

# Susceptibility to viral infection in honey bees driven by genotypic differences in tolerance and resistance

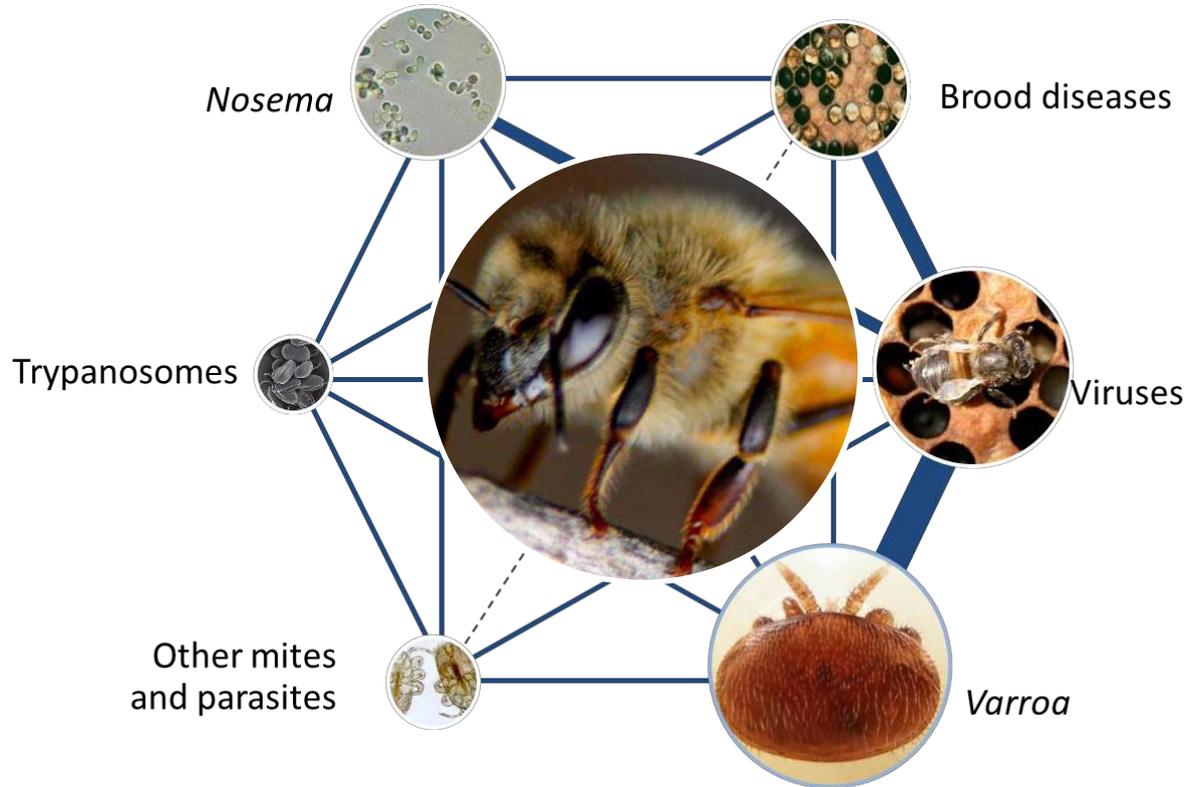
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# Web of parasites and pathogens:

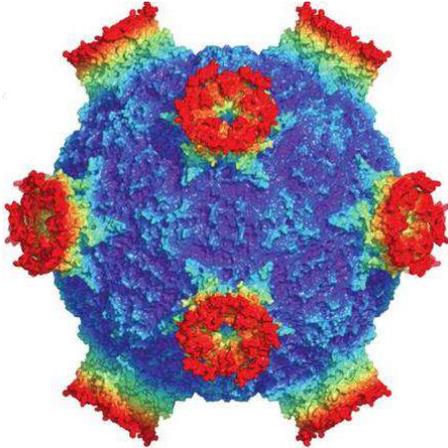
## Biotic stressors impacting bee health



# A focus on Deformed wing virus:

## Using DWV as a starting point

ssRNA I flavivirus with overt symptomology



Škubník et al. 2017, PNAS

# A focus on Deformed wing virus:

## Using DWV as a starting point

- DWV implicated as major driver of colony losses and overwintering death (often with *Varroa*)

Highfield et al. 2009, *App. Env. Microbiol.*

Dainat et al. 2012, *App. Env. Microbiol.*

Dainat et al. 2012, *PLoS ONE*

Francis et al. 2013, *PLoS ONE*

Kielmanowicz et al. 2015, *PLoS Pathogens*

- DWV may suppress honey bee immune system, increasing reproduction of *Varroa*

Di Prisco et al. 2016, *PNAS*

# A focus on Deformed wing virus:

## The main questions

- Are certain genotypes more susceptible to DWV?
- What are mechanisms of resistance?
- Ultimate goal: breeding for viral resistance.



# Understanding differential response to viruses: Resistance vs. tolerance

- Possible virus resistance or tolerance naturally developed or seen in mite-resistant stock

## Defining resistance

- Exhibiting no overt symptoms, preventing or reducing viral replication and maintaining low viral titer

## Defining tolerance

- Exhibiting no overt symptoms, but maintaining high viral replication and titer

# Dynamics of DWV across populations:

## Hints of resistance and/or tolerance

- Lines selected for mite resistance also exhibit lower viral titers

Emsen et al. 2015, PLoS ONE; Toufailia et al. 2014, J. Apicult. Res.

- Isolated populations can survive with high titers

Locke et al. 2014, PLoS ONE

- Russian and Pol-line/VSH pupae may have reduced increase in DWV after mite infestation and fewer symptoms when fed virus

Khongphinitbunjong et al. 2015, J. Invert Physiol.; 2015, J. Asia-Pacific Ent

# A series of experimental tests:

Influence of genotype and life stage on viral susceptibility

1. Pupal inoculation
2. Adult inoculation
3. Queen inoculation



# Pupal inoculation: Simulating mite vectored DWV

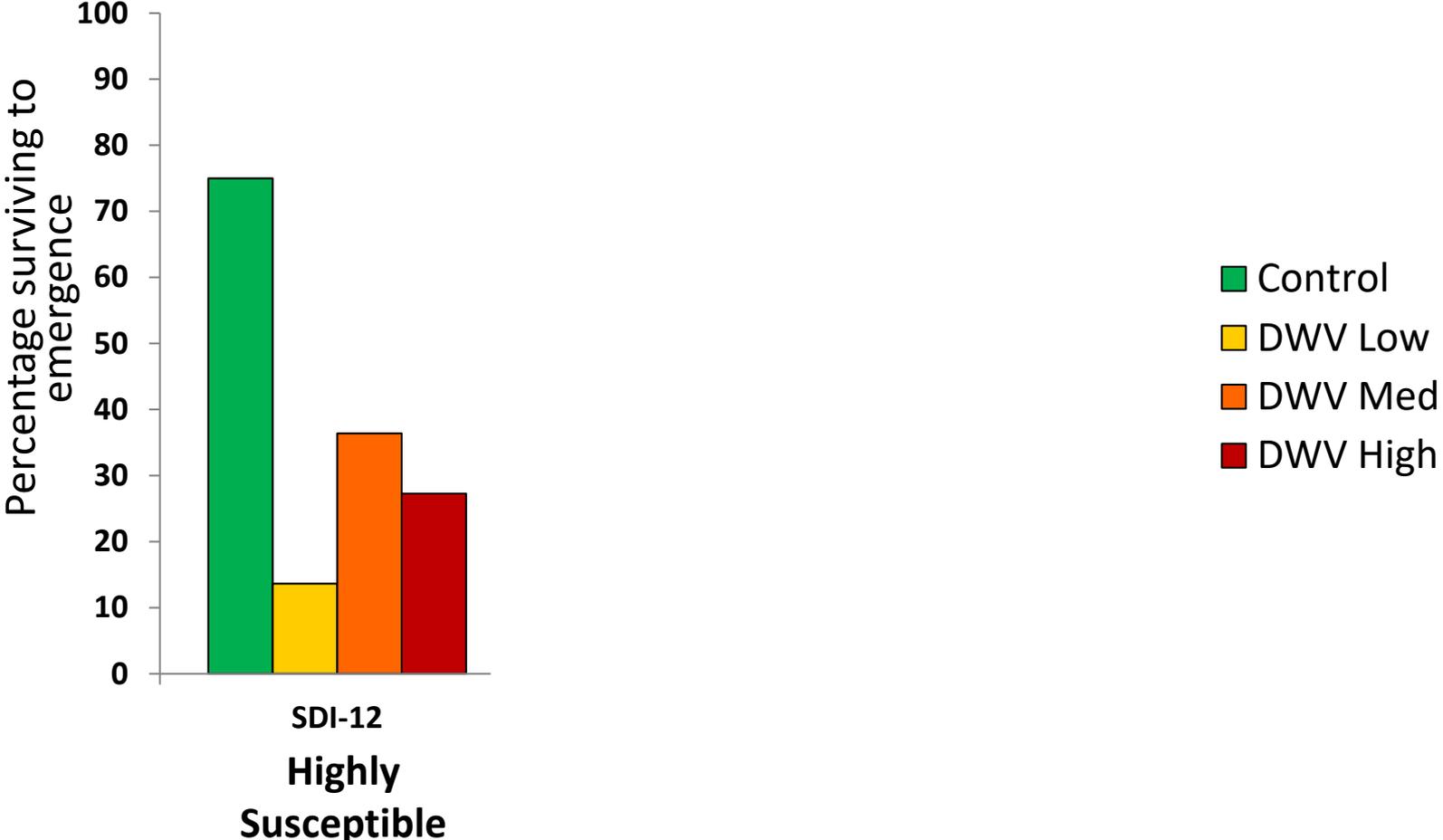
1. Inject pupae with viral solutions  
(control-PBS, low  $10^4$ , med  $10^7$ , high  $10^{10}$ )
2. Sample pupae and developed bees

Tested colonies headed by single-drone  
inseminated queens

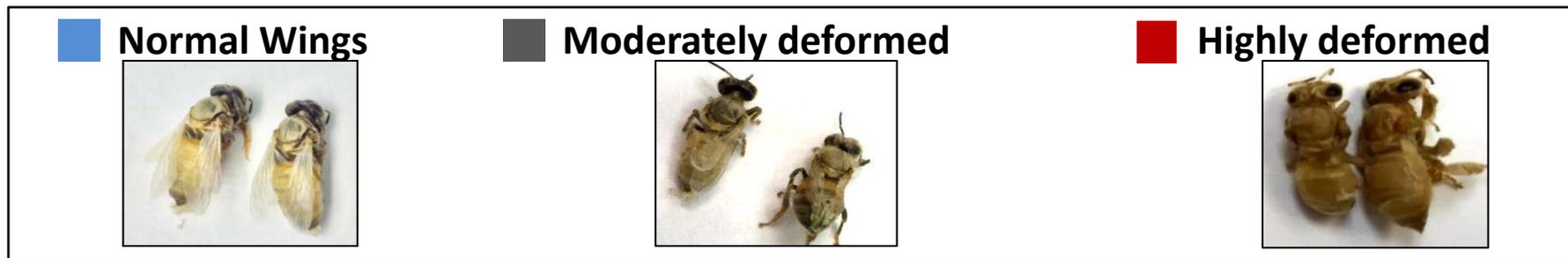
Over 12,000 pupae injected  
~5,400 emerged bees collected



# Colony variation in *survival* after DWV injection

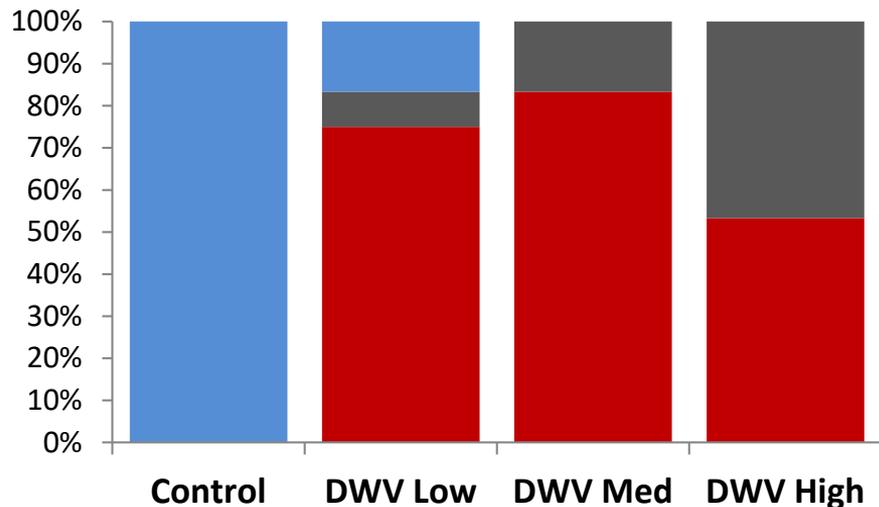


# Differential expression of DWV symptoms



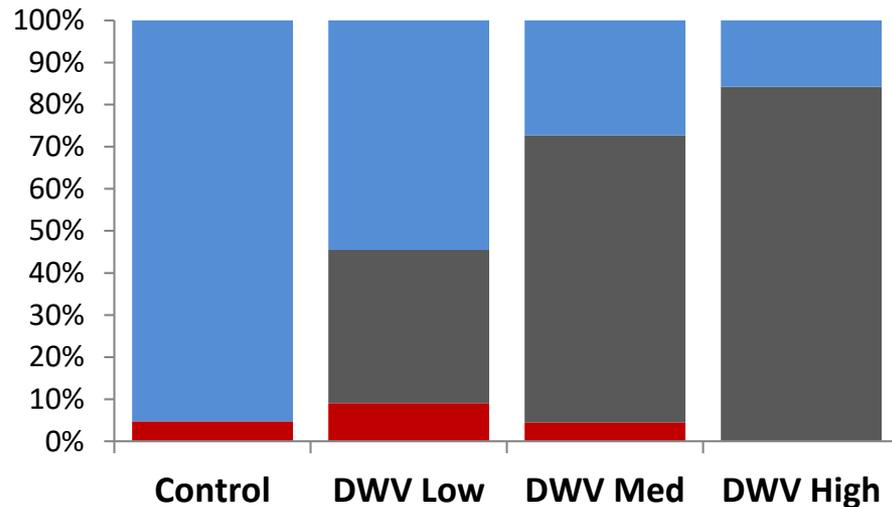
## Colony SDI-11

(Good survival but poor health)



## Colony SDI-18

(A better candidate for study)



# A series of experimental tests:

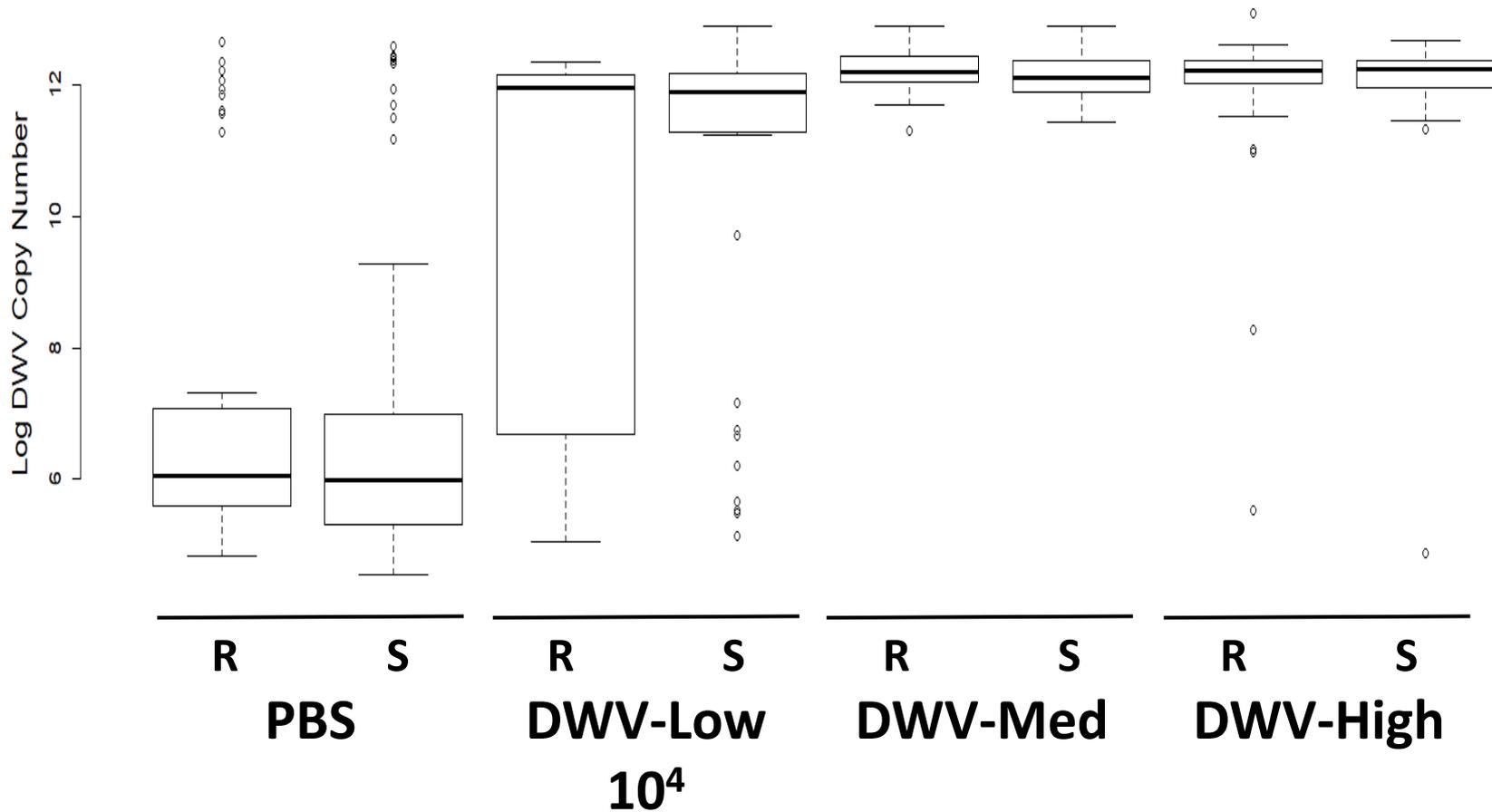
## Influence of genotype and life stage on viral susceptibility

### 1. Pupal inoculation

- Viral levels and gene expression measured 5d post-injection from **8 colonies differing in susceptibility based on survival and symptomology**
- Viral levels assessed of emerged bees (7-9 days post-injection)



# Differential susceptibility driven by tolerance



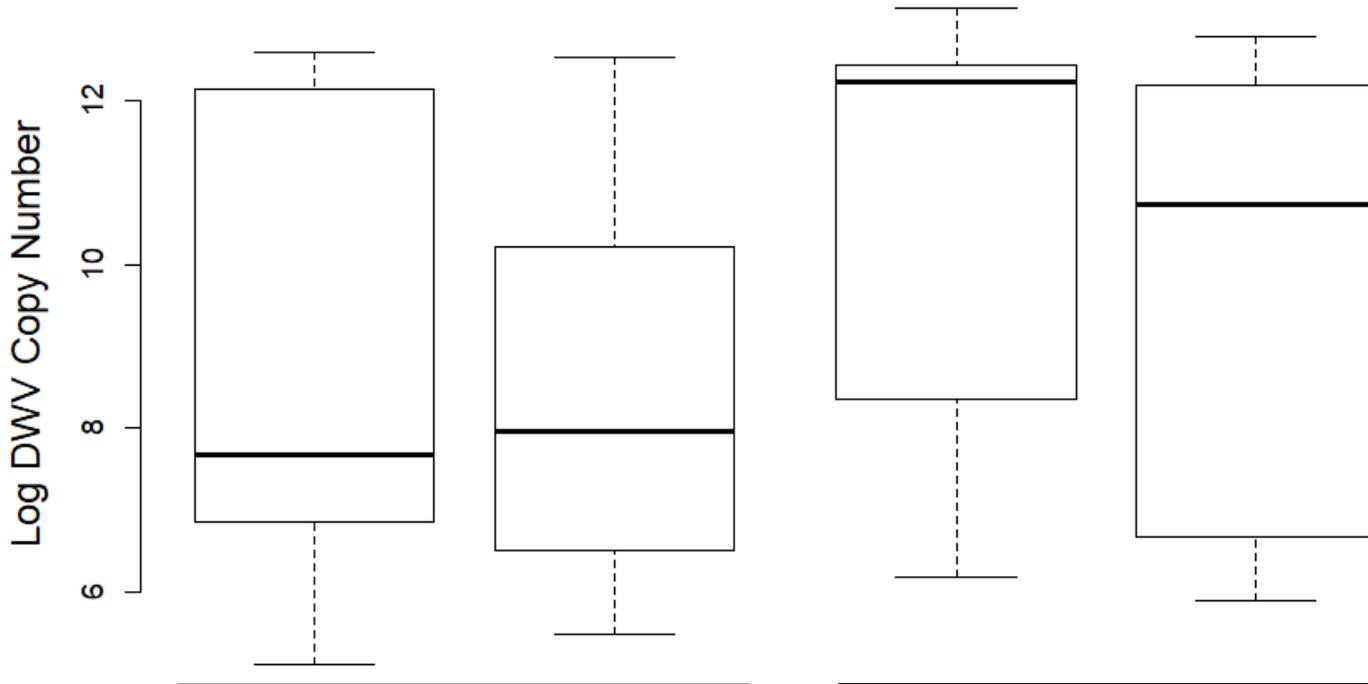
# Asymptomatic vs. Symptomatic bees

- At different doses, why do only some bees from the same colony develop symptoms?



- Susceptible vs. ~~resistant~~ tolerant colonies
- Examine titers after emergence

# More evidence for tolerance: viral titers and symptoms



**Tolerant    Susceptible**  
**Normal Wings**



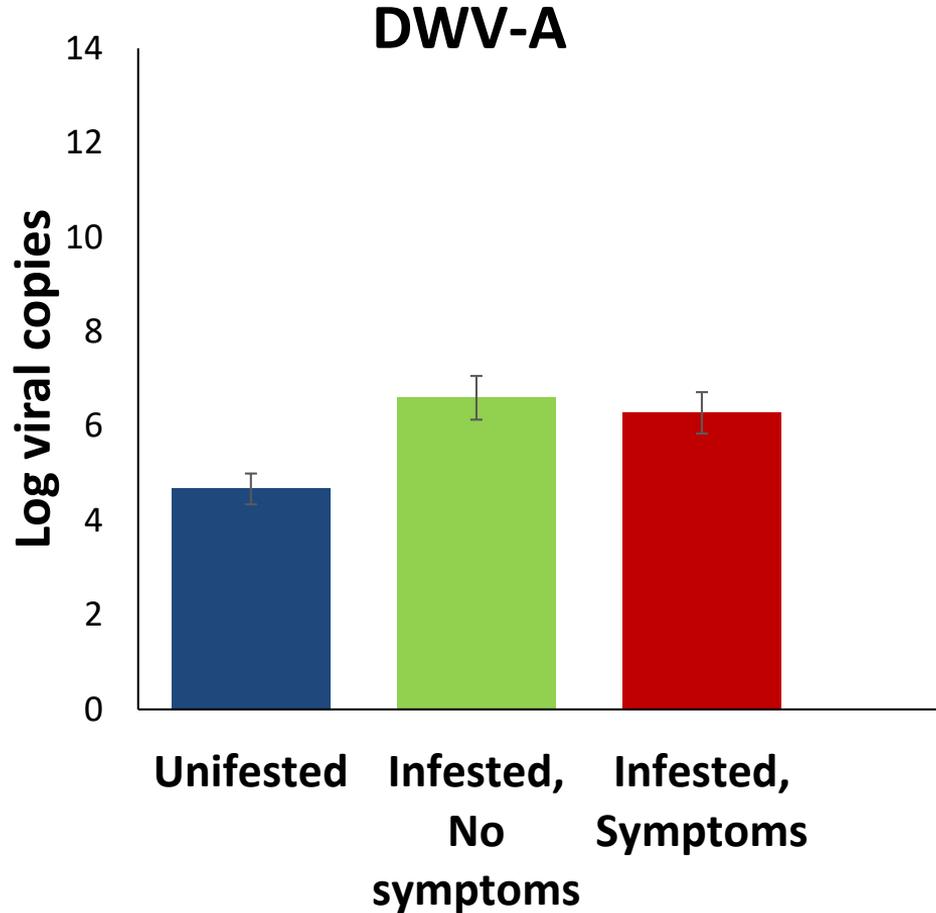
**Tolerant    Susceptible**  
**Deformed Wings**

# Viral genotype and symptoms in a mite tolerant stock

- Collected emerging bees from a mite-tolerant colony bred from beekeeper identified mite-tolerant stock
- Analyzed viral levels from uninfested and mite-infested pupae to determine if viral response influences mite tolerance



# Viral genotype and symptoms in a mite tolerant stock



# A series of experimental tests:

## Influence of genotype and lifestage on viral susceptibility

### 2. Adult inoculation

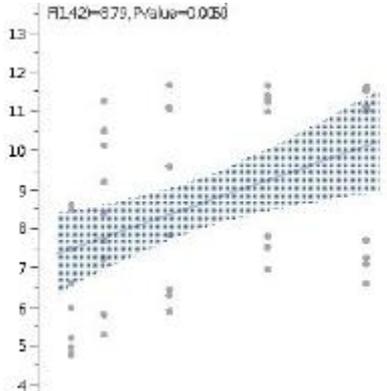
- Inject newly emerged bees with  $10^5$  mixed DWV-A/B
- 3 colonies each of five different stocks
- Maintain in cages and collect over 10 days
- Examine different body parts for viral dissemination



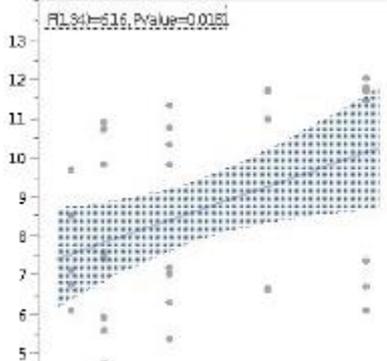
# Genotype may influence viral replication in adults

Carniolan

Log DWV-B (GLAND)



Log DWV-B (LEG)



Day 1-10 



# Finding ~~resistance~~ tolerance to viruses:

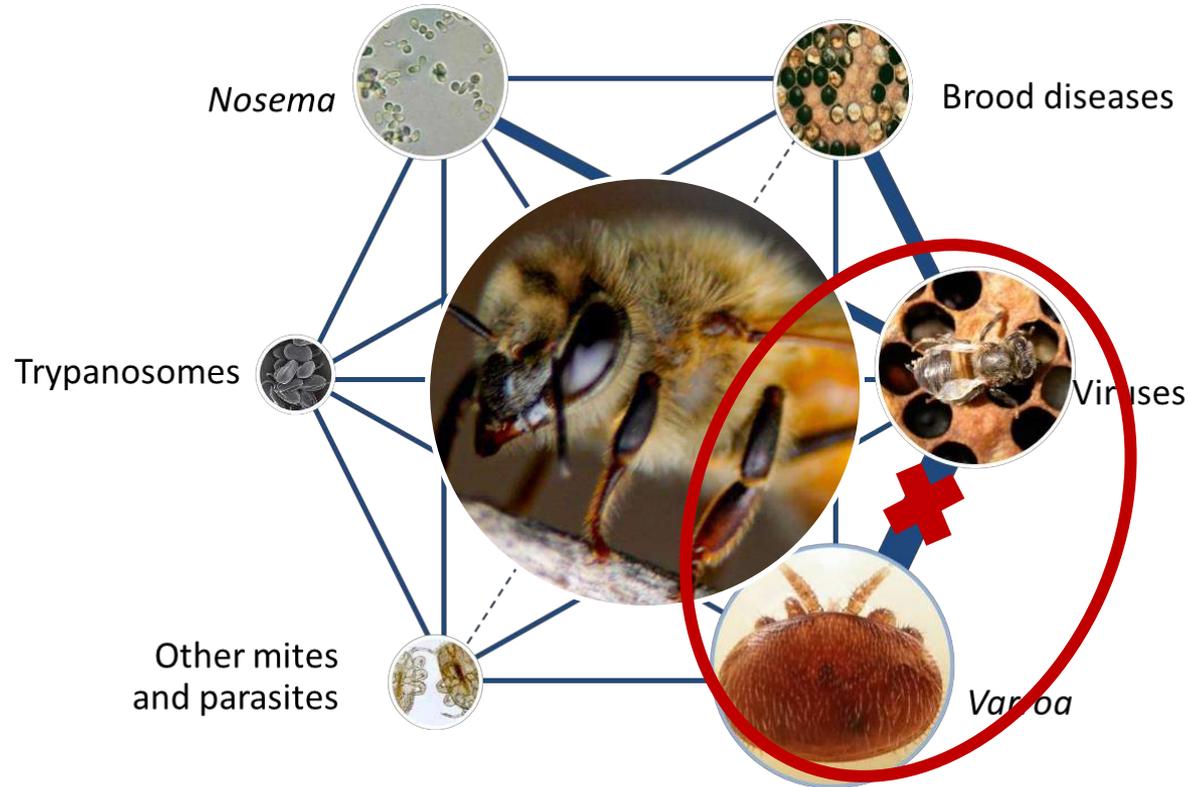
## Moving forward

- Significant variation across and within stocks of honey bees
- Mechanism of tolerance may to be related to immune/antiviral responses
- Is tolerance to viral infection a desirable trait for breeding?
- Complexities of viral infection—  
transmission route, host and viral genotypes



# Web of parasites and pathogens:

## Disentangling the connections





# Questions and comments?

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