

1 **First detection of bee viruses in hoverfly (syrphid) pollinators**

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1 **Abstract**

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3 Global declines of insect pollinators jeopardise the delivery of pollination services in both agricultural and
4 natural ecosystems. The importance of infectious diseases has been documented in honey bees, but there is
5 little information on the extent to which these diseases are shared with other pollinator orders. Here, we
6 establish for the first time the presence of three important bee viruses in hoverfly pollinators (Diptera:
7 Syrphidae): black queen cell virus (BQCV), sacbrood virus (SBV), and deformed wing virus strain B
8 (DWV-B). These viruses were detected in two *Eristalis* species, which are behavioural and morphological
9 bee mimics and share a foraging niche with honey bees. Nucleotide sequences of viruses isolated from the
0 *Eristalis* species and *Apis mellifera* were up to 99 and 100% identical for the two viruses, suggesting that
1 these pathogens are being shared freely between bees and hoverflies. Interestingly, while replicative
2 intermediates (negative strand virus) were not detected in the hoverflies, viral titres of SBV were similar to
3 those found in *A. mellifera*. These results suggest that syrphid pollinators may play an important but
4 previously unexplored role in pollinator disease dynamics.
5

6 Introduction

7 Emerging infectious diseases (EIDs) are a global problem for biodiversity and human health [1]. Their
8 occurrence has been associated with anthropogenic pressures, such as the global transport of managed
9 animals and plants [1,2], which introduce diseases into novel hosts and alter natural disease dynamics [3].
0 EIDs can be particularly problematic for small and declining populations where ‘spillover’ from large
1 managed populations can occur repeatedly, potentially resulting in the eventual extinction of the native
2 population [3].

3

4 The positive-stranded RNA viruses found in managed honey bees (*Apis mellifera* and *Apis ceranae*)
5 represent a key complex of potential EIDs that are shared with other wild bee pollinators [4,5]. These
6 viruses have been implicated in the declines of wild bee populations, leading to concern for the economic
7 and ecological value of associated ecosystem services [6,7]. Viruses originally thought to be honey bee-
8 specific are now known to occur in and infect a wide range of wild bee species [8]. Interspecific transfer of
9 these viruses, and other parasites, is thought to occur when individuals forage at the same flowers [4,9,10].
0 While many other taxa commonly share floral resources with bees, information on the presence of these
1 diseases in taxa other than bees is poor [11]. To understand and manage disease pressure on pollinator
2 populations, the role played by other taxa of flower visitors in the transmission of ‘bee’ viruses needs to be
3 evaluated.

4

5 Hoverflies (Diptera: Syrphidae) regularly share flowers with bees and are important providers of pollination
6 services [12,13]. Here we investigate whether four abundant taxa of hoverflies act as hosts or potential
7 vectors for six common bee viruses.

8

9 Materials and methods

0 **Sample collection**

1 During 16-22 July 2016, 20 individuals each of honey bees and four of the most common UK species of
2 hoverfly (*Episyrphus balteatus* (De Geer, 1776), *Platycheirus albimanus* (Fabricius, 1781), *Eristalis tenax*
3 (Linnaeus, 1758), and *Eristalis arbustorum* (Linnaeus, 1758)) were collected with permission from
4 grassland and open woodland habitats at Wytham Woods, Oxfordshire, United Kingdom (51.77°N, -
5 1.33°W). Flies were identified while alive, then killed and stored at -80°C.
6

7 **Molecular Analysis**

8 Total RNA was extracted from bee and hoverfly abdomens using a Direct-zol™ RNA MiniPrep kit (Zymo
9 Research). cDNA was synthesized from 2µg of the RNA using M-MLV Reverse Transcriptase (Promega)
0 with 0.5 µg random hexamers (Invitrogen). Further details are given in the supplementary methods.
1

2 Presence or absence of six common bee viruses (Acute Bee Paralysis virus ABPV, Black Queen Cell Virus
3 BQCV, Deformed Wing Virus strain A DWV-A, and strain B DWV-B, Slow Bee Paralysis Virus SBPV,
4 and Sacbrood Virus SBV) was determined by RT-PCR (supplementary methods, primers in Table S1).

5 Positive samples identified by the amplification of the correct-sized product were verified by amplification
6 in an independent RT-PCR reaction and subsequent Sanger sequencing (by Source Bioscience, Cambridge)
7 to confirm they mapped to the virus of interest in the National Center for Biotechnology Information
8 (NCBI) database. All amplicons of the correct size showed high sequence identity to the virus of interest
9 (Table S2). All sequences are available at NCBI Genbank with the accession numbers MG737448-
0 MG737473.
1

2 Viral titres of SBV and BQCV were quantified using qRT-PCR (see supplementary methods, primers in
3 Table S1). To detect the negative strand of SBV and BQCV, which is indicative of virus replication, the
4 protocol of de Miranda et al. [14; section 10.2.8.1] was followed using Superscript III (Invitrogen). A
5 combined exonuclease and restriction digest was carried out on tagged cDNA to reduce the chance of false-
6 positives and non-specific priming during PCR (supplementary methods).

7

8 **Statistical analyses**

9 Analyses were carried out in R version 3.4.1 [15]. Viral titres were compared between *Apis* and hoverflies
0 using Welch's *t*-tests following log-transformation. To compare virus incidence among species, we used chi-
1 squared tests in the coin package [16]. An approximated null distribution using 9999 replicate Monte Carlo
2 simulations was used to account for zero/low counts.

3

4 **Results**

5 **Detection of bee viruses by RT-PCR**

6 Viruses were detected in both *A. mellifera* and hoverflies (Table 1; Figure 1). When considering positive
7 results verified by independent amplification and sequencing (supplementary results), the most commonly
8 detected virus in our samples was BQCV. BQCV was detected significantly more frequently in *A. mellifera*
9 samples (13/20 samples) than in the hoverfly samples, *Er. tenax* (2/20) and *Er. arbustorum* (2/20;
0 approximate test for differences among species: $\chi^2 = 42.2$, $p < 0.001$). BQCV was not detected in *P.*
1 *albimanus* or *Ep. balteatus*, but there was no evidence that the proportion of samples with BQCV differed
2 significantly among hoverfly species ($\chi^2 = 4.2$, $p = 0.32$). SBV was also frequently detected in *A. mellifera*
3 (6/20), *Er. tenax* (4/20) and *Er. arbustorum* (1/20), but not in *P. albimanus* or *Ep. balteatus*. There was a
4 significant difference in the proportion of SBV-positive samples across all species ($\chi^2 = 14.7$, $p = 0.007$),
5 and across hoverfly species ($\chi^2 = 19.2$, $p = 0.05$).

6

7 When assaying for the DWV complex, results from hoverfly samples were highly inconsistent for most sets
8 of primers, and we were unable to verify the presence of DWV-A in our samples using two different primer
9 sets (see supplementary results; Figure S1). DWV-B results were also difficult to verify, so detection of this
0 virus in only one hoverfly sample may underestimate its true incidence.

1

2

3 **Variation in BQCV and SBV sequences**

4 Analysis of a 345 bp section of SBV capsid gene from *A. mellifera* and hoverfly sequences indicated that the
5 strains of virus present in these individuals were highly similar (ranging from 95 to 99% nucleotide identity
6 between hoverfly sequences and *A. mellifera* sequences; Table S3). Similarly, analysis of a 696 bp section of
7 BQCV RNA-dependent RNA polymerase gene from *A. mellifera* and hoverfly sequences indicated high
8 virus similarity (87 to 100% nucleotide identity of hoverfly sequences to *A. mellifera* sequences; Table S4).

0 **Viral titres of BQCV and SBV**

1 For BQCV, *A. mellifera* samples contained $3.7 \times 10^6 \pm 2.1 \times 10^6$ genome equivalents per abdomen (mean \pm
2 SE; n = 13). This was significantly higher than in hoverflies ($t(5.4) = 5.0$, $p = 0.003$), where all samples fell
3 outside of our standard curve (a threshold equivalent to roughly 1×10^4 viral equivalents per sample) but
4 were extrapolated to contain $3.9 \times 10^3 \pm 2.3 \times 10^3$ genome equivalents per abdomen (n = 4; Figure 2). For
5 SBV, viral titres were not significantly different across *A. mellifera* and hoverfly samples ($t(7.5) = 0.8$, $p =$
6 0.43), at $1.3 \times 10^5 \pm 7.1 \times 10^4$ (n = 6) and $7.4 \times 10^4 \pm 5.0 \times 10^4$ (n = 5) per abdomen respectively.

8 **Evidence of replication of BQCV and SBV**

9 Negative strand-specific RT-PCR of BQCV and SBV positive samples indicated possible replication of
0 BQCV in 2/13 *A. mellifera* workers and replication of SBV in 3/6 *A. mellifera* workers. Replication
1 intermediates of SBV or BQCV were not detected in any hoverfly samples (5 and 4 individuals
2 respectively), suggesting lack of viral replication.

4 **Discussion**

5 Our study is the first to detect bee viruses in hoverfly pollinators. In contrast, an earlier study found no
6 evidence for the presence of DWV in three hoverfly species [17]. Our results add further evidence that
7 viruses traditionally considered ‘bee’ diseases are not restricted to Hymenoptera [11], and highlight the

8 importance of understanding the role of non-bee pollinators in pathogen transmission. Interestingly, bee
9 viruses were only detected in hoverfly species in the genus *Eristalis*, which mimic *A. mellifera* in both
0 morphology and behaviour [18]. This presumed foraging niche overlap between *Eristalis* and *A. mellifera*
1 may have increased the probability of exposure to bee pathogens via shared floral resources. In contrast, *Ep.*
2 *balteatus* and *P. albimanus* are both generalist floral visitors that do not mimic bees [19].

3
4 Only viruses that were detected in co-foraging honey bees were detected in our hoverfly samples, and these
5 were always at higher or equal prevalence in honey bees. Combined with high sequence similarity between
6 isolates, this is consistent with spillover of these viruses into hoverflies, as has previously been suggested for
7 bumblebees [4,5]. However, the detection of bee viruses in a sample does not imply infection and could be
8 explained by vectoring. There was no evidence of viral replication for either BQCV or SBV in the
9 hoverflies. But, given the low titres detected and subsequent likelihood of false negatives, we cannot rule out
0 that these were true infections. While BQCV viral titres were much higher in honey bees, SBV titres in
1 *Eristalis* were similar to those in honey bees, suggesting that *Eristalis* may potentially be acting as a host to
2 SBV.

3
4 Regardless of whether hoverflies are active hosts or passive vectors of the pathogens [10], our results
5 suggest that hoverfly flower visitors may play an important but previously unexplored role in pollinator
6 disease networks. As abundant flower visitors sharing resources with both honey bees and wild bees,
7 hoverflies may be capable of moving these pathogens around the landscape, facilitating transmission
8 between susceptible bee species. *Er. tenax* is capable of extensive, long-distance migration [20], suggesting
9 the potential for supra-national networks of pathogen transmission among pollinators. This is particularly
0 concerning for emerging pathogens such as DWV-B, a recently discovered, highly virulent strain of the
1 deformed wing virus [21]. Further work is now needed to investigate the role of hoverflies as both hosts and
2 vectors for a wider range of pathogens, and the extent to which use of shared floral resources leads to
3 spillover and transmission among species.

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5

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8

9 **Author contributions**

0 KRD collected and identified the samples; EJB, KRD, JB & LR carried out the molecular lab work; EJB &
1 KRD led the writing of the manuscript; MJFB & OL conceived and designed the study; MJFB, OL and EJB
2 coordinated the study; all authors helped draft the manuscript, gave final approval for publication and agree
3 to be accountable for its content.

4

5 **Data accessibility**

6 Supporting datasets: <https://doi.org/10.17637/rh.5706154>

7 DNA sequences: Genbank accessions MG737448-MG737473

8

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1 BB/N000668/1 awarded to MJFB from BBSRC.

2

3 **Competing interests**

4 We have no competing interests.

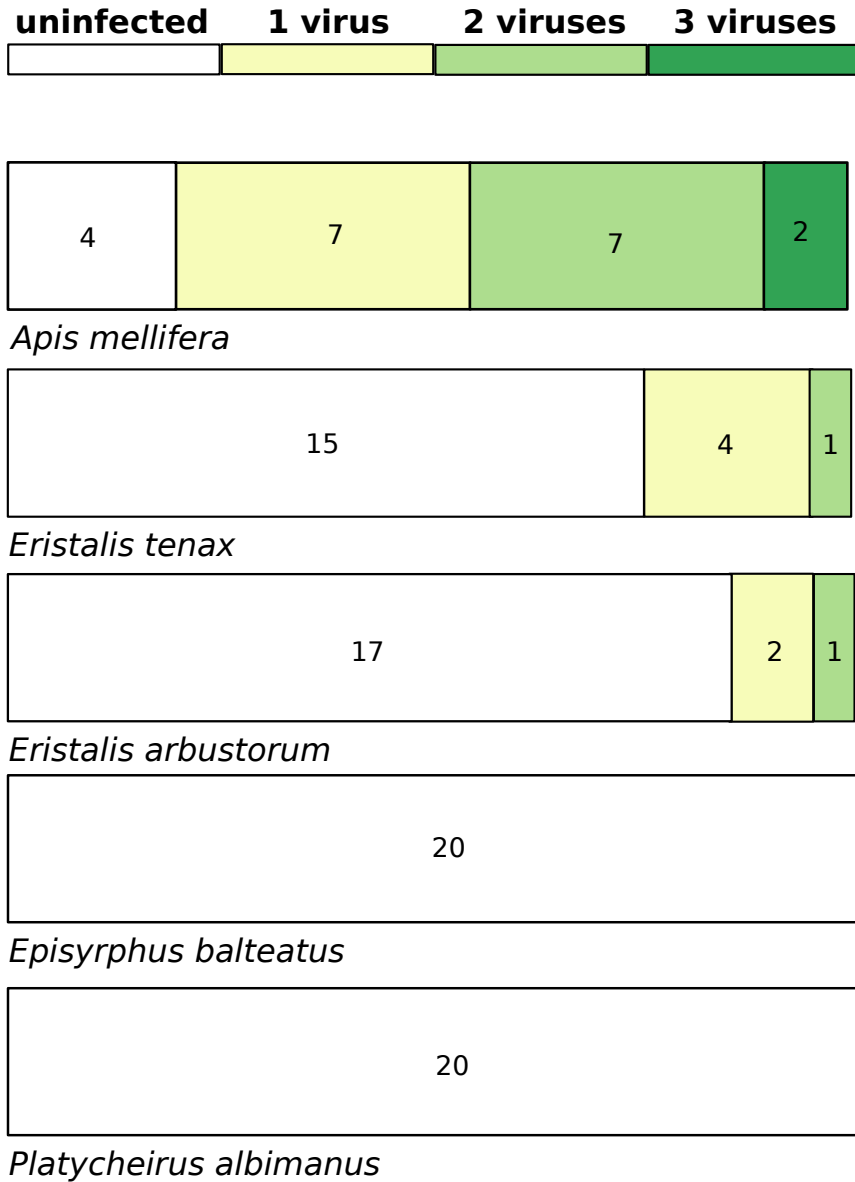
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6 **Ethical statement**

7 Work complied with local ethical requirements.

8 **Figure legends**

9 **Figure 1** - The number of viruses detected within an individual for each species. Bar width represents
0 proportion of samples, numbers on bars are number of individuals.

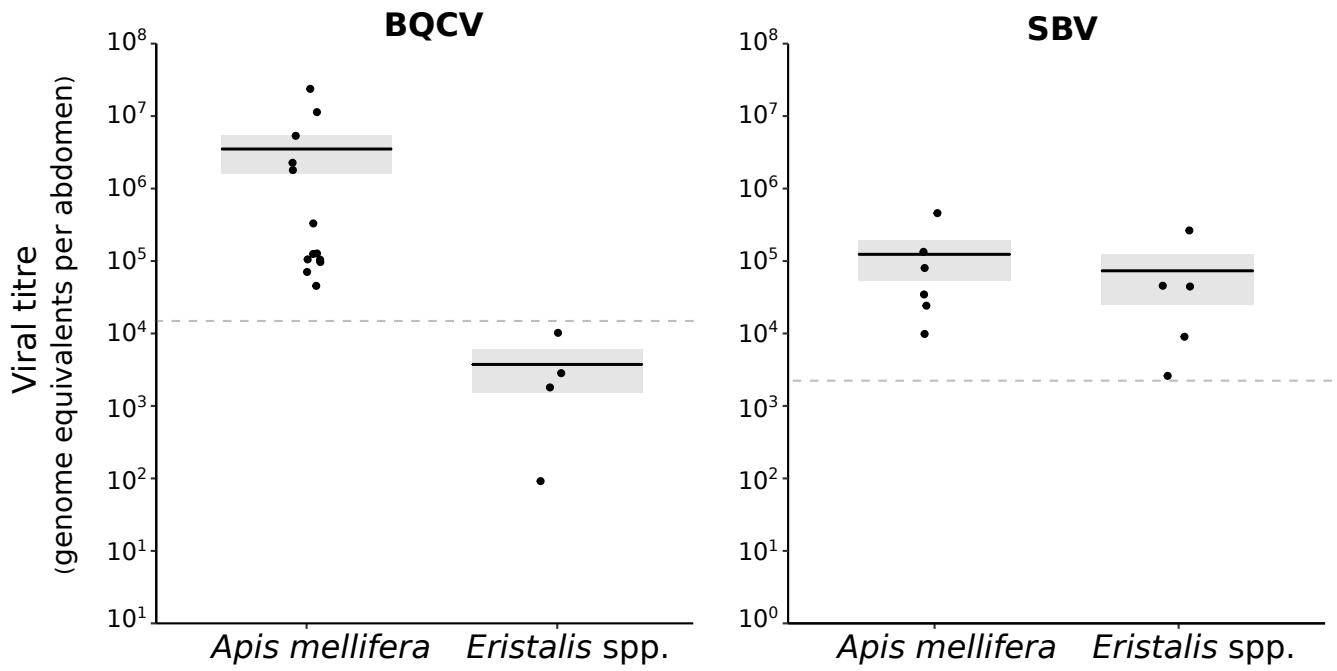


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2 **Figure 2** - The viral titres (grey boxes represent SE, black line = mean) of honey bee and hoverfly

3 abdomens. The dotted line represents the limit of the standard curve. Filled circles are individual data points.

4 (left) BQCV titres; extrapolated for hoverflies (right) SBV titres.



5

6 **Table 1** - The number of individuals for each species where virus was verified to be present by RT-PCR.

Species	BQCV	ABPV	SBV	SBPV	DWV-B	n
<i>Apis mellifera</i>	13	1	6	0	7	20
<i>Eristalis tenax</i>	2	0	4	0	0	20
<i>Eristalis arbustorum</i>	2	0	1	0	1	20
<i>Episyrphus balteatus</i>	0	0	0	0	0	20
<i>Platycheirus albimanus</i>	0	0	0	0	0	20

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8