Notes from the Lab: The Latest Bee Science Distilled

by Scott McArt

Tropical stingless bees can pick up disease from sick honey bees

Stingless bees aren't present in the United States, but they're native to tropical areas such as Mexico and Central America. These social bees come in many shapes and sizes and can be very beautiful (see Photos 1-3).

Some species of stingless bees, including *Melipona beecheeii* (see Photo 4), have been domesticated since at least Mayan times (i.e., ~4,000 years ago) and are managed for their hive products. Their honey is a prized commodity, often selling for 4-5 times as much as honey produced by the western honey bee, *Apis mellifera*. I've tried honey from several stingless bees and can confirm it's delicious, though some varieties are only for the adventurous. All varieties taste quite different from *Apis* honey.

The western honey bee was introduced to North America in 1622 and to Mexico and Central America a bit later, probably the mid to late 1800s (Carpenter & Harpur 2021). So western honey bees and native stingless bees have existed together in the tropics for about 150 years. As mentioned in previous *Notes from the Lab* columns, there's currently lots of interest among beekeepers, conservationists, and land managers regarding the impact that managed honey bees can potentially have on native bees via competition for resources and sharing of diseases. See for example the November 2021 and September 2022 columns, 161(11):1225-1227 and 162(9):1025-1027, respectively.

Nearly all research on disease spillover from honey bees to native bees has focused on temperate bumble bees (*Bombus spp.*) as the disease recipient. But what about other bees from other regions? Do tropical stingless bees share viruses with honey bees? Do these viruses "spill over" from honey bees and cause elevated mortality in stingless bees? If so, what can beekeepers do to minimize impacts on native stingless bees? These are the topics for the sixty-seventh *Notes from the Lab*, where I summarize ***"Trouble***

David Sossa

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Photos 1-2 *Workers guarding and entering a* Tetragonisca angustula *stingless bee hive in South America*

David Sossa

Photo 3 *Workers entering a* Trigona sp. *stingless bee hive in South America*

in the tropics: Pathogen spillover is a threat for native stingless bees," written by Fernando Fleites-Ayil and colleagues and published in the journal ***Biological Conservation*** **[2023].**

For their study, Fleites-Ayil and colleagues collected honey bees (*Apis mellifera*) and stingless bees (*Melipona beecheeii*) from twelve locations throughout the Yucatan Peninsula (see Figure 1). They brought the bees back to the lab and screened 9-10 of each species at each site (234 bees total) for six RNA viruses: acute bee paralysis virus (ABPV), black queen cell virus (BQCV), deformed wing virus (DWV genotypes A and B), sacbrood virus (SBV) and slow bee paralysis virus (SBPV).

To check the genetic identity of BQCV, the most prevalent virus in their bees, the authors sequenced a viral fragment from all bees that were positive for BQCV from seven locations. In total, virus genotypes were sequenced from 11 *Apis* and 13 *Melipona* adults.

Finally, to assess whether the viruses caused *Melipona* to get sick and die, the authors 1) injected pupae with BQCV, DWV-A or DWV-B and tested whether each virus caused an active infection, and 2) fed adults an inoculum of BQCV, DWV-A or DWV-B and monitored whether the bees died more quickly compared to bees fed an uncontaminated control inoculum.

So, what did they find? Did stingless bees share viruses with honey bees? Yes. Across all sites, the virus with highest prevalence in both *Melipona* and *Apis* bees was BQCV (see Figures 1 & 2). Both species of bees also had some individuals who were positive for DWV-A, but only two *Apis* bees from the entire study were positive for DWV-B (see Figure 2 center and right panels).

When looking at quantitative loads, BQCV viral titres were higher in *Apis* bees (range 10^{3}-10^{7} genome equivalents per bee) compared to *Melipona* bees (10^{2}-10^{5} genome equivalents per bee). DWV-A titres in *Apis* bees were variable (10^{2}-10^{8} genome equivalents) and the only *Melipona* bee positive for DWV-A had a titre of 10^{5} genome equivalents. The titres of the two *Apis* bees positive with DWV-B were low (10^{3} genome equivalents).

Were the same genotypes of BQCV present in stingless bees and honey bees? Yes and no, depending on the location. At some locations, the BQCV genotypes found in *Melipona* were identical to the genotypes found in *Apis*. At other sites, distinct genotypes were found in each species.

Can stingless bees be infected with BQCV, DWV-A, and DWV-B and do these viruses have negative impacts on them? Unfortunately, yes, yes, yes, and yes. Each virus was able to quickly replicate and reach 10^{6}-10^{7} genome equivalents in *Melipona* pupae within 3-5 days post-inoculation. When *Melipona* adults were fed inoculum containing BQCV, DWV-A, and DWV-B, they died 14% faster (see Figure 3; survival of virus-inoculated bees = 6 days on average, compared to survival of control-inoculated bees = 7 days on average).

Well that's troubling. What does all of this mean for spillover of disease from honey bees to stingless bees, and what can beekeepers do about it? Through multiple lines of evidence, the study by Fleites-Ayil suggests that spillover of viruses from *Apis* to *Melipona* is occurring and *Melipona* is being harmed. Prevalence and loads of viruses were greater in *Apis* than *Melipona*, which is consistent with a pattern of spillover from *Apis* to *Melipona*. Some BQCV genotypes were identical in both *Apis* and *Melipona*, indicating sharing of virus strains, while other genotypes were unique in *Melipona*, suggesting viral evolution within *Melipona* post-transmission. Finally, *Melipona* became sick and died faster when exposed to BQCV, DWV-A, and DWV-B that were sourced from *Apis*.

Obviously none of this is good news for *Melipona*. But do not despair, there is hope! The main way that honey bees get sick with viruses is from inadequate control of the varroa mite. Deformed wing virus (DWV)

Rob Paxton

Photo 4 *A* Melipona beecheeii *stingless bee colony. Note the vertical brood cells (center) and wax covering (periphery) of the colony.*

David Sossa

Photo 5 *A beekeeper in South America extracts larval food (a mix between pollen and nectar) from a* Tetragonisca angustula *brood comb.*

and acute bee paralysis virus (ABPV) can be directly transmitted by varroa, but the mites also feed on bee fat bodies, which weakens their immune system and makes them susceptible to other viruses and diseases. So, if beekeepers adequately control varroa, the threat of disease spillover to native bees is greatly reduced. In fact, a Ph.D. student in my lab has recently shown that disease spillover is nearly eliminated when beekeepers adequately control varroa (stay tuned for the forthcoming publication on this topic).

If you're a beekeeper and reading this article, when was the last time you checked varroa levels in your colonies? Here at the Dyce Lab, we do monthly checks of 3-6 colonies in each apiary, which is what we recommend to all beekeepers. We recommend applying a treatment if varroa levels in any colony within an apiary are above 2 mites per 100 bees in the spring and summer, or 3 mites per 100 bees in the fall. For a step-by-step varroa monitoring guide, IPM treatment guide for varroa, and templates for creating your own monitoring and treatment plans, please check out our website and pass along the link to anyone who may be interested! **https://cals.cornell.edu/pollinator-network/beekeeping/managing-pests-parasites-disease**

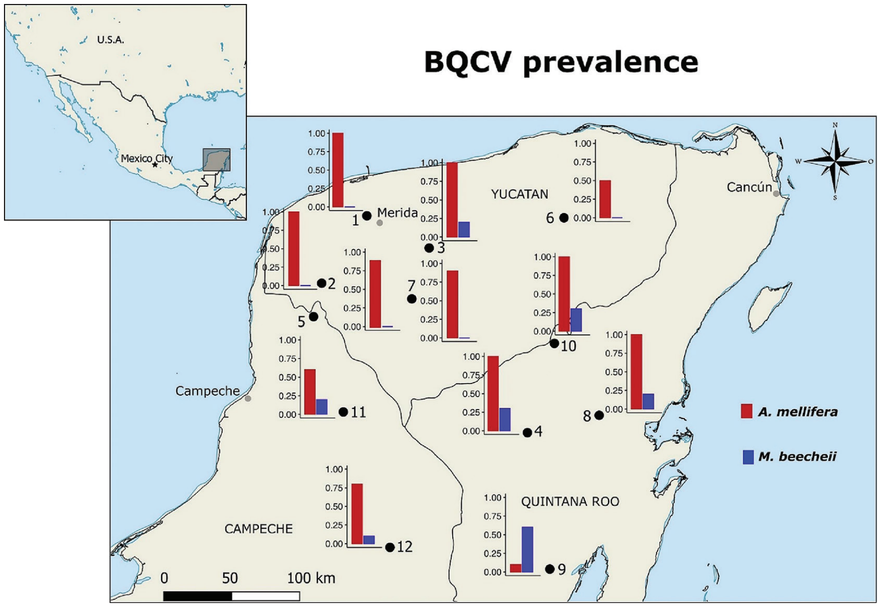

Fig. 1 *Map of the study region in the Yucatan Peninsula (Mexico) indicating black queen cell virus (BQCV) prevalence by location. Location codes: 1. UADY, 2. Maxcanú, 3. Hocabá, 4. Polyuc, 5. Calkiní, 6. Espita, 7. Mama, 8. Felipe Carrillo Puerto, 9. Bacalar, 10. Tihosuco, 11. Hopelchén, and 12. Calakmul.*

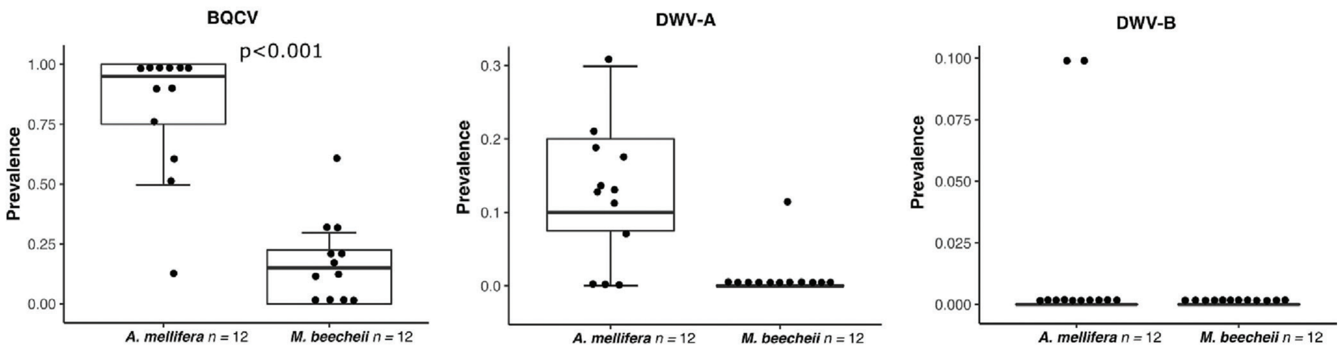

Fig. 2 *Viral prevalence (BQCV, DWV-A and DWV-B) in the stingless bee,* Melipona beecheii, *and western honey bee (*Apis mellifera*) from the Yucatan Peninsula*

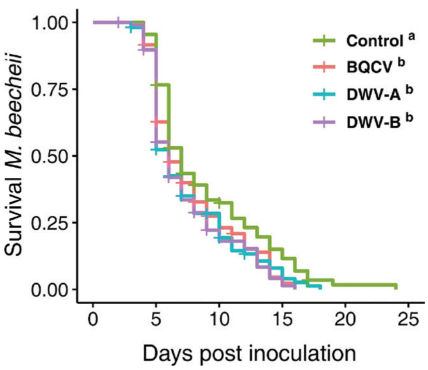

Fig. 3 *Kaplan-Meier curves showing reduced survival of* M. beecheii *adult worker bees exposed by feeding with BQCV, DWV-A and DWV-B; Cox proportional hazard model, $\chi^2 = 43.5$, $p < 0.001$; different lowercase letters following a treatment show significance of differences in survival ($p < 0.05$).*

At the end of the day, everyone — beekeepers, conservationists, and the public — wants all bees to be healthy. That includes native wild bees, domestic honey bees, and other domestic bees such as stingless bees. There are many ways to keep bees healthy, but for all of us who own domestic honey bees, staying on top of varroa is perhaps the most important, both for our own bees and the other bees with which they share diseases.

Until next time, bee well and do good work.

Scott McArt

References:

Carpenter, M. H. and B. A. Harpur. 2021. Genetic past, present, and future of the honey bee (*Apis mellifera*) in the United States of America. *Apidologie* 52:63-79 **https://doi.org/10.1007/s13592-020-00836-4**

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