Trouble in the tropics: Pathogen spillover is a threat for native stingless bees

Fernando A. Fleites-Ayil [a,*], Luis A. Medina-Medina [b], José Javier G. Quezada Euán [b], Eckart Stolle [c], Panagiotis Theodorou [a,d], Simon Tragust [a], Robert J. Paxton [a,d]

[a] *Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany*
[b] *Departamento de Apicultura Tropical, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán, México, Km 15.5 carr. Mérida-Xmatkuil, Merida, Yucatan CP 97100, Mexico*
[c] *Leibniz Institute of Animal Biodiversity, Center of Molecular Biodiversity Research, Zoological Research Museum Alexander Koenig, Bonn, Germany*
[d] *German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Puschstrasse 4, 04103 Leipzig, Germany*

A B S T R A C T

Pathogen spillover is a major threat to biodiversity. Insect pollinators, important providers of the ecosystem service of pollination that are in global decline, are no exception to this threat, with mounting evidence of pathogen spillover from managed into wild bee species in temperate regions. The phenomenon is likely global in scope, though poorly documented, and its consequences for recipient species are largely unknown. To address these knowledge gaps, we investigated viral spillover in the neotropics from the honey bee (*Apis mellifera*), where it is a managed and invasive species, into native stingless bees, a biodiverse taxon of pollinators. We furthermore exposed stingless bees to honey bee viruses to test for their impact on host survival. High viral prevalence in honey bees and low prevalence of identical viral haplotypes in stingless bees supports ongoing spillover from managed to native species. The survival of native stingless bees was reduced when inoculated with virus by feeding, a plausible route of natural infection. We conclude that viral spillover from managed to wild insect pollinators is likely a global phenomenon and poses a serious threat worldwide to native insect species. If negative impacts are detected in the field, conservation management needs to be developed to reduce spillover, including better control of pathogens in managed species and legislation on their movement.

1. Introduction

Pathogen spillover is a major threat to wild and domestic animals and human well-being (Daszak, 2000). A case in point is the recent Covid-19 pandemic in which the SARS-CoV-2 virus jumped from a wild host into the human population in late 2019 (Li et al., 2021). Insect pollinators are not exempt from the threat of pathogen spillover. Of the major causes of pollinator decline, including global change phenomena such as climate change, intensification in land use, reduced habitat and resource availability and pesticide misuse, a widely acknowledged though poorly investigated factor is pathogen spillover (Dicks et al., 2021; Potts et al., 2016). Given the importance of insect pollinators for the ecosystem service of pollination and crop production (Klein et al., 2007) yet their ongoing decline (Potts et al., 2016), including the worldwide decline in wild bee species (Zattara and Aizen, 2021), there is a pressing need to understand the extent to which pathogen spillover

contributes to insect pollinator decline.

Managed species may be reservoir hosts and a source of pathogens that jump between species e.g. the rinderpest virus in cattle that infected wild African ruminants in the late 19th and 20th Centuries (Kock et al., 1999). Among temperate region insect pollinators, pathogen spillover from managed to wild bumble bees (*Bombus* spp.) has been well documented; protozoan pathogens spill over from managed to native *Bombus impatiens* Cresson in North America (Colla et al., 2006), from managed to native *Bombus terrestris* L. in Europe (Murray et al., 2013) and from the managed, exotic *B. terrestris* to native *Bombus* spp. in South America (Schmid-Hempel et al., 2014).

The western honey bee (*Apis mellifera* L.) is also thought to be a reservoir host and major source of pathogens, particularly RNA viruses, to which wild non-*Apis* bee species are exposed (Tehel et al., 2016). *Apis mellifera* dominates as a managed flower-visitor in diverse agricultural and semi-natural terrestrial biomes in temperate and tropical regions

* Corresponding author.
  E-mail address: fernando.fleites-ayil@student.uni-halle.de (F.A. Fleites-Ayil).

(Hung et al., 2018). At the same time, its populations throughout the world harbour many RNA viruses (Beaurepaire et al., 2020), of which deformed wing virus (DWV) is considered an emerging infectious disease (Martin et al., 2012; Wilfert et al., 2016). In temperate regions, RNA viruses of *A. mellifera*, including DWV, have been found in wild bumble bee species (*Bombus* spp.) in both North America (Alger et al., 2019), South America (e.g. Bravi et al., 2019; Gamboa et al., 2015; Reynaldi et al., 2013) and Europe (Fürst et al., 2014; McMahon et al., 2015), and in wild solitary bee species of Eurasia (Radzevičiūtė et al., 2017). Support for spillover comes from the higher viral prevalence in honey bees than in wild bees at the same field site, where both host taxa share the same viral variant (Fürst et al., 2014; McMahon et al., 2015; Radzevičiūtė et al., 2017). Given that *A. mellifera* is the world's most numerous managed insect pollinator (Osterman et al., 2021), pathogen spillover from it to wild bee species is likely a global phenomenon, including in the tropics, where spillover has received scant attention.

The tropics contain a rich diversity of eusocial stingless bee species (Hymenoptera: Apidae: Meliponini) that are important native pollinators with >390 spp. in the neotropics alone (Michener, 2007; Quezada-Euán, 2018). Their populations are considered to be threatened by a variety of factors impacting bee species in temperate regions, including pathogens (Quezada-Euán et al., 2022; Toledo-Hernández et al., 2022). Honey bee-associated viruses, including DWV, have been detected in stingless bees of Latin America e.g. Argentina (Alvarez et al., 2018), Brazil (de Souza et al., 2019; Guimarães-Cestaro et al., 2020; Ueira-Vieira et al., 2015) and Mexico (Guzmán-Novoa et al., 2016; Morfin et al., 2021), supporting the idea that also in the tropics virus spills over from *A. mellifera* to stingless bees. Following the emergence of DWV in (tropical) Hawaii's European managed honey bees in 2007 after they became infested with the ectoparasitic *Varroa destructor* mite (Martin et al., 2012), DWV was detected in native Hawaiian insects such as ants and wasps associated with honey bee apiaries (Brettell et al., 2020). As European honey bees are particularly susceptible to *V. destructor*, which is a vector for numerous viruses such as DWV (Yañez et al., 2020), it is unclear whether viral spillover observed in Hawaii occurs in other tropical regions where *A. mellifera* is native (Africa) or an exotic invasive (the neotropics, where it is termed Africanized) and is not so susceptible to *V. destructor* (Traynor et al., 2020). Pathogen spillover from *A. mellifera* may therefore be not only a temperate region problem but also potentially one of global extent.

Invasive species may be a source of particularly pernicious pathogens that mediate displacement of native species ahead of competition for resources or habitat, particularly when the invasive is an exotic species, as are Africanized honey bees in the neotropics, because native species are naïve to the invasive's pathogens. Examples include the decline of the South American native bumble bee *Bombus dahlbomii* Guérin-Méneville, attributed to the introduction and spread through Chile and Argentina of the Eurasian bumble bee *B. terrestris* and its protozoan pathogens (Schmid-Hempel et al., 2014). Whilst there is growing evidence for pathogen spillover from honey bees to other insects, the subsequent effects of viral spillover on native, non-*Apis* insect pollinators are less well characterised. Earlier studies in Europe using native honey bees and *Bombus* spp. demonstrated that inoculation with DWV leads to reduced survival of *B. terrestris* (Fürst et al., 2014; Graystock et al., 2016), though subsequent experiments in the laboratory and the field have suggested limited impact of honey bee viruses on commercially sourced *B. terrestris* (Tehel et al., 2022; Streicher et al., 2022). We lack studies on the impact of viral spillover on populations of wild non-*Apis* bee species from tropical regions, where honey bees and wild bees also share floral resources and spillover is likely to be prevalent and where, for the neotropics, *A. mellifera* is an exotic invasive species.

Here, in the first test of the extent and impact of viral spillover from *A. mellifera* to non-*Apis* insect pollinators in the tropics, we evaluated the prevalence of so-called honey bee RNA viruses in *A. mellifera* and a native stingless bee, *Melipona beecheii* Bennett, using a structured survey across the neotropical Yucatan Peninsula of Mexico. In laboratory

experiments we then tested for potential negative impacts of these RNA viruses on this emblematic stingless bee species. Our results have implications for the global reach of pathogen spillover from honey bees among communities of insect pollinators.

2. Material and methods

2.1. Field locations and sampling

Sampling of bees was carried out from January to April 2019 during the flowering season at 12 locations (SM Table S1) in the Yucatan Peninsula, Mexico (Fig. 1). We chose locations with meliponaries, traditional places where *M. beecheii* and other stingless bee colonies are managed for bee conservation and the production of bee products. Honey bees are widely distributed and are essentially found everywhere at high density (Moritz et al., 2013), both as managed colonies in beehives as well as feral colonies in natural cavities. Sampling locations were >15 km apart from one another to ensure independence, given the typical foraging ranges of 2–5 km for honey bees and, based on its similar body size, *M. beecheii* (Greenleaf et al., 2007). Full details of sampling are in the Supplementary Methods (and see SM Fig. S1).

2.2. RNA extraction, virus detection, and absolute quantification

We screened bees for acute bee paralysis virus (ABPV), black queen cell virus (BQCV), DWV (genotypes A and B), sacbrood virus (SBV) and slow bee paralysis virus (SBPV). These RNA viruses have a worldwide distribution in *A. mellifera* (Beaurepaire et al., 2020), suggesting the western honey bee is their reservoir host. Many of them have been detected in Africanized *A. mellifera* from South America (Tibatá et al., 2021).

RNA extraction, viral detection and absolute viral quantification by qPCR were performed on individual bees using standard methods developed for honey bees (De Miranda et al., 2013) and following Tehel et al. (2019). We randomly selected 10 *M. beecheii* bees (total $n = 120$) and ca. 10 honey bees (total $n = 114$) per location for viral screening. As quality controls, we included technical duplicate qPCRs to confirm viral presence, inclusion of positive and negative controls on every 96-well qPCR plate, amplification of a bee species-specific reference gene to confirm successful RNA extraction and cDNA synthesis, a melt curve analysis at the end of each qPCR to ensure the correct product had been amplified, and a dilution series of a qPCR product for absolute quantification of viral titre. Full details are provided in the SM.

2.3. Sequencing of BQCV to test for host specificity

To check the genetic identity of BQCV, the most prevalent virus (see results), we cloned and Sanger sequenced a viral fragment (294 bp) from bees that were qPCR positive for this virus from seven locations (for methods, see SM), comprising in total 48 unique BQCV amplicons derived from 11 honey bees and 13 *M. beecheii* adults. All amplicons corresponded to the BQCV reference sequence (NC_003784) with >98 % identity. To determine if the same viral variant was shared by host species and across the seven locations, we constructed Median-Joining haplotype networks in PopART v1.7 (Leigh and Bryant, 2015). Due to the low number of *M. beecheii* infected with DWV-A ($N = 2$ bees) and the absence of DWV-B in *M. beecheii*, we did not perform the same analysis for these viruses.

2.4. Viral impact on M. beecheii

To test the virulence of the three prevalent viruses in Yucatecan honey bees (BQCV, DWV-A and DWV-B; see results) in *M. beecheii*, we performed a viral exposure experiment using the pure viral inocula of Tehel et al. (2019) that were derived from honey bees (see SM).
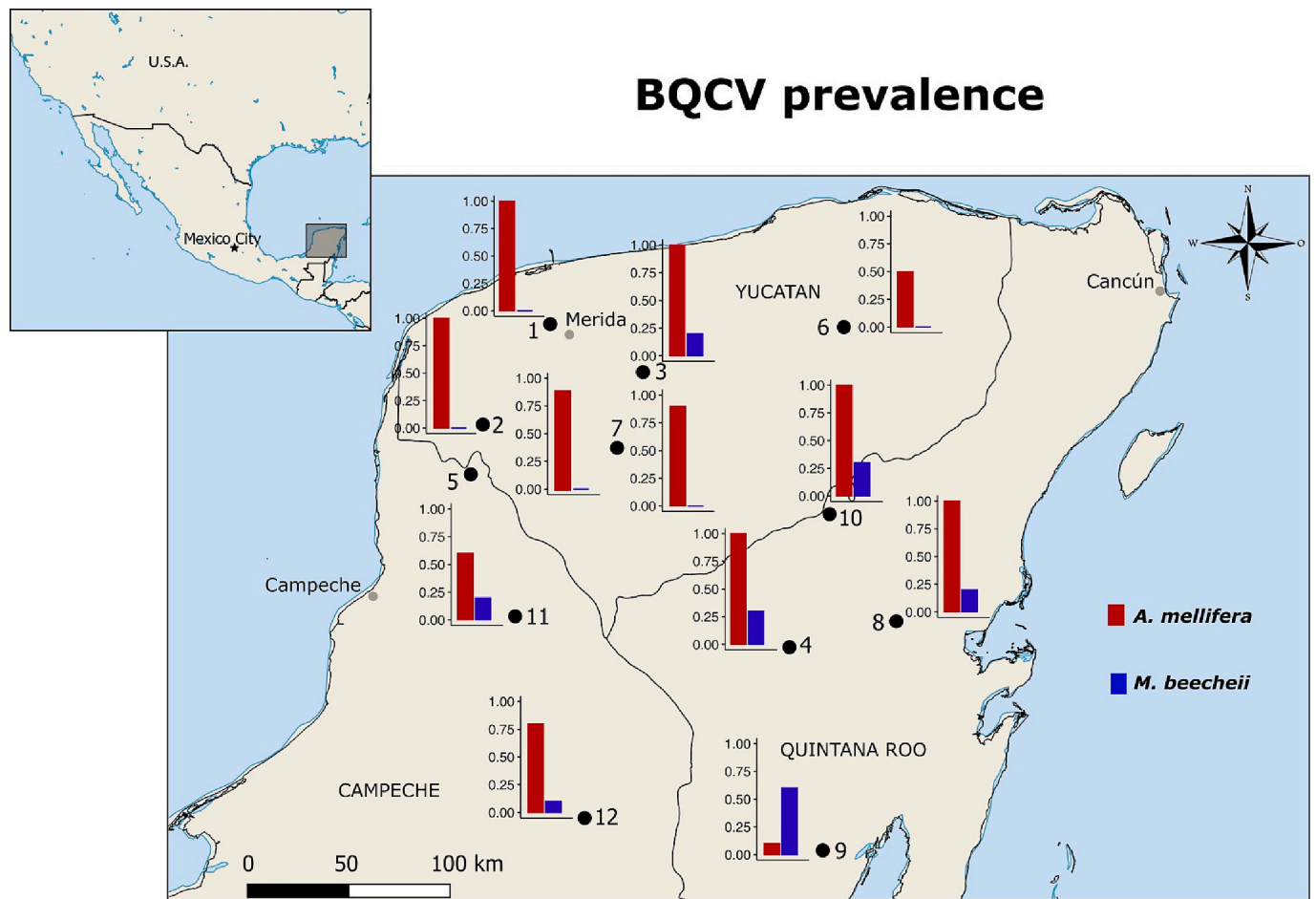
Fig. 1. BQCV prevalence by location in the Yucatan Peninsula. Location codes: 1. UADY, 2. Maxcanú, 3. Hocabá, 4. Polyuc, 5. Calkiní, 6. Espita, 7. Mama, 8. Felipe Carrillo Puerto, 9. Bacalar, 10. Tihosuco, 11. Hopelchén and 12. Calakmul.

2.4.1. Testing the competence of M. beecheii to support viral replication

To evaluate whether *M. beecheii* is a competent host for BQCV, DWV-A and DWV-B derived from honey bees, we removed white-eyed pupae from five colonies of *M. beecheii* housed in a traditional meliponary at the Autonomous University of Yucatán and transferred them to a CT chamber (32 °C, 70 % RH). We then injected 1 µl of 10^5 viral GEs into the abdomens of white-eyed *M. beecheii* pupae (15 pupae per virus) and included a control group ($n = 15$ pupae) injected with 1 µl of 0.5 M cold PPB (pH 8.0). After five days, pupae from all three treatments and control were collected individually in vials and stored at −80 °C for subsequent measurement of viral titre. Injections were performed as described for honey bee pupae (see SM).

2.4.2. Experimental viral inoculation by feeding M. beecheii adult worker bees

To determine the impact of viral inocula on *M. beecheii* worker bees, simulating viral spillover in the field at flowers, brood combs from five *M. beecheii* colonies were stored under the same environmental conditions as white-eyed pupae in the laboratory (32 °C, 70 % RH) until worker emergence. Newly emerged *M. beecheii* worker bees (within 24 h of emergence) were starved individually in 1.5 ml vials for 1 h. Each bee was then individually fed with 10 µl of a viral inoculum consisting of 1 µl of 10^8 viral genomes equivalents (GEs) of either DWV-A or DWV-B, or 10^6 viral GEs of BQCV mixed with 9 µl of sucrose solution (50 % *w/v*). Viral doses were chosen to reflect viral loads detected in bees in the field in this study (BQCV: 10^3 - 10^7 GEs; DWV-A: 10^2 - 10^8 GEs; see Results below) and by Alger et al. (2019) in bumble bees in the USA (BQCV: 10^6–10^8 GEs; DWV: 10^4–10^9 GEs) whilst ensuring that the dose was

sufficient for 100 % of bees to become infected (for a honey bee inoculated with BQCV, DWV-A and DWV-B: 10^7 GEs; for a bumble bee inoculated with BQCV, DWV-A and DWV-B: 10^9 GEs; see Tehel et al., 2020). Bees that did not consume the entire 10 µl inoculum were rejected. After individual feeding, bees were observed to ensure they did not regurgitate the inoculum and then transferred in groups of 10 bees per treatment to plastic cages (15 cages per treatment) with a removable base, multiple ventilation holes, and ad libitum access to two vials containing a 50 % w/v sucrose solution (Evans et al., 2009) to record mortality. Control bees were fed with 10 µl of sucrose solution (50 % w/v). Though inoculations were undertaken across 7 days, an equal number of bees was inoculated for all four treatments on any one day and by the same person. The survival of adult *M. beecheii* was recorded for 24 days, by which time all bees had died.

To identify changes in viral load of inoculated bees over time, we removed one bee per cage at three time points (two, four, and six days) post-inoculation (d.p.i) to quantify viral titre in control and all viral treatment groups. Before processing, bees were stored individually at −80 °C to avoid RNA degradation. Eight bees per treatment (including the control) and time point were then processed for RNA extraction, cDNA synthesis, virus screening and absolute viral quantification by qPCR, as described above ($n = 24$ bees per virus and $n = 72$ control bees). None of the bees from our control treatment showed a viral signal by qPCR strongly suggesting that all freshly emerged *M. beecheii* were devoid of virus.

Ethical approvals were not required for experiments on insects in Germany or Mexico. The insects used in Germany (honey bees) and in Mexico (honey bees, *Melipona beecheii*) are not under conservation

protection because they are managed.

2.5. Statistical analyses

All analyses were performed in R v. 4.1.3 (R Core Team). Model assumptions were checked using the R package "DHARMa" (Hartig, 2020) and were found to conform to expectations (residuals were normally distributed and homogeneity of variances was observed).

To test whether viral prevalence varied across host species, we used generalized linear mixed models (GLMMs) with binomial error structure. Species identity was used as a fixed factor and sampling location was included as a random factor. To investigate whether pathogen prevalence in *M. beecheii* was related to pathogen prevalence in honey bees at the same locality, we also used GLMMs with binomial error structure in which honey bee pathogen prevalence was used as a fixed effect and sampling location was included as a random factor. The analyses were implemented using the function *glmer* within the R package *lme4* (Bates et al., 2015). Differences in viral titre between honey bees and *M. beecheii* were also investigated using GLMMs with a Poisson error structure using *lme4*. GLMMs were performed for each virus separately.

Survival analysis of adult *M. beecheii* after oral virus exposure was performed with a Cox proportional hazards model using the R package *coxme* (Therneau et al., 2003). Experimental treatment (control, BQCV, DWV-A or DWV-B) was used as a fixed factor and cage as a random factor. To test for differences between experimental treatments, Tukey post-hoc tests were implemented with the R package *multcomp* (Hothorn et al., 2008), adjusting the family-wise error rate. For each virus, differences in viral titre across time of experimentally inoculated bees were investigated with a GLMM with a Poisson error structure and 'cage' as a random factor.

3. Results

3.1. RNA viruses in honey bees and stingless bees in the Yucatan Peninsula

Three of the six screened RNA viral targets were detected in honey bees: BQCV, DWV-A and DWV-B. In *A. mellifera* the most prevalent was BQCV at 80 % (91 of 114 bees), and was detected at all 12 locations (Fig. 1), followed by DWV-A at 13 % (14 of 114 bees) detected at nine locations (SM Fig. S2), then DWV-B at 2 % (2 of 114 bees) at two locations (SM Fig. S3).

In *M. beecheii*, we detected BQCV (prevalence 15 %, 19 of 120 bees) at 7 locations (Fig. 1) thought DWV-A at only one location (prevalence 1 %, 1 of 120 bees; SM Fig, S2). These are far lower average prevalence than in honey bees (BQCV: GLMM, z = −3.08; Tukey's HSD, $p = 0.001$; DWV-A: $\chi_1^2 = 12.76$ p $= 0.001$; Fig. 2,). We note one exceptional location (location 9, Bacalar) at which the prevalence of BQCV in *M. beecheii* was higher (60 %) than in *A. mellifera* (10 %, Fig. 1). DWV-B was not detected in *M. beecheii* (SM Figs. S2 & S3).
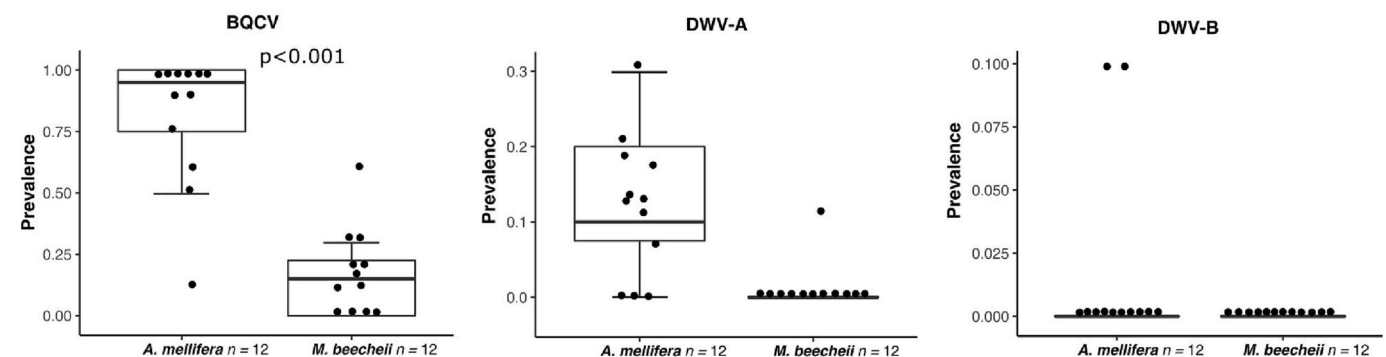
Across locations, the prevalence of BQCV in *M. beecheii* was unrelated to that in honey bees (GLMM, $\chi_1^2 = 2.55$ $p = 0.11$; SM Fig. S4).

BQCV viral titres were significantly higher in qPCR-positive honey bees ($n = 91$; median 10^5, range 10^3–10^7 GEs per bee) compared to qPCR-positive *M. beecheii* ($n = 19$; median 10^4, range 10^2–10^5 GEs per bee) (GLMM, $\chi_1^2 = 349$ p $= 0.001$; SM Fig. S5). DWV-A viral titres in qPCR-positive honey bees were quite variable (10^2–10^8 GEs, $n = 14$). The only *M. beecheii* sample qPCR-positive for DWV-A had a titre of 10^5 GEs. The viral titres of the two honey bees infected with DWV-B were low, and at the threshold of detection ($\sim 10^3$ GEs).

3.2. BQCV sequences analysis

Haplotype network analysis of BQCV revealed two main clusters (Fig. 3), one shared by *A. mellifera* and *M. beecheii* at the same locality (SM Fig. S6) and one restricted to *M. beecheii*. The latter haplotype cluster included *M. beecheii* isolates from locality Bacalar (locality 9), at which the prevalence of BQCV in *M. beecheii* was also high (Fig. 1). Coded by geographic origin, the haplotype network revealed that BQCV variants from both clusters were widely distributed across two or all three states of the Yucatan Peninsula (SM Fig. S7).

3.3. Experimental inoculation of RNA viruses in M. beecheii pupae

Injection of 10^5 GEs of BQCV, DWV-A or DWV-B into *M. beecheii* pupae revealed that this stingless bee species is a competent host for all three viral targets. Three to five days after viral injection, *M. beecheii* pupae contained 6×10^7 GEs BQCV, 10^7, DWV-A, and 6×10^6 DWV-B (SM Fig. S8). Control *M. beecheii* pupae that were injected with buffer were devoid of BQCV, DWV-A, DWV-B or other viruses (ABPV, SBV, and SBPV), demonstrating that pupae were not infected at the start of this infection experiment.
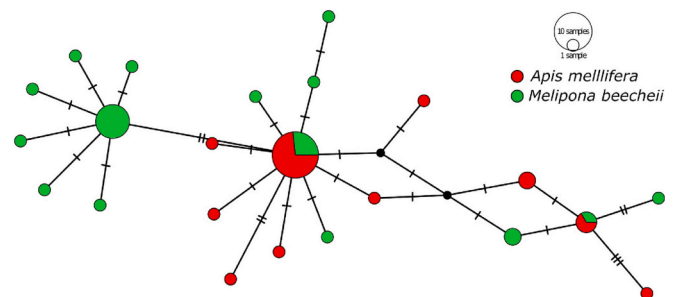

Fig. 3. Median-Joining haplotype network of BQCV sequences from *A. mellifera* ($n = 11$ bees) and *M. beecheii* ($n = 13$ bees) at seven locations. The size of the circle representing a haplotype is proportional to the haplotype's frequency. Hatch marks indicate mutational steps and black dots represent inferred haplotypes.

Fig. 2. Viral prevalence (BQCV, DWV-A and DWV-B) in *M. beecheii* and honey bees from the Yucatan Peninsula.

3.4. Experimental inoculation of RNA viruses in M. beecheii adults

The survival of *M. beecheii* adults was significantly reduced when experimentally fed with BQCV, DWV-A or DWV-B compared to control bees (*coxme*, $\chi^2 = 43.47$, df = 3 $p < 0.001$; Control vs. BQCV: Hazard Ration (HR) 2.13, Control vs. DWV-A: HR 2.28, Control vs. DWV-B: HR 2.40; Fig. 4 and SM Table S4). The median survival of control bees was 7 days (95 % CI: 6–8) versus 6 days for all viral treatments (BQCV, 6 ± 6–8; DWV-A, 6 ± 5–7; DWV-B, 6 ± 5–7), maximum longevity was 23 days for control bees and 16–18 days for virus-inoculated bees (Fig. 4). *Melipona beecheii* inoculated with virus by feeding had detectable titres of the inoculum's respective virus at two, four and six days post inoculation, which indicates a successful viral inoculation. Though viral titres decreased slightly over time (SM Fig. S9).

4. Discussion

Our study demonstrates that honey bee-associated RNA viruses are found in neotropical stingless bees, likely due to spillover from honey bees, and that these viruses may be harming their populations. BQCV and DWV have a worldwide distribution in honey bees and have been reported in honey bees and stingless bees from elsewhere in neotropical Latin America (Ueira-Vieira et al., 2015; Guzmán-Novoa et al., 2016), suggesting that spillover of virus from managed or feral honey bees may be as common a phenomenon in the tropics as in temperate regions, potentially contributing to the decline of tropical non-*Apis* bees.

Our data support the notion that *M. beecheii* often acquires virus through spillover from honey bees because the prevalence and titre of BQCV and DWV-A in *M. beecheii* were generally lower than those in honey bees at the same location. This pattern in viral prevalence and titre is also seen in honey bees and *Melipona colimana* from the west of Mexico (Morfin et al., 2021). Though the higher BQCV prevalence in *A. mellifera* at 11 of 12 Yucatecan sites suggests that virus spillover is predominantly from honey bees to stingless bees, we did not find a statistically significant relationship between BQCV prevalence in honey bees and *M. beecheii* across the Yucatan Peninsula, contrary to the pattern seen in temperate regions between wild bees (bumble bees) and honey bees (Fürst et al., 2014; McMahon et al., 2015). Indeed, at one Yucatecan location (number 9, Bacalar), BQCV prevalence was higher in *M. beecheii* compared to honey bees. These data suggest more complex

pattern of viral sharing, with spillover from honey bees to *M. beecheii* and onward transmission within *M. beecheii*.

Analysis of viral haplotypes support this more complex pattern of viral spillover and onward transmission of BQCV within *M. beecheii*. On the one hand, we found that one widespread BQCV haplotype was shared among *A. mellifera* and *M. beecheii*, supporting a dominant role for spillover from honey bees in driving the epidemiology of virus in *M. beecheii*. Similar patterns of viral haplotype sharing between honey bees and wild bee species have been recorded for DWV-A, DWV-B and BQCV in Europe and Asia (Fürst et al., 2014; Radzevičiūtė et al., 2017; Manley et al., 2019), supporting ongoing sharing of virus by different species at the same site. On the other hand, we also found a widespread BQCV haplotype that was apparently restricted to *M. beecheii*, suggesting local adaptation of a BQCV variant in *M. beecheii* and its onward intra-specific transmission.

Despite considerable support for spillover of virus from honey bees to wild bee species (Fürst et al., 2014; McMahon et al., 2015; Radzevičiūtė et al., 2017; Alger et al., 2019), it is still an open question as to whether RNA viruses associated with honey bees are detrimental to wild bees populations. We now address this knowledge gap for an emblematic and declining neotropical stingless bee species (Quezada-Euán, 2018). Firstly, we confirm that *M. beecheii* workers (pupae) are competent hosts for BQCV, DWV-A, and DWV-B. These pathogens may well be generalist insect viruses as they have also been shown to replicate readily in bumble bees (Fürst et al., 2014; Gusachenko et al., 2020; Tehel et al., 2020). Secondly, and more importantly, we observed a negative impact of virus on the survival of adult *M. beecheii* worker bees after oral exposure, mimicking a realistic mode of viral transmission in the field at flowers (Burnham et al., 2021). Our data suggest that viral spillover from honey bees could have a negative impact on *M. beecheii* populations.

Interspecific interactions at flower patches, whereby honey bees and wild bees sequentially or simultaneously visit the same flower to collect resources such as pollen and nectar, is considered a plausible scenario for viral sharing among bee species (McArt et al., 2014; Graystock et al., 2015; Alger et al., 2019; Burnham et al., 2021; Dalmon et al., 2021). *Melipona beecheii* and honey bees are very likely to visit the same flowers in the Yucatan Peninsula because of the high density of managed honey bees for honey production (Echazarreta et al., 1997) as well as the high density of feral Africanized honey bee colonies (Quezada-Euán, 2007), which would support floral transmission of virus.

On the other hand, as colonies of *M. beecheii* are often traditionally managed, we do not discard other potential inter- or intraspecific routes of transmission through human management. The use of honey bee products in meliponiculture (Teixeira et al., 2020) and the indiscriminate transport of stingless bee species for commercial purposes (Quezada-Euán et al., 2022; Carvalho, 2022) could lead to viral spread within and among stingless bees and other wild bee species. Future research should focus on exploring these potential intraspecific and interspecific routes of viral transmission whilst conservation policy should be aimed at improving hygiene in stingless bee management and legislation on colony movement. Improved disease control of honey bee colonies is also likely to lower viral titres in managed *A. mellifera* and reduce spillover, though we note that tropical *A. mellifera* largely survives as unmanaged wild (Africa) or feral (neotropics) populations (Quezada-Euán, 2007) that are not accessible to disease control measures.

The results of our research add support to a growing number of studies demonstrating viral sharing and pathogen spillover among honey bees and wild bees. Our evidence of a negative impact of honey bee-associated RNA viruses on *M. beecheii* highlights the potential risk of these pathogens for stingless bees, of which 15 species have been considered for management worldwide (Osterman et al., 2021), and other wild bee populations of the neotropics and elsewhere. Our results also underscore the importance of further research using experimentally controlled and field-realistic conditions to reveal the impact of spillover pathogens on non-*Apis* bee species. Generating this information will
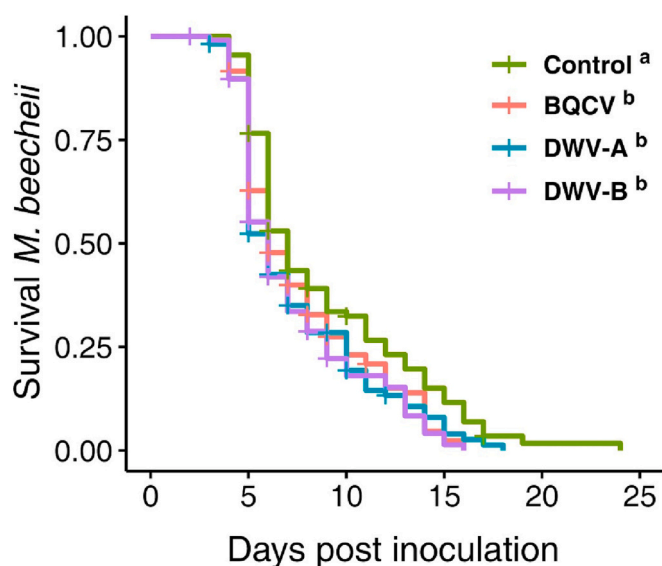

Fig. 4. Kaplan-Meier curves showing reduced survival of *M. beecheii* adult worker bees exposed by feeding with BQCV, DWV-A and DWV-B; Cox proportional hazard model, $\chi^2 = 43.47$, df = 3 $p < 0.001$; different lower case letters following a treatment show significance of differences in survival ($p < 0.05$).

contribute to the conservation and sustainable management of stingless bees, valuable components of terrestrial biodiversity that contribute to the health of tropical ecosystems as well as the economic and cultural development of societies that depend on this important pollinator group.

CRediT authorship contribution statement

F.A.F-A. conceived the study with L.A. M-M, J.J.G. Q-E and R.J.P.; F. A.F-A., S.T., E.S. and R.J.P. designed the experiments; F.A.F-A. wrote the original draft ms. S.T., P.T. and F.A.F-A. undertook data analysis and visualisation. All the authors reviewed and contributed to the final version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

All raw data will be made available via Mendeley data repository: DOI: 10.17632/p3j85ytnk3.1.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2023.110150.

References

Alger, S.A., Burnham, P.A., Brody, A.K., 2019. Flowers as viral hot spots: honey bees (*Apis mellifera*) unevenly deposit viruses across plant species. PLoS One 14 (9), 1–16. https://doi.org/10.1371/journal.pone.0221800.

Alvarez, L.J., Reynaldi, F.J., Ramello, P.J., Garcia, M.L.G., Sguazza, G.H., Abrahamovich, A.H., Lucia, M., 2018. Detection of honey bee viruses in Argentinian stingless bees (Hymenoptera: Apidae). Insect. Soc. 65 (1), 191–197. https://doi.org/10.1007/s00040-017-0587-2.

Bates, D., Mächler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/JSS.V067.I01.

Beaurepaire, A., Piot, N., Doublet, V., Antunez, K., Campbell, E., Chantawannakul, P., Chejanovsky, N., Gajda, A., Heerman, M., Panziera, D., Smagghe, G., Yañez, O., De Miranda, J.R., Dalmon, A., 2020. Diversity and global distribution of viruses of the western honey bee, *Apis mellifera*. Insects. 11 (4), 1–25. https://doi.org/10.3390/insects11040239.

Bravi, M.E., Alvarez, L.J., Lucia, M., Pecoraro, M.R.I., García, M.L.G., Reynaldi, F.J., 2019. Wild bumble bees (Hymenoptera: Apidae: Bombini) as a potential reservoir for bee pathogens in northeastern Argentina. J. Apic. Res. 58 (5), 710–713. https://doi.org/10.1080/00218839.2019.1655183.

Brettell, L.E., Schroeder, D.C., Martin, S.J., 2020. RNAseq of deformed wing virus and other honey bee-associated viruses in eight insect taxa with or without varroa infestation. Viruses. 12 (11), 1229. https://doi.org/10.3390/v12111229.

Burnham, P.A., Alger, S.A., Case, B., Boncristiani, H., Hébert-Dufresne, L., Brody, A.K., 2021. Flowers as dirty doorknobs: deformed wing virus transmitted between *Apis mellifera* and *Bombus impatiens* through shared flowers. J. Appl. Ecol. 58 (10), 2065–2074. https://doi.org/10.1111/1365-2664.13962.

Carvalho, A.F., 2022. Illegalities in the online trade of stingless bees in Brazil. Insect Conserv. Divers. 15 (6), 673–681. https://doi.org/10.1111/ICAD.12590.

Colla, S.R., Otterstatter, M.C., Gegear, R.J., Thomson, J.D., 2006. Plight of the bumble bee: pathogen spillover from commercial to wild populations. Biol. Conserv. 129 (4), 461–467. https://doi.org/10.1016/j.biocon.2005.11.013.

Dalmon, A., Diévart, V., Thomasson, M., Fouque, R., Vaissière, B.E., Guilbaud, L., Le Conte, Y., Henry, M., 2021. Possible spillover of pathogens between bee communities foraging on the same floral resource. Insects. 12 (2), 1–31. https://doi.org/10.3390/insects12020122.

Daszak, P., 2000. Emerging infectious diseases of wildlife–threats to biodiversity and human health. Science. 287, 443–449. https://doi.org/10.1126/science.287.5452.443.

De Miranda, J.R., Bailey, L., Ball, B.V., Blanchard, P., Budge, G.E., Chejanovsky, N., Chen, Y.P., Gauthier, L., Genersch, E., De Graaf, D.C., Ribière, M., Ryabov, E., De Smet, L., Van Der Steen, J.J.M., 2013. Standard methods for virus research in *Apis mellifera*. J. Apic. Res. 52 (4), 1–56. https://doi.org/10.3896/IBRA.1.52.4.22.

de Souza, F.S., Kevill, J.L., Correia-Oliveira, M.E., de Carvalho, C.A.L., Martin, S.J., 2019. Occurrence of deformed wing virus variants in the stingless bee *Melipona subnitida* and honey bee *Apis mellifera* populations in Brazil. J. Gen. Virol. 100 (2), 289–294. https://doi.org/10.1099/jgv.0.001206.

Dicks, L.V., Breeze, T.D., Ngo, H.T., Senapathi, D., An, J., Aizen, M.A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L.A., Gemmill-Herren, B., Howlett, B.G., Imperatriz-Fonseca, V.L., Johnson, S.D., Kovács-Hostyánszki, A., Kwon, Y.J., Lattorff, H.M.G., Lungharwo, T., Seymour, C.L., Potts, S.G., 2021. A global-scale expert assessment of drivers and risks associated with pollinator decline. Nat. Ecol. Evol. 5, 1453–1461. https://doi.org/10.1038/s41559-021-01534-9.

Echazarreta, C.M., Quezada-Euán, J.J.G., Medina, L.M., Pasteur, K.L., 1997. Beekeeping in the Yucatan peninsula: development and current status. Bee World. 78 (3), 115–127. https://doi.org/10.1080/0005772X.1997.11099346.

Evans, J.D., Chen, Y.P., Di Prisco, G., Pettis, J., Williams, V., 2009. Bee cups: single-use cages for honey bee experiments. J. Apic. Res. 48 (4), 300–302. https://doi.org/10.3896/IBRA.1.48.4.11.

Fürst, M.A., McMahon, D.P., Osborne, J.L., Paxton, R.J., Brown, M.J.F., 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. Nature. 506 (7488), 364–366. https://doi.org/10.1038/nature12977.

Gamboa, V., Ravoet, J., Brunain, M., Smagghe, G., Meeus, I., Figueroa, J., Riaño, D., De Graaf, D.C., 2015. Bee pathogens found in *Bombus atratus* from Colombia: a case study. J. Invertebr. Pathol. 129, 36–39. https://doi.org/10.1016/J.JIP.2015.05.013.

Graystock, P., Goulson, D., Hughes, W.O.H., 2015. Parasites in bloom: flowers aid dispersal and transmission of pollinator parasites within and between bee species. Proc. R. Soc. B Biol. Sci. 282 (1813) https://doi.org/10.1098/rspb.2015.1371.

Graystock, P., Blane, E.J., McFrederick, Q.S., Goulson, D., Hughes, W.O.H., 2016. Do managed bees drive parasite spread and emergence in wild bees? Int. J. Parasitol. Parasites Wildlife 5 (1), 64–75. https://doi.org/10.1016/j.ijppaw.2015.10.001.

Greenleaf, S.S., Williams, N.M., Winfree, R., Kremen, C., 2007. Bee foraging ranges and their relationship to body size. Oecologia. 153 (3), 589–596. https://doi.org/10.1007/S00442-007-0752-9.

Guimarães-Cestaro, L., Martins, M.F., Martínez, L.C., Alves, M.L.T.M.F., Guidugli-Lazzarini, K.R., Nocelli, R.C.F., Malaspina, O., Serrão, J.E., Teixeira, É.W., 2020. Occurrence of virus, microsporidia, and pesticide residues in three species of stingless bees (Apidae: Meliponini) in the field. Sci. Nat. 107 (3) https://doi.org/10.1007/s00114-020-1670-5.

Gusachenko, O.N., Woodford, L., Balbirnie-Cumming, K., Ryabov, E.V., Evans, D.J., 2020. Evidence for and against deformed wing virus spillover from honey bees to bumble bees: a reverse genetic analysis. Sci. Rep. 10 (1) https://doi.org/10.1038/s41598-020-73809-3.

Guzmán-Novoa, E., Md Hamiduzzaman, M., Anguiano-Baez, R., Correa-Benítez, A., Castañeda-Cervantes, E., Arnold, N.I., 2016. First detection of honey bee viruses in stingless bees in North America. J. Apic. Res. 54 (2), 93–95. https://doi.org/10.1080/00218839.2015.1100154.

Hartig, F., 2020. DHARMa: residual diagnostics for hierarchical (multi- level/mixed) regression models. R package version 0.3.1. https://CRAN.R-project.org/package=DHARMa.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50 (3), 346–363. https://doi.org/10.1002/BIMJ.200810425.

Hung, K.L.J., Kingston, J.M., Albrecht, M., Holway, D.A., Kohn, J.R., 2018. The worldwide importance of honey bees as pollinators in natural habitats. Proc. R. Soc. B Biol. Sci. 285 (1870) https://doi.org/10.1098/RSPB.2017.2140.

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274 (1608), 303–313. https://doi.org/10.1098/RSPB.2006.3721.

Kock, R.A., Wambua, J.M., Mwanzia, J., et al., 1999. Rinderpest epidemic in wild ruminants in Kenya 1993–1997. Vet. Rec. 145, 275.

Leigh, J.W., Bryant, D., 2015. POPART: full-feature software for haplotype network construction. Methods Ecol. Evol. 6 (9), 1110–1116. https://doi.org/10.1111/2041-210X.12410.

Li, J., Lai, S., Gao, G.F., Shi, W., 2021. The emergence, genomic diversity and global spread of SARS-CoV-2. Nature. 600, 408–418. https://doi.org/10.1038/s41586-021-04188-6.

Manley, R., Temperton, B., Doyle, T., Gates, D., Hedges, S., Boots, M., Wilfert, L., 2019. Knock-on community impacts of a novel vector: spillover of emerging DWV-B from varroa-infested honeybees to wild bumblebees. Ecol. Lett. 22 (8), 1306–1315. https://doi.org/10.1111/ele.13323.

Martin, S.J., Highfield, A.C., Brettell, L., Villalobos, E.M., Budge, G.E., Powell, M., Nikaido, S., Schroeder, D.C., 2012. Global honey bee viral landscape altered by a parasitic mite. Science. 336 (6086), 1304–1306. https://doi.org/10.1126/SCIENCE.1220941.

McArt, S.H., Koch, H., Irwin, R.E., Adler, L.S., 2014. Arranging the bouquet of disease: floral traits and the transmission of plant and animal pathogens. Ecol. Lett. 17 (5), 624–636. https://doi.org/10.1111/ELE.12257.

McMahon, D.P., Fürst, M.A., Caspar, J., Theodorou, P., Brown, M.J.F., Paxton, R.J., 2015. A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. J. Anim. Ecol. 84 (3), 615–624. https://doi.org/10.1111/1365-2656.12345.

Michener, C.D., 2007. The Bees of the World, 595. Johns Hopkins University Press, Baltimore.

Morfin, N., Gashout, H.A., Macías-Macías, J.O., De la Mora, A., Tapia-Rivera, J.C., Tapia-González, J.M., Contreras-Escareño, F., Guzman-Novoa, E., 2021. Detection, replication and quantification of deformed wing virus-A, deformed wing virus-B, and black queen cell virus in the endemic stingless bee, *Melipona colimana*, from Jalisco, Mexico. Int. J. Trop. Insect Sci. 41, 1285–1292. https://doi.org/10.1007/s42690-020-00320-7.

Moritz, R.F.A., Kraus, F.B., Huth-Schwarz, A., Wolf, S., Carrillo, C.A.C., Paxton, R.J., Vandame, R., 2013. Number of honeybee colonies in areas with high and low beekeeping activity in Southern Mexico. Apidologie. 44 (1), 113–120. https://doi.org/10.1007/s13592-012-0163-8.

Murray, T.E., Coffey, M.F., Kehoe, E., Horgan, F.G., 2013. Pathogen prevalence in commercially reared bumble bees and evidence of spillover in conspecific populations. Biol. Conserv. 159, 269–276. https://doi.org/10.1016/J.BIOCON.2012.10.021.

Osterman, J., Aizen, M.A., Biesmeijer, J.C., Bosch, J., Howlett, B.G., Inouye, D.W., Jung, C., Martins, D.J., Medel, R., Pauw, A., Seymour, C.L., Paxton, R.J., 2021. Global trends in the number and diversity of managed pollinator species. Agric. Ecosyst. Environ. 322, 107653 https://doi.org/10.1016/J.AGEE.2021.107653.

Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., Dicks, L.V., Garibaldi, L.A., Hill, R., Settele, J., Vanbergen, A.J., 2016. Safeguarding pollinators and their values to human well-being. Nature. 540 (7632), 220–229. https://doi.org/10.1038/nature20588.

Quezada-Euán, J.J.G., 2007. A retrospective history of the expansion of Africanized honeybees in Mexico. J. Apic. Res. 46 (4), 295–300. https://doi.org/10.1080/00218839.2007.11101412.

Quezada-Euán, J.J.G., 2018. Stingless Bees of Mexico: The Biology, Management and Conservation of an Ancient Heritage. Springer Cham, NY.

Quezada-Euán, J.J.G., May-Itzá, W.J., de la Rúa, P., Roubik, D.W., 2022. From neglect to stardom: how the rising popularity of stingless bees threatens diversity and meliponiculture in Mexico. Apidologie. 53 (70) https://doi.org/10.1007/s13592-022-00975-w.

Radzevičiūtė, R., Theodorou, P., Husemann, M., Japoshvili, G., Kirkitadze, G., Zhusupbaeva, A., Paxton, R.J., 2017. Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan. J. Invertebr. Pathol. 146, 14–23. https://doi.org/10.1016/j.jip.2017.04.002.

Reynaldi, F.J., Sguazza, G.H., Albicoro, F.J., Pecoraro, M.R., Galosi, C.M., 2013. First molecular detection of co-infection of honey bee viruses in asymptomatic *Bombus atratus* in South America. Braz. J. Biol. 73 (4), 797–800. https://doi.org/10.1590/S1519-69842013000400016.

Schmid-hempel, R., Eckhardt, M., Goulson, D., Heinzmann, D., Lange, C., Plischuk, S., Escudero, L.R., Salath, R., Scriven, J.J., Schmid-Hempel, P., 2014. *T*he invasion of southern South America by imported bumblebees and associated parasites. J. Anim. Ecol. 83 (4), 823–837. https://doi.org/10.1111/1365-2656.12185.

Streicher, T., Tehel, A., Tragust, S., Paxton, R.J., 2022. Experimental viral spillover can harm *Bombus terrestris* workers under field conditions. Ecol. Entomol. 48 (1), 81–89. https://doi.org/10.1111/EEN.13203.

Tehel, A., Brown, M.J.F., Paxton, R.J., 2016. Impact of managed honey bee viruses on wild bees. Curr. Opin. Virol. 19, 16–22. https://doi.org/10.1016/j.coviro.2016.06.006.

Tehel, A., Vu, Q., Bigot, D., Gogol-Döring, A., Koch, P., Jenkins, C., Doublet, V., Theodorou, P., Paxton, R., 2019. The two prevalent genotypes of an emerging infectious disease, deformed wing virus, cause equally low pupal mortality and equally high wing deformities in host honey bees. Viruses. 11 (2) https://doi.org/10.3390/v11020114.

Tehel, A., Streicher, T., Tragust, S., Paxton, R.J., 2020. Experimental infection of bumblebees with honeybee- associated viruses: no direct fitness costs but potential future threats to novel wild bee hosts. R. Soc. Open Sci. 7 (7) https://doi.org/10.1098/rsos.200480.

Tehel, A., Streicher, T., Tragust, S., Paxton, R.J., 2022. Experimental cross species transmission of a major viral pathogen in bees is predominantly from honeybees to bumblebees. Proc. R. Soc. B Biol. Sci. 289, 20212255. https://doi.org/10.1098/rspb.2021.2255.

Teixeira, É.W., Ferreira, E.A., da Luz, C.F.P., Martins, M.F., Ramos, T.A., Lourenço, A.P., 2020. European foulbrood in stingless bees (Apidae: Meliponini) in Brazil: old disease, renewed threat. J. Invertebr. Pathol. 172, 107357 https://doi.org/10.1016/J.JIP.2020.107357.

Therneau, T.M., Grambsch, P.M., Pankratz, V.S., 2003. Penalized survival models and frailty. J. Comput. Graph. Stat. 12, 156–175. https://doi.org/10.1198/1061860031365.

Tibatá, V.M., Sanchez, A., Palmer-Young, E., Junca, H., Solarte, V.M., Madella, S., Ariza, F., Figueroa, J., Corona, M., 2021. Africanized honey bees in Colombia exhibit high prevalence but low level of infestation of varroa mites and low prevalence of pathogenic viruses. PLoS One 16 (5), e0244906. https://doi.org/10.1371/JOURNAL.PONE.0244906.

Toledo-Hernández, E., Peña-Chora, G., Hernández-Velázquez, V.M., Lormendez, C.C., Toribio-Jiménez, J., Romero-Ramírez, Y., León-Rodríguez, R., 2022. The stingless bees (Hymenoptera: Apidae: Meliponini): a review of the current threats to their survival. Apidologi. 53 (1), 1–23. https://doi.org/10.1007/S13592-022-00913-W.

Traynor, K.S., Mondet, F., de Miranda, J.R., Techer, M., Kowallik, V., Oddie, M.A.Y., Chantawannakul, P., McAfee, A., 2020. *Varroa destructor*: a complex parasite, crippling honey bees worldwide. Trends Parasitol. 36 (7), 592–606. https://doi.org/10.1016/j.pt.2020.04.004.

Ueira-Vieira, C., Almeida, L.O., de Almeida, F.C., Amaral, I.M.R., Brandeburgo, M.A.M., Bonetti, A.M., 2015. Scientific note on the first molecular detection of the acute bee paralysis virus in Brazilian stingless bees. Apidologie. 46 (5), 628–630. https://doi.org/10.1007/s13592-015-0353-2.

Wilfert, L., Long, G., Leggett, H.C., Schmid-Hempel, P., Butlin, R., Martin, S.J.M., Boots, M., 2016. Honeybee disease: deformed wing virus is a recent global epidemic in honeybees driven by varroa mites. Science. 351 (6273), 594–597. https://doi.org/10.1126/science.aac9976.

Yañez, O., Piot, N., Dalmon, A., de Miranda, J.R., Chantawannakul, P., Panziera, D., Amiri, E., Smagghe, G., Schroeder, D., Chejanovsky, N., 2020. Bee viruses: routes of infection in Hymenoptera. Front. Microbiol. 11 https://doi.org/10.3389/fmicb.2020.00943.

Zattara, E.E., Aizen, M.A., 2021. Worldwide occurrence records suggest a global decline in bee species richness. One Earth 4 (1), 114–123. https://doi.org/10.1016/j.oneear.2020.12.005.