Presence of *Apis mellifera* pathogens in different developmental stages of wild Hymenoptera species

Giovanni Cilia\(^1\), Simone Flamino\(^1,2\), Rosa Ranalli\(^1,3\), Laura Zavatta\(^1\), Antonio Nanetti\(^1\), Laura Bortolotti\(^1\), Gherardo Bogo\(^1\)

\(^1\)CREA Research Centre for Agriculture and Environment, Bologna, Italy
\(^2\)Laboratory of Zoology, University of Mons, Mons, Belgium
\(^3\)ZooPlantLab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy

**Abstract**

Pollinators are vitally important for the maintenance of ecosystems and the reproduction of most spontaneous and cultivated plants. However, in the last decades, they are suffering from an alarming decline, with the spread of pathogens and parasites being one of the main causes. *Apis mellifera* L. is the best-studied pollinator, and several studies have highlighted the presence of some of its pathogens in many other insect species. However, until now we have little or no indication of the effects these pathogens may have on other species. Here, we analysed the prevalence and load of several honey bee pathogens in different developmental stages of the non native species, giant resin bee, *Megachile sculpturalis* Smith. Also, newly emerged adults of three wild hymenopteran species, *M. sculpturalis*, *Heriades truncorum* (L.) and *Ancistrocerus* sp., nesting in a bee hotel were analysed. We found *Nosema ceranae* and CBPV in all three species analysed, and in *M. sculpturalis* with an increasing trend in parasite and virus levels from early to late stages. In addition, in free-ranging *M. sculpturalis* adults, we also found DWV, BQCV and AmiFV. All viruses found were replicative. Our results indicate that honey bee viruses and parasites can replicate in wild Hymenoptera and their pre-imaginal developmental stages. Therefore, the considered viruses and parasites can be considered multi-host viruses and parasites, and wild pollinators could act not only as a reservoir but also as amplification hosts, highlighting an important threat not only for wild but also for managed bees, because they may result in spillback infections.

**Key words** wild pollinators, *Megachile sculpturalis*, developmental stages, *Nosema ceranae*, CBPV.

**Introduction**

Pollinators play a pivotal role in terrestrial ecosystems, in that they allow the reproduction of both wild plants and crops. It is estimated that 87.5% of entomophile angiosperms depend on them (Ollerton *et al.*, 2011), and more than three-quarters of the major types of global food crops benefit from animal pollination (Klein *et al.*, 2007; Rader *et al.*, 2016; Rhodes, 2018). The recent decades witnessed a pollinator decline due to different factors contributing to the reduction of their populations, biodiversity, abundance, and distribution (Szabo *et al.*, 2012; Ollerton *et al.*, 2014; Powney *et al.*, 2019). Together with pesticides, land-use change and global warming, pests and pathogens are deemed major causes of pollinator decline (Goulson *et al.*, 2015).

*Apis mellifera* L. is the most studied and known pollinator, mainly due to its commercial value. The same also applies to the pathogens and pests affecting this species. It is well-known that *A. mellifera* is exposed to several viruses, bacteria, fungi, protists, and mites, which are responsible for severe effects at the individual and social levels. The negative effects of these pathogens may include altered homeostasis and renewal of intestinal tissues, consequent malnutrition, impaired behaviour and orientation, suppressed immune function, problems collecting nectar and pollen and, finally, colony losses (Gisder and Genersch, 2017; McMahon *et al.*, 2018; Beareprea et al., 2020; Boncristiani *et al.*, 2020).

Those pathogens do not threaten honey bees only but are reported to spread in the environment and infect wild bees also (Martinez-Lopez *et al.*, 2021; Nanetti *et al.*, 2021a; Piot *et al.*, 2022). The health of managed honey bees, wild bees and other arthropods is interconnected, which implies the implementation of a One Health approach to honey bee health (Mahefarisoa *et al.*, 2021; Wilfert *et al.*, 2021). This approach is essential to generate suitable ecosystems for pollinators and other arthropods contributing to human livelihoods, promoting environmental health, and preventing the transmission of pathogens and pests to protect managed and wild insect populations (Manley *et al.*, 2015).

So far, spillover of honey bee parasites and viruses were documented more frequently for deformed wing virus (DWV), Kashmir bee virus (KBV), Israeli acute paralysis virus (IAPV), sac brood virus (SBV), and *N. ceranae* (Dalmon *et al.*, 2021; Nanetti *et al.*, 2021a). In addition, the majority of the studies regarded mostly bumblebees, mason bees and leafcutter bees as host species, probably as for honey bees, due to their economic value (Tehel *et al.*, 2016; Ngor *et al.*, 2020).

Although parasites and viruses can cause severe damage in apiaries at both individual and colony levels, their effects on other insect hosts remain largely unknown. A notable exception to that is DWV infections in bumblebees, where the presence of the replicative virus was associated with wings deformities in *Bombus terrestris* (L.) and *Bombus pascuorum* (Scopoli) (Genersch *et al.*, 2006; Cilia *et al.*, 2021) and in wild bee longevity (Fürst *et al.*, 2014; Graystock *et al.*, 2016; Tehel *et al.*, 2022).

Honey bee parasites and viruses can be transmitted by different routes. In arthropods sharing the same environment as the honey bees, some parasites and viruses may occur by orofaecal route (Cilia *et al.*, 2018b; de Landa et
al., 2020; Nanetti et al., 2021b), by ingestion of contaminated pollen, and direct contact (Singh et al., 2010). Foraging activity could also promote spillover, as pollen, nectar, and floral organs may get contaminated by infected honey bee foragers so spreading the pathogens to other flower visitors (Mazzei et al., 2014; Alger et al., 2019; Burnham et al., 2021). Hornets, wasps and scavengers may get infected as a consequence of honey bee predation and carcass cannibalization (Sébastien et al., 2015; Forzan et al., 2017; Mazzei et al., 2018, 2019).

Honey bee colonies may represent pathogen sources to other pollinator populations and the latter could act as reservoirs (Ravoet et al., 2014). However, to the best of our knowledge, the previous studies on the presence of honey bee viruses and parasites in wild insects were limited to adult stages, showing a severe gap in our understanding of the preimaginal susceptibility to infections.

The aim of this work was to study some aspects related to the presence and circulation of honey bee viruses and parasites in wild hymenopterans, in particular: i) we analysed the pathogen presence in different developmental stages of the giant resin bee Megachile sculpturalis Smith (Hymenoptera Megachilidae), and ii) we investigated the presence of these viruses and parasites in different newly emerged individuals of three cavity-nesting hymenopterans, including M. sculpturalis.

Materials and methods

Sample collection

The study was conducted in April-July 2021 at CREAA, Bologna, Italy (44°31'26.8”N 11°21'04.5”E), in a single bee hotel. The bee hotel (artificial nesting site for wild solitary Hymenoptera species) is located in an area of roughly 500 m², coexisting with an experimental apiary included approximately forty Apis mellifera ligustica Spinola colonies.

The bee hotel consisted of assembled wooden cubes (cavities of 0.2-1.4 cm of diameter, 10-15 cm deep), cut reeds (Arundo donax L. of various diameters and lengths), and trunk segments from local wood species (cavities of 0.4-1.0 cm of diameter, 11-12 cm deep). Since its establishment in 2016, the bee hotel was used by M. sculpturalis as a nesting site. This species, native to Southeast Asia, was accidentally introduced first to North America in the 1990s, and then to many countries in Europe (Lanner et al., 2021). It is a cavity-nesting solitary bee that builds its nests in pre-existing cavities in wooden rods, dead wood, or reed stems, as well as in artificial holes including bee hotels (Guarento et al., 2019). In April, M. sculpturalis individuals were sampled pre-emergence from nests in reeds, identified based on their peculiar features (i.e., larval cells composed mostly of resin mixed with vegetable debris and the closing plug by a mixture of resin, mud and wood fibres) (Agudo et al., 2018). We collected samples belonging to different developmental stages (i.e. prepupae, pupae, and pre-emerged adults). In addition, in the same periods, wooden cubes and trunk segments were placed in mesh cages to allow the subsequent collection of newly emerged individuals of all Hymenoptera species emerging in the same period of study.

Between the end of June and the beginning of July, 69 newly emerged individuals of three different Hymenoptera species (including M. sculpturalis) were collected for taxonomic identification and pathogen analyses. At the same time, 100 newly emerged M. sculpturalis adults were marked with water-based colours, released open-air and recollected two weeks later (hereafter: “free-ranging adults”). All the samples were stored in individual tubes at –80 °C until analysis.

Extraction of total nucleic acids

Before extraction, all samples were washed with 95% ethanol to remove external microbial contaminations.

Wild hymenopterans were analysed individually to clear assess their infections. Each sample was placed in a 2 mL microtube with 500 µL of DNA/RNA Shield (Zymo Research, Irvine, CA, USA) and crushed with a TissueLyser II (Qiagen, Hilden, Germany) for 3 minutes at 30 Hz, as previously reported (Cilia et al., 2019; Nanetti et al., 2021b). The obtained suspensions were split into two aliquots, from which DNA and RNA were separately extracted.

The above-described procedures were accomplished by using respectively Quick DNA Microprep Plus Kit (Zymo Research) and Quick RNA Microprep Plus Kit (Zymo Research) following the modified manufacturer’s instructions for solid tissue processing (Mazzei et al., 2019; Nanetti et al., 2021c).

The obtained nucleic acids were eluted in 50 µL of Dnase-Rnase-free water and the extracts were stored at –80 °C until the qPCR assays.

qPCR assays to detect parasites and viruses

The extracted nucleic acids were analysed using Real-Time PCR to quantify the abundance of detected parasites and viruses in the samples, using the primers reported in table 1. For each target gene, a total reaction volume of 15 µL was prepared as previously described (Cilia et al., 2020, 2021) using PowerUp™ SYBR™ Green Master Mix (ThermoFisher, Waltham, MA, USA) for DNA microorganism and Power SYBR™ Green Cells-to-CT™ Kit (ThermoFisher Scientific) for RNA viruses. The real-time PCR assay was performed on an Applied Biosystems® 7500 Real-Time PCR (ThermoFisher Scientific), following the protocols for each gene sequence (Dobbelrae et al., 2001; Martin-Hernandez et al., 2007; Roetschi et al., 2008; Arisendi et al., 2016; Cilia et al., 2018a; Xu et al., 2018). DNA and RNA previously extracted from positive honey bee samples were used as positive controls for each investigated parasite and virus.

For each target gene, a standard curve was generated by amplifying serially diluted recombinant plasmids containing the pathogen-specific DNA and RNA fragments from 1 × 10¹ to 1 × 10⁸ copies in a qPCR assay on an Applied Biosystems® 7500 Real-Time PCR (ThermoFisher Scientific), as previously reported (Mazzei et al., 2019; Cilia et al., 2020, 2021; Nanetti et al., 2021c), following the amplification and quantification protocols (Dobbelrae et al., 2001; Chantawannakul et al., 2006; Martin-Hernandez et al., 2007; Roetschi et al., 2008; de Miranda et al., 2021b).
Table 1. List of primers used to detect parasites and viruses.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer Name</th>
<th>Sequence (5’-3’)</th>
<th>Reference</th>
</tr>
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<tr>
<td>Nosema ceranae</td>
<td>Hsp70_F</td>
<td>GGGTTACAAAGGGTGCTAGAGGGTATT</td>
<td>Cilia et al., 2018a</td>
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<td></td>
<td>Hsp70_R</td>
<td>TGGTACAGCCATAGCAAGGCTGTTA</td>
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<tr>
<td>Nosema apis</td>
<td>321APIS_F</td>
<td>GGGGGCAGATGTCCTTGGTCGCTAGTGA</td>
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<tr>
<td></td>
<td>321APIS_R</td>
<td>GGGGGGCGTTTTAATGGTAAACACACTAGT</td>
<td>Martin-Hernandez et al., 2007</td>
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<tr>
<td>Pauenibacillus larvae</td>
<td>AFB-F</td>
<td>CTTTGTGTCTCTTCGGGAGCAGCCA</td>
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<tr>
<td></td>
<td>AFB-R</td>
<td>TCTTATAGGCCACCTCTTCGGG</td>
<td></td>
</tr>
<tr>
<td>Melissococcus plutinius</td>
<td>Melissor</td>
<td>ATGCTAGTGATAGTAGGCAAATCAAT</td>
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<tr>
<td>Crithidia mellifica</td>
<td>Cyt_b_F</td>
<td>GAAATTTGTTGGATACCGCTATGATTGGTATG</td>
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<tr>
<td></td>
<td>Cyt_b_R</td>
<td>ACCCAAAAGTACGGAAGGAA</td>
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<tr>
<td>Lotmaria passim</td>
<td>Lp2F 459</td>
<td>GGGTTACAAAGGGTGCTAGAGGGTATT</td>
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<td></td>
<td>Lp2R 459</td>
<td>TGGTACAGCCATAGCAAGGCTGTTA</td>
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<tr>
<td>KBV</td>
<td>KBV 83F</td>
<td>ACCAAGGATATTTCTACCTTGTAAG</td>
<td>Chantawannakul et al., 2006</td>
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<td>KBV 161R</td>
<td>TGGGACCTGTGTCGTCGGTCGCAG</td>
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<td>DWV Fw 8450</td>
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<td>DWV Rev 8953</td>
<td>CTGTCGAGGCTGCAGTTTCCTGCC</td>
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<tr>
<td>ABPV</td>
<td>APV 95F</td>
<td>TCTCATATCGGCAGGAGAAGAACCAA</td>
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<td></td>
<td>APV 159R</td>
<td>GCGCTTAAATCTACCTCAATAGTAAG</td>
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<tr>
<td>BQCV</td>
<td>IAPV B4S0427_R130M</td>
<td>RCRGCTAGCTGTCGTCGTCG</td>
<td>Kajobe et al., 2010</td>
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<td>IAPV B4S0427_L17M</td>
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<td>SBV</td>
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<td></td>
<td>SBV 380R</td>
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<td>CBPV</td>
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<td>CPV 371R</td>
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<td>SBPV major</td>
<td>SPV 8383F 81</td>
<td>TGGATTGCAGCTGGCTTGGCTA</td>
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<td></td>
<td>SPV 8456R</td>
<td>CAAAATTTGCTACCTTTTTCGGAGGT</td>
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<td>SBPV minor</td>
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<td>ATAGGGCTGTTGTTGTTACCTGCA</td>
<td>Martin et al., 2012</td>
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<td></td>
<td>SPV Minor R1</td>
<td>CTGGAATTGACCTACCATCCCATG</td>
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<tr>
<td>AmFV</td>
<td>AmFV2-F</td>
<td>ACCCAACCTTTGCGGAGGTT</td>
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<td></td>
<td>AmFV2-R</td>
<td>ATGGGCCTTGTCGCGGTTAACCACA</td>
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</table>

KBV: Kashmir bee virus; DWV: deformed wing virus; ABPV: acute bee paralysis virus; IAPV: Israeli acute paralysis virus; BQCV: black queen cell virus; SBV: sac brood virus; CBPV: chronic bee paralysis virus; SBPV: slow bee paralysis virus; AmFV: Apis mellifera filamentous virus.

2010; Kajobe et al., 2010; Martin et al., 2012; Hartmann et al., 2015; Arismendi et al., 2016; Cilia et al., 2018a; Mazzei et al., 2018; Xu et al., 2018).

Strand-specific RT-PCR

The active replication of viruses was evaluated through strand-specific RT-PCRs using specific primers to detect positive and negative RNA strands, as previously described (Mazzei et al., 2018; Nanetti et al., 2021b). Reactions were performed for all RNAs extracted using QuantiTect Reverse Transcription Kit (Qiagen). For DWV, CBPV and BQCV, the obtained cDNAs were amplified by PCR for the related viral targets, and the amplicons were visualized on a 2% agarose gel. On the other hand, to assess the replicative activity of AmFV, an aliquot of 10 μl from extracted RNA was treated with DNase and, after, the obtained cDNA was amplified by PCR and visualized as reported above. Replicative viruses previously extracted from positive honey bees were used as the positive control for each investigated virus. Subsequently, the amplicons were sequenced (BMR Genomics, Padua, Italy) and analysed using BLAST (Altschul et al., 1990).

Statistical analysis

Pathogen prevalence among the three wild Hymenoptera species and the different developmental stages of M. sculpturalis were analysed by chi-square test with Bonferroni correction. Data on pathogen abundance were first tested for normality using Shapiro-Wilk test. The abundance of the different viruses and parasites in positive samples calculated by averaging each of the two replicates of qPCR was analysed by Kruskal-Wallis H-test, and Mann-Whitney U-test for the investigated viruses and parasites. The significance threshold was set at 0.05.

All data on pathogen abundance were presented as mean ± SE. Analyses were performed and figures were drawn in R version 4.1.2 using ggplot2 package (Wickham, 2009; R Core Team, 2021).

Results

Raw data on pathogen abundance, sample sizes of individuals collected and samples positive to viruses and parasites are reported in supplemental material table S1.

M. sculpturalis developmental stages

We analysed a total of 79 individuals of M. sculpturalis belonging to five different developmental stages: prepupae (N = 12), pupae (N = 15), pre-emerged adults (N = 17), newly emerged adults (N = 23), and free-ranging adults (N = 12). We found that 49.4% of all analysed samples were positive to N. ceranae and 31.7% to CBPV (figure 1) and we did not find statistical differences in pathogen prevalence among stages (N. ceranae: χ² = 1.70, p = 0.790;
CBPV: $\chi^2_{2} = 1.92$, $p = 0.751$). In addition, although non-significant ($N. ceranae$: $H = 3.16$, $p = 0.531$; CBPV: $H = 4.00$, $p = 0.405$), there was an increasing trend in the abundance and range variability from pre-imaginal to newly emerged stages, especially as regards CBPV (figure 2). The strand-specific PCR demonstrated active viral replication of CBPV in all PCR-positive samples. Among free-ranging $M. sculpturalis$ adults, we found DWV in 50% of the samples, and $AmiFV$ and $BQCV$ in one sample each. All these three viruses were replicative in all samples. Concerning viral sequences, the analysis highlighted the complete homology of CBPV (supplemental material figure S1) among all stages of $M. sculpturalis$.

Finally, considering all the developmental stages, we found nine individuals presenting a co-infection (supplemental material table S2). Of these, five individuals simultaneously presented $N. ceranae$ and CBPV, two $N. ceranae$ and DWV, one CBPV and DWV, and one $N. ceranae$ and BQCV.

**Newly emerged hymenopterans**

Collected newly emerged individuals were identified as the individuals $Heriades truncorum$ (L.) (N = 19), $M. sculpturalis$ (N = 23), and $Ancistrocerus$ sp. (N = 27). The first two species belong to the family Megachilidae, while the latter belongs to the family Vespidae (subfamily Eumeninae). Concerning the newly emerged individuals of the three Hymenoptera species, we obtained similar results to those of the different developmental stages of $M. sculpturalis$: 53.6% of individuals were positive to $N. ceranae$ and 27.5% to CBPV and the prevalence did not statistically differ depending on the species ($N. ceranae$: $\chi^2_{22} = 0.068$, $p = 0.967$; CBPV: $\chi^2_{22} = 0.146$, $p = 0.930$; figure 3). All individuals were negative to the other investigated viruses and parasites.

We did not find a significant interspecific difference in the abundance of $N. ceranae$ ($F_{3,43} = 8.20$, $p < 0.001$; figure 4a), and in the abundance of CBPV ($F_{3,25} = 7.12$, $p = 0.001$; figure 4b).

A BLAST analysis highlighted the complete homology of the CBPV viral sequences found in the three species (supplemental material figure S2). The strand-specific PCR demonstrated active viral replication of CBPV in all PCR-positive samples.

![Figure 1](image.png)

**Figure 1.** Percentage of $M. sculpturalis$ samples at different developmental stages that were positive to $N. ceranae$ and CBPV.

![Figure 2](image.png)

**Figure 2.** The abundance of $N. ceranae$ (a) and CBPV (b) samples of $M. sculpturalis$ at different developmental stages. Means are showed as black dots, medians, interquartiles and ranges (or 95% CIs) are shown.

Finally, we found six samples (8.7%) co-infected by $N. ceranae$ and CBPV (supplemental material table S1). One of those samples belonged to $H. truncorum$, two to $M. sculpturalis$ (already mentioned in the analysis among different developmental stages), and three to $Ancistrocerus$ sp.
Discussion

We found *N. ceranae* and CBPV in all the developmental stages of *M. sculpturalis* and also in the other two wild Hymenoptera species nesting in the same bee hotel. In addition, in *M. sculpturalis* free-ranging adults we also found pathogens absent in the bee hotel (i.e. DWV, *AmiFV*, and BQCV). Although honey bee viruses and parasites had already been found in wild pollinators (Graystock et al., 2013; Ngor et al., 2020; Pritchard et al., 2021; Cilia et al., 2022a), our results showed that the viruses found were all replicative, indicating that they can be considered multi-host viruses and parasites.

The abundance of the viruses and parasites identified in the investigated species were lower than the threshold value measured in symptomatic honey bee (> 1 × 10^6 copies) in previous studies (Chen et al., 2006; Mazzei et al., 2014; Martín-Hernández et al., 2018). This is the only kind of comparison that can be made, because unfortunately no threshold values for these infections have yet been defined for wild bees and, especially, no correlation between copy number and disease symptoms has been seen. This is one of the most important gaps because possibly even low levels of infection of honey bee viruses and parasites can be harmful to wild bees and other hymenopterans (Dolezal et al., 2016).

Regarding *N. ceranae*, however, we must point out that this microsporidium parasite could have not truly infected the species analysed. Although some samples resulted infected by more than 1 × 10^7 microsporidian copies, the other ones showed a lower level of infection presumably not enough to trigger the infection in the midgut (Gisder et al., 2020; Higes et al., 2020).

*M. sculpturalis* developmental stages

To the best of our knowledge, this is the first identification of DWV, CBPV, *AmiFV*, BQCV and *N. ceranae* in *M. sculpturalis* individuals, and several developmental stages of a wild pollinator. Previously, only SBV were found in *M. sculpturalis* adults (Cilia et al., 2022b).

We hypothesise that contaminated pollen generated infections in the larvae (Singh et al., 2010; Alger et al., 2019), that remained active throughout the subsequent developmental stages.

Although non-significant, the abundance of CBPV in pupae than in prepupae could be linked to the time elapsed since the interruption of defecation, which occurs just before the stage of prepupa and could have slightly decreased the viral load; during the time elapsed between defecation and pupal development, the level of infection has continued to grow, until the complete formation of the individual. Another possible explanation may be a general immune deficiency of pupae. On the other hand, the slightly lower load in free-ranging adults could be linked to the resumption of defecation in the environment, which is the main vehicle for spreading the infection. Moreover, the hatching of already infected individuals could be due to the vertical transmission of these viruses and parasites that could consistently increase individual positive rates within a specific population, improving the disease spreading.

Considering the other viruses found in free-ranging adults, the most plausible cause is contact with contaminated pollen via flowers.

**Newly emerged individuals**

The viruses DWV and CBPV have never been identified before in *H. truncorum*, while *N. ceranae* has been previously detected in individuals of the same species sampled in Belgium (Ravoet et al., 2014). Concerning
eumene wasps, to date, only ABPV and SBV were detected in Ancistrocerus auctus (F.) in Argentina (Alvarez et al., 2018). This is not the first case in which honey bee viruses and parasites are shared between individuals using the same nests or living in the same area (Radzvečiūtė et al., 2017; Keller et al., 2018; Purkiss and Lach, 2019; Macias-Macias et al., 2020).

The detected viruses were replicative in the investigated individuals, highlighting their role as “new” hosts for these honey bee viruses and parasites. Furthermore, the occurrence of replicative DWV and CBPV or one replicative virus with N. ceranae in the same individuals indicates the possibility of pathogen co-infections in the hymenopteran species, as already reported in A. mellifera and other insect species (de Miranda et al., 2010; Nanetti et al., 2021b).

Moreover, the active replication in species other than A. mellifera could represent an important ecological threat not only for wild bees but also for managed honey bees, because they may occur in re- or new infection (Tapia-González et al., 2019; Piot et al., 2020; Yañez et al., 2020; Burnham et al., 2021; Morfin et al., 2021). This is also demonstrated by the presence of AmiTV and BQCV in free-ranging M. sculpturalis adults. In addition, the fact that this species is rapidly spreading (Lanner et al., 2021) may have an impact on bee disease dynamics in the future.

Conclusion

The spillover of viruses and parasites from managed honey bees to wild bees and other hymenopterans represents an important threat to the health of pollinators with a potentially high impact on individual and population fitness (Graystock et al., 2013; Gisder and Genersch, 2017; Nanetti et al., 2021a).

Unfortunately, there is a lack of information on this topic. Few studies analysed the pathogen replication, thus verifying the occurrence of an effective spillover. In addition, only very few studies analysed the effects of these viruses and parasites on wild insects, focusing mainly on mortality and no other crucial aspects of their fitness as flower-visiting behaviour, mating and reproductive success, nesting, pollen provisioning, and larval development.

Despite the lack of replication of our study (only one bee hotel and one season considered), further studies are needed to evaluate the risk of pathogen mutual transmission between wild and managed bees, which may create a ‘vicious infection circle’.

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The authors declare no competing interests.

References


Cilia G., Zavattà L., Ranalli R., Nanetti A., Bortolotti L., 2021.- Replicative deformed wing virus found in the head of adults from symptomatic commercial bumblebee (Bombus terrestris) colonies. - Veterinary Sciences, 8: 117.


Genscher E., Yue C., Fries I., De Miranda J. R., 2006.- Detection of deformed wing virus, a honey bee viral pathogen, in bumble bees (Bombus terrestris and Bombus pascuorum) with wing deformities.- Journal of Invertebrate Pathology, 91: 61-63.


Hartmann U., Forsgren E., Charrière J. D., Neumann P., Gauthier L., 2015.- Dynamics of Apis mellifera filamentous virus (AmFV) infections in honey bees and relationships with other parasites.- Viruses, 7: 2654-2667.


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Aeric and quantification of deformed wing virus ORFIN WIN RWIN FT.

Aethina tumida Parasitology T., 2019.

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Eruca sativa AXTON G., 2021a.

Giovanni.cilia@crea.gov.it

Pathogens R., 20150610.

C., 2014.

Nature Communications, 10: 1018.


Authors' addresses: Giovanni Cilia (corresponding author: giovanni.cilia@crea.gov.it), Simone Flamino, Rosa Ranalli, Laura Zavatta, Antonio Nanetti, Laura Bortolotti, Gherardo Bogo, CREA Research Centre for Agriculture and Environment, via di Corticella 133, 40128 Bologna, Italy; Simone Flamino, Laboratory of Zoology, University of Mons, avenue du Chesnay de Mars 6, B7000 Mons, Belgium; Rosa Ranalli, ZooPlantLab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, piazza della Scienza 2, 20126 Milano, Italy.

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