CWV	Cloudy wing virus
dNTPs	Deoxynucleotide solution mix
dsDNA	double-strand DNA
dsRNA	double-strand RNA
DWV	Deformed wing virus
IAPV	Israel acute paralysis virus
ICTV	International Committee on Taxonomy of Viruses

ICTV	International Committee on Taxonomy of Viruses (ICTV)
kb	Kilobase
KBV	Kashmir bee virus
KI	Kangaroo Island
KSBV	Korean sacbrood virus
LD50	Lethal dose 50% or median lethal dose
m^2	Square meters
$MgCl_2$	Magnesium chloride
Ml	Millilitre
mM	Millimolar
NCBI	National Center for Biotechnology Information
NH_4	Ammonium
NP	National Park
nt	Nucleotides
NZ	New Zealand
ORF	Open Reading Frame
RdRp	RNA-dependent RNA-polymerase region
RNAi	RNA interference
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
s	Seconds
SA	South Australia
SBPV	Slow bee paralysis virus

SBV	Sacbrood virus
siRNA	Small interfering RNA
SNP	Single Nucleotide Polymorphism
ssDNA	single-strand DNA
ssRNA	single-strand RNA
TAS	Tasmania
TSBV	Thai sacbrood virus
UK	United Kingdom
USA	United States of America
UV	Ultra-violet
M	Molar
μM	Micromola