

Honey bee (*Apis mellifera*) hygienic behavior linked to innate defenses against an adult bee pathogen, *Vairimorpha* (*Nosema*) *ceranae*

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The honey bee colony (*Apis mellifera*) as a superorganism, functions with a dual immune system operating at the individual and social level. Hygienic behavior as a social mechanism of disease resistance plays a key role in limiting the transmission of pathogens within the colony. This behavior has been linked to reduced brood diseases like chalkbrood, foulbrood, and *Varroa* infestations, and has been a major focus in artificial selection programs for disease resistance. *Vairimorpha* (*Nosema*) *ceranae*, a microsporidian parasite known to infect adult bees, is not directly targeted by the removal of diseased brood. However, using the Unhealthy Brood Odor (UBeeO) assay, we observed that hygienic colonies had lower *V. ceranae* loads over time compared to unhygienic colonies. We inoculated individuals from hygienic and unhygienic colonies with *V. ceranae* to assess their innate defenses against the pathogen. Our results suggest that individuals from hygienic colonies tend to avoid *V. ceranae*-contaminated food sources, exhibit upregulated vitellogenin gene expression, and have a greater probability of surviving *V. ceranae* infection. Using mark-recapture techniques, we also investigated the colony acceptance rate of infected individuals in hygienic and unhygienic colonies. Fewer infected individuals were recovered from hygienic colonies by day 3, suggesting that hygienic colonies may be better able to distinguish infected from non-infected individuals, and less likely to accept *V. ceranae*-infected individuals. Here, we present new findings that link hygienic behavior to general odor detection and individual tolerance to *V. ceranae*, along with co-occurring social defense mechanisms, that confer colony-level resistance to *V. ceranae*.

Honey bee hygienic behavior linked to innate defenses against *Vairimorpha ceranae*.

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Thank you to collaborators and funding sources

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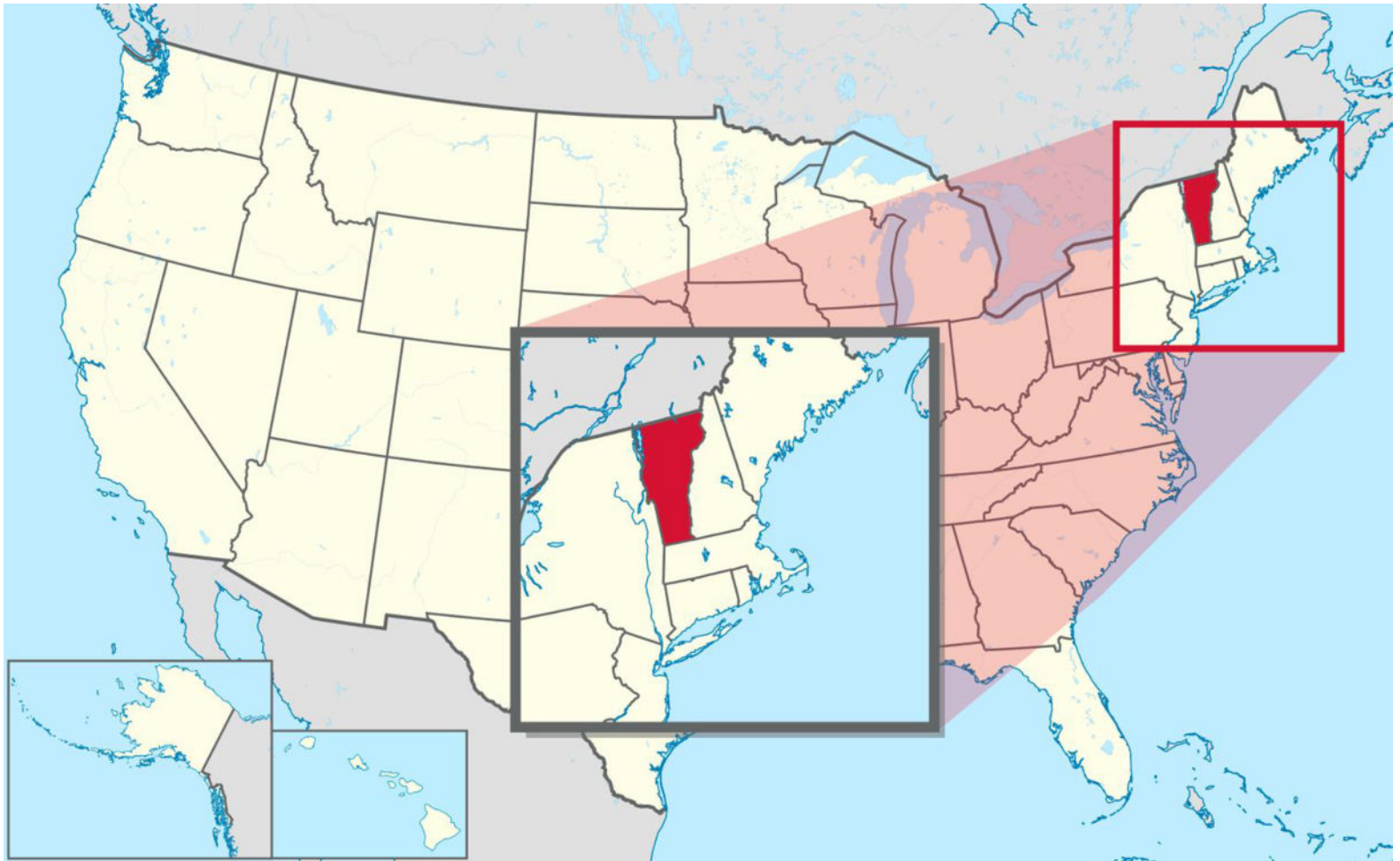
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**VERMONT SPECIALTY CROP
BLOCK GRANT PROGRAM**







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Current Programming

- Supporting VT Bee Breeders with UBeeO (2022- current)
- Diagnostic Clinic (2019-current)
- National Honey Bee Survey (since 2015)
- VT Pesticide Monitoring Project (2021-current)



Support Bee Producers with UBeeO

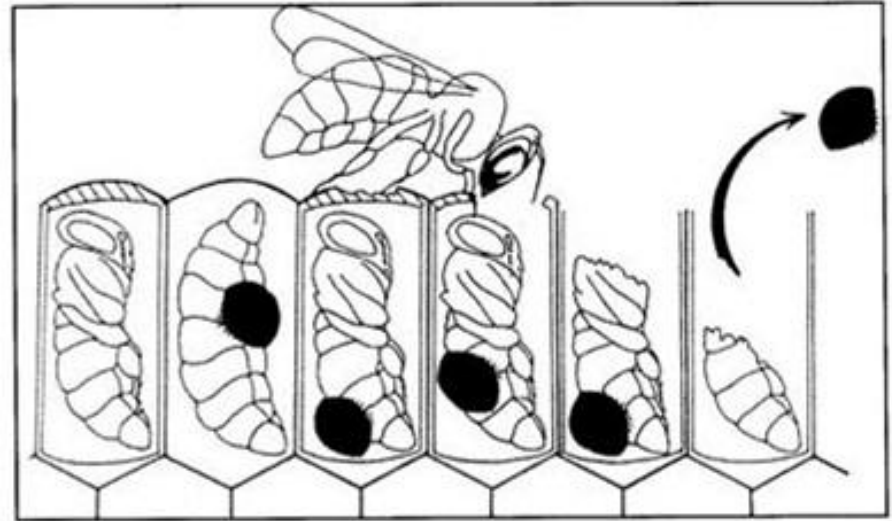
Our Goals:

- Leverage our lab's resources to improve the quality and availability of locally adapted, pest/pathogen resistant honey bee stock in Vermont.
- Supporting bee producers with novel hygienic behavior testing (UBeeO) and disease monitoring.



Hygienic behavior in honey bees

- Regulated by chemical communication
- Developing brood signals health status to adult nurse bees and this triggers hygienic uncapping and removal of brood
- Selective breeding can offer a sustainable, long-term solution for Varroa control



▲ **Figure 15.1** The process of removal, or hygienic, behavior. A bee detects a cell containing a parasitized pupa. She uncaps the cell, and the pupa is removed from the cell. The mother mite may escape from the cell, but her offspring would be destroyed by the bees during the removal process. Drawing by O. Boecking.

Measuring Hygienic Behavior

Methods of measuring hygienic behavior have been labor intensive or does not always confer Varroa resistance (Leclercq et al., 2018, Spivak and Danka, 2021)



Photo by SARE



Hygiene-Eliciting Brood Semiochemicals as a Tool for Assaying Honey Bee (Hymenoptera: Apidae) Colony Resistance to *Varroa* (Mesostigmata: Varroidae)

K Wagoner ✉, J G Millar, J Keller, J Bello ✉, P Waiker, C Schal, M Spivak, O Rueppell

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Unhealthy Brood Odor Assay (UBeeO)



Low UBO Score



High UBO Score

UBee0 advantages

UBee0 tests are now available to purchase at Optera.

Advantages:

- Testing for response to 'stressed' brood, not dead brood
- Predicts a wide range of pests and pathogens (Alger et al., 2025)
- Quicker (2 hr vs. 48 hr)
- Doesn't require liquid nitrogen

Participating Bee Producers

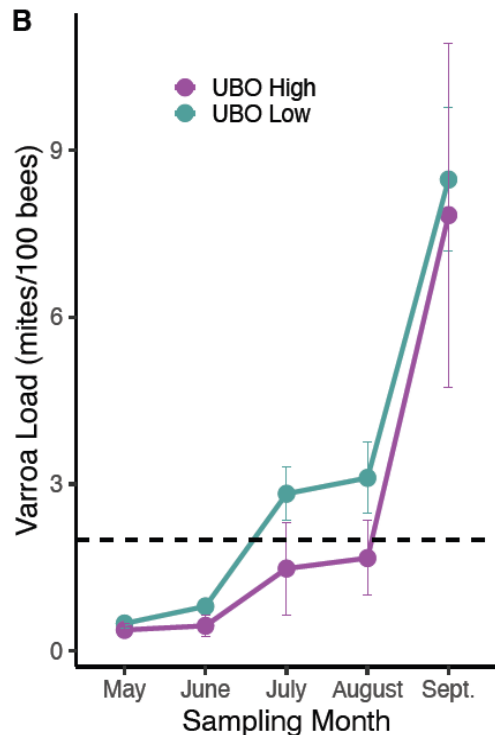
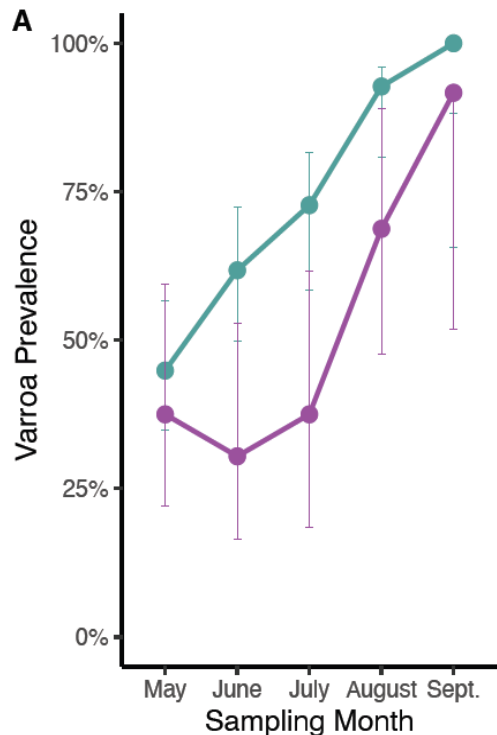
- French Hill Apiaries- Mike Palmer
- Lemonfair Honey Works- Andrew Munkres
- Hundred Acre Woods Apiary- Jack Rath

150 - 1,000 hives, 300-1,500 queens produced
Open Mated and Instrumentally Inseminated



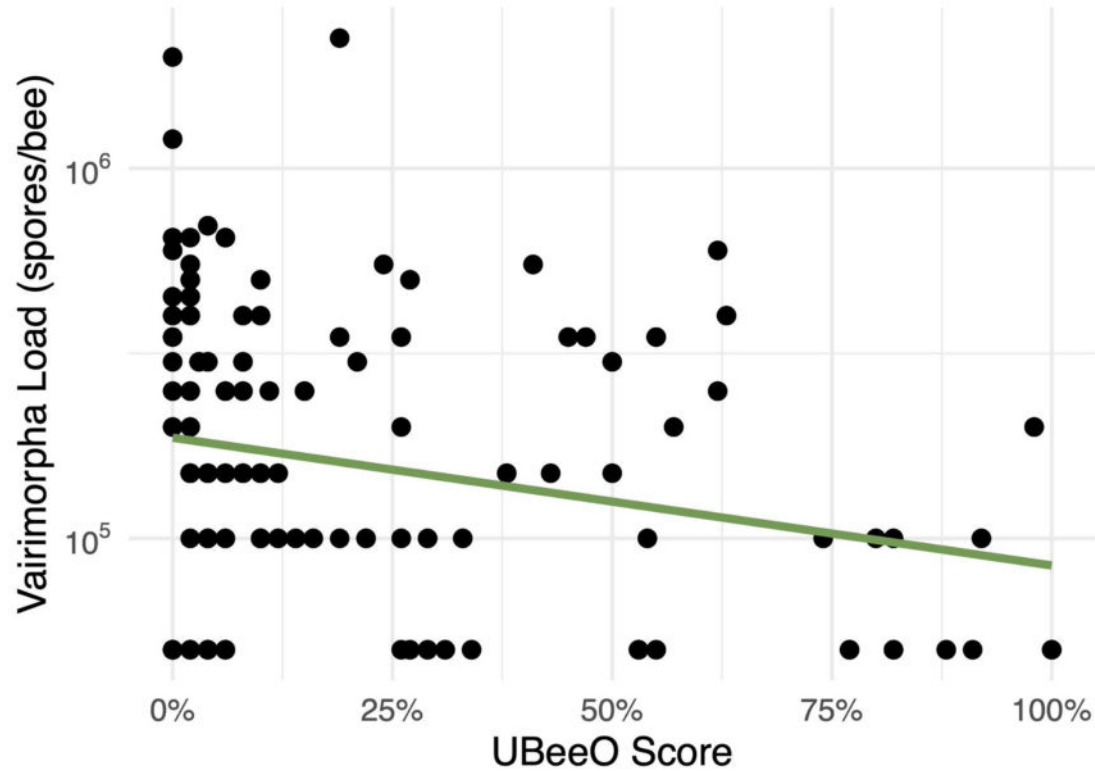
Hygienic colonies have lower Varroa prev. and load

UBO: $p = 0.01$
Time: $p < 0.001$
UBO * Time: $p = 0.26$



UBO: $p = 0.03$
Time: $p < 0.001$
UBO * Time: $p = 0.01$

Higher UBee0 scores predict lower Nosema load



How do high-scoring UBeeO 'hygienic' bees control *Vairimorpha (Nosema) ceranae*?

We evaluated the potential mechanisms of reduced *V. ceranae* loads in high-scoring UBeeO colonies.

Honey bees express two levels of immune function:

- **Innate Immunity:** The bee's internal physiological immune system
- **Social Immunity:** Colony-level defenses that reduce disease spread among individuals

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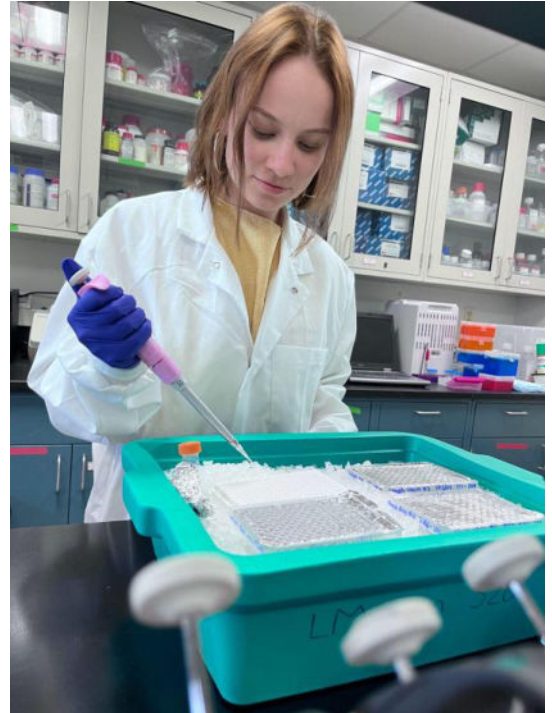
Methods

- 4 High UBeeO colonies ($\geq 80\%$)
- 4 Low UBeeO colonies ($< 10\%$)
- 3 doses of *N. ceranae* inoculant:
 - Control: 0 spores/bee
 - Low dose: 10,000 spores/bee
 - High dose: 50,000 spores/bee



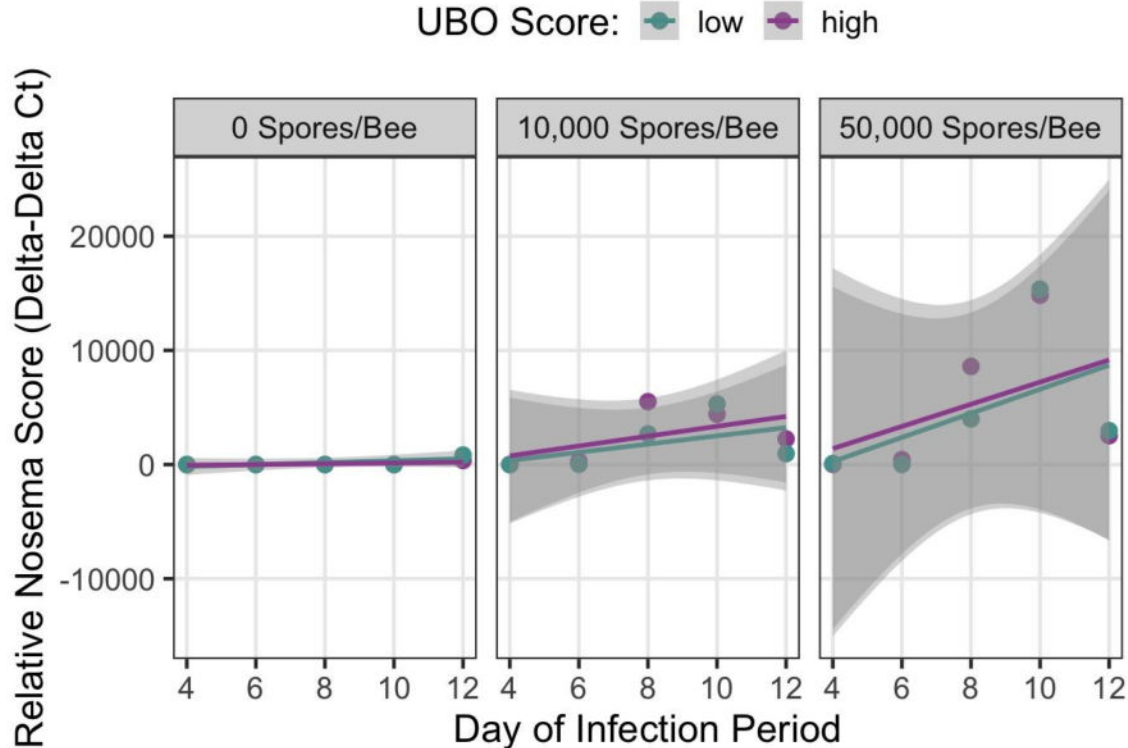
Methods

- Newly emerged adults were reared *in vitro*
- Bees were group-fed *V. ceranae* inoculum for 24hrs (30 bees/cup)
- Four bees were extracted every 2 days, during the 12 day infection period
- Used RT-PCR to assess the level of active *V. ceranae* infection and immune gene expression in individual worker bees (relative quantification)



V. ceranae infection levels increased over time

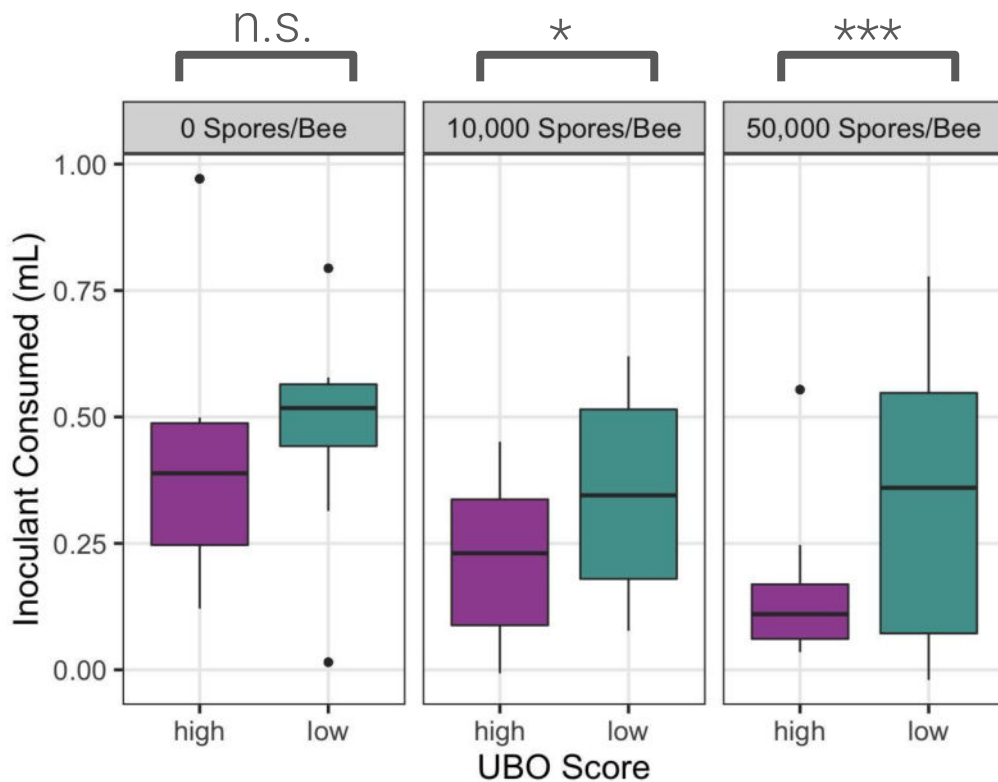
$p = 0.003$



High UBee0 bees consumed less *V. ceranae* inoculum

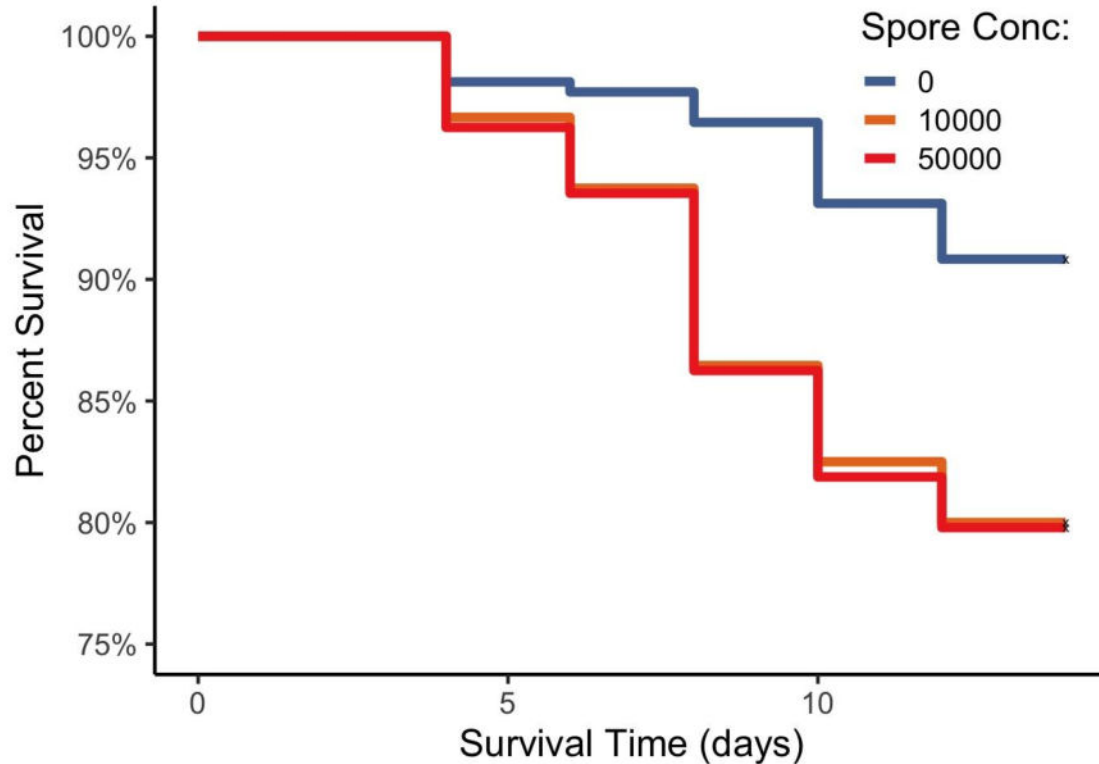
* $p < 0.001$

** $p < 0.0001$



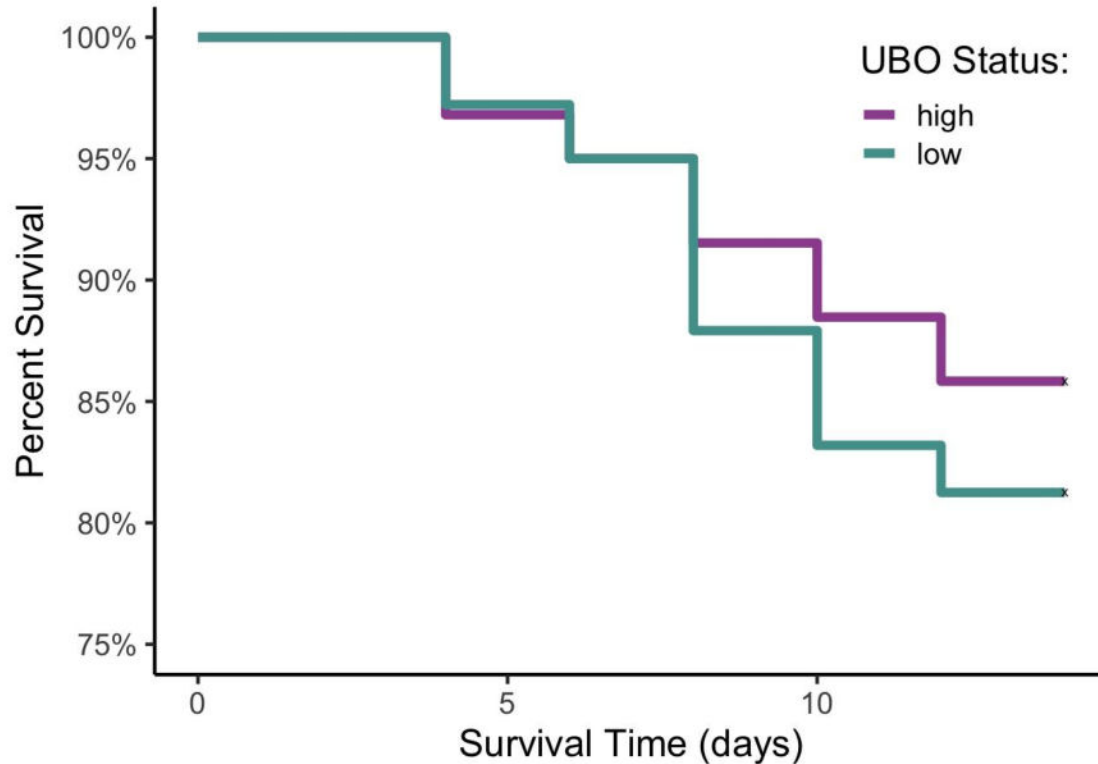
V. ceranae dosage impacted probability of bee survival

$p = 0.0003$

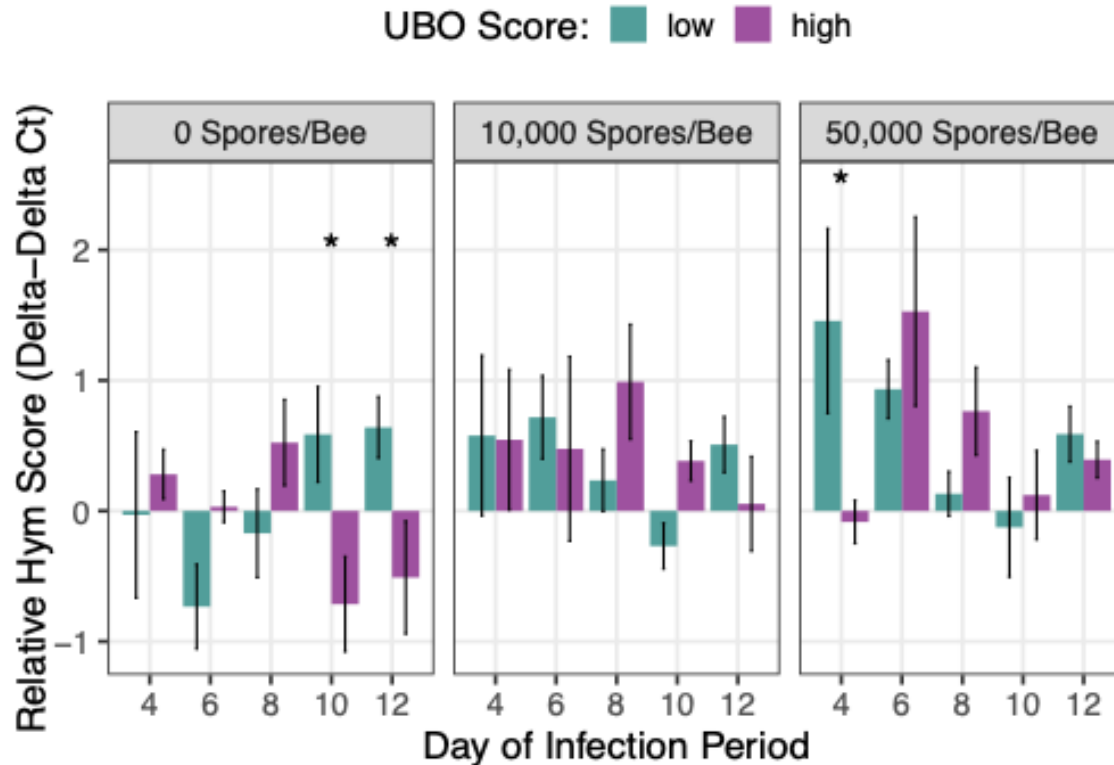


High UBO bees were more likely to survive longer

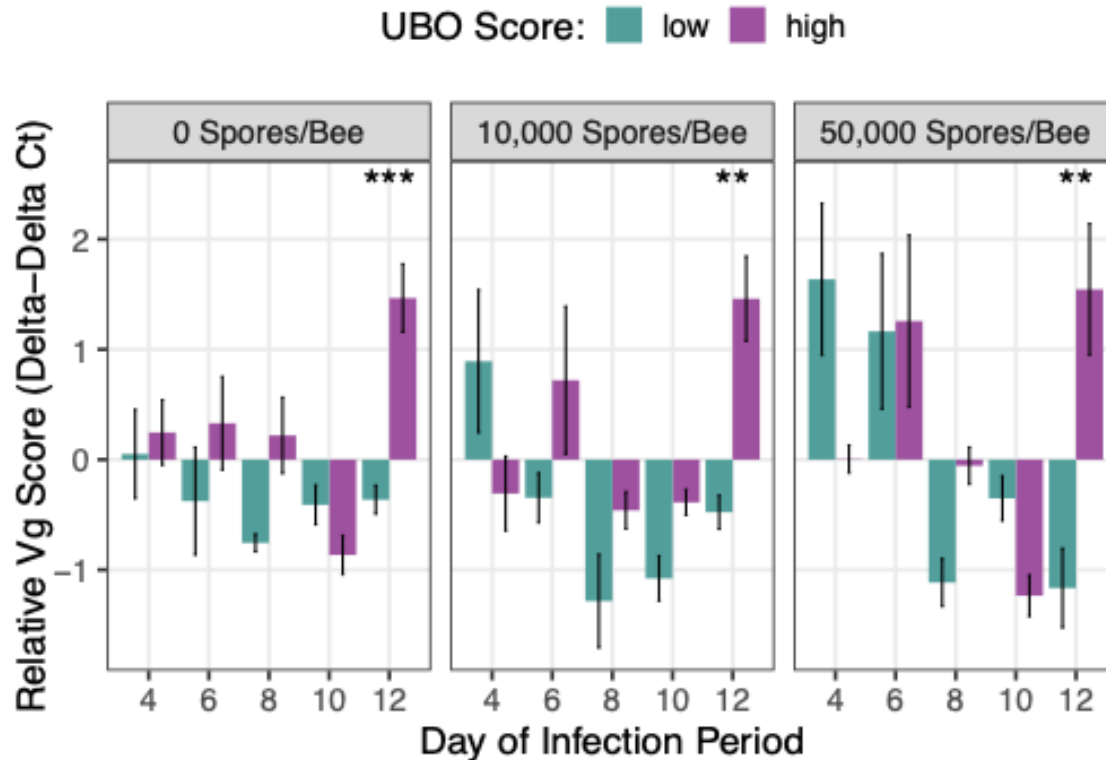
$p = 0.018$



High UBee0 bees differed in their Hymenoptaecin expression



High UBee0 bees showed upregulated Vitellogenin expression at peak *V. ceranae* infection



Multiple co-occurring innate defense mechanisms

Our findings suggest that:

- Bees from hygienic colonies may be better able to **detect and avoid contaminated food sources**.
- **Upregulated vitellogenin** in bees from hygienic colonies may limit the health impact of Nosema infection.
- **Differing hymenoptaecin expression** may suggest less investment in innate immunity under healthy conditions.
- Bees from hygienic colonies may have an **enhanced tolerance to *V. ceranae*** (rather than resistance).

Thank you!

Questions?



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