



Recently identified bee viruses and their impact on bee pollinators

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Bees are agriculturally and ecologically important plant pollinators. Recent high annual losses of honey bee colonies, and reduced populations of native and wild bees in some geographic locations, may impact the availability of affordable food crops and the diversity and abundance of native and wild plant species. Multiple factors including viral infections affect pollinator health. The majority of well-characterized bee viruses are picorna-like RNA viruses, which may be maintained as covert infections or cause symptomatic infections or death. Next generation sequencing technologies have been utilized to identify additional bee-infecting viruses including the Lake Sinai viruses and Rhabdoviruses. In addition, sequence data is instrumental for defining specific viral strains and characterizing associated pathogenicity, such as the recent characterization of Deformed wing virus master variants (DWV-A, DWV-B, and DWV-C) and their impact on bee health.

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Introduction

Bees are important pollinators of plant species in natural and agricultural ecosystems. Recent high annual losses of honey bee colonies and reduced populations of native and wild bees involve multiple factors including pathogens, agrochemical exposure, and inadequate habitat and nutritional resources [1–8,9^{*},10,11^{*},12–14]. Although no single abiotic or biotic factor is responsible for recent bee deaths, viruses have been associated with honey bee colony losses [12,15–21] and individual mortality and morbidity in native and wild bees [22–25,26^{**},27^{*},28^{*},29,30] (reviewed

in [31^{**}]). Bee-infecting viruses are primarily positive sense single-stranded RNA viruses (+ssRNA) in the order *Picornavirales* [32,33]. Common bee viruses include: the Dicistroviruses (Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), Acute bee paralysis virus (ABPV), and Black queen cell virus (BQCV)); the Iflaviruses (Deformed wing virus (DWV), Kakugo virus, Varroa destructor virus-1/DWV-B, Sacbrood virus (SBV), and Slow bee paralysis virus (SBPV)); and taxonomically unclassified viruses (Chronic bee paralysis virus (CBPV) and the Lake Sinai viruses (LSVs)) (reviewed in [32,33]). Recently identified +ssRNA viruses include Bee macula-like virus (BeeMLV) in the *Tymoviridae* family, *Apis mellifera* flavivirus and *Apis mellifera* nora virus 1 [34,35^{**}]. The first bee-infecting negative sense single-stranded RNA viruses (–ssRNA) were also recently described, specifically *Apis mellifera* rhabdovirus-1 (ARV-1) and *Apis mellifera* rhabdovirus-2 (ARV-2) [35^{**}], also known as Bee rhabdovirus (BRV) [36]. To date, only a single honey bee-infecting double-stranded DNA virus, *Apis mellifera* filamentous virus (AmFV), has been sequenced and characterized [37–39].

Bee virus discovery has been facilitated and accelerated by next generation sequencing technologies and it is likely that additional bee-associated viruses will be discovered. Virus identification and characterization are important first steps toward understanding the role of viruses in bee health. Typically, bee viruses are defined by the organism from which they were first identified (e.g., honey bee viruses), though many bee viruses have wide host ranges and are transmitted between genera [23,25,30,40,41^{*},42,43,44^{*},45–48] (reviewed in [31^{**}]). Virus nomenclature varies; a virus may be named after the symptoms or diseases associated with infection, the host from which it was isolated, or for the geographic regions or features (e.g., mountains, rivers, and so on) near where the virus was first identified. Viruses are assigned to families based on the type and sequence of nucleic acid that makes up their genome. The International Committee on the Taxonomy of Viruses (ICTV; URL: <https://talk.ictvonline.org/taxonomy/>) is responsible for maintaining a taxonomic catalog of viruses, though the most up to date resource for virus sequences is the National Center for Biotechnology Information (NCBI) non-redundant nucleic acid data base [49]. Importantly, recent identification of a virus does not necessarily make it an emerging virus, and identification of viruses in additional hosts is not indicative of spillover from a reservoir population into a novel host population. The

directionality of virus transmission is difficult to discern, but field-based studies indicate bee viruses are transmitted from both managed bees to wild bees (e.g., DWV [41[•]]) and from wild bees to managed bees (e.g., ABPV [25,41[•]]). *Varroa destructor*, an ectoparasitic mite that commonly infests honey bee colonies and feeds on developing and adult bees, is an active vector for numerous viruses, including DWV [50–52,53[•],54[•],55,56], KBV [57], IAPV [58], and CBPV [59], and may be a passive vector or host of many more (e.g., ABPV (reviewed in [60]), BeeMLV [34], LSV [61], VDV-2 and VDV-3 [62^{••}], Moku Virus [63[•]], BQCV [64,65], and SBV [64–66]). Furthermore, mite-mediated DWV transmission has been shown to bottleneck virus populations [67] and result in enhanced replication of recombinant DWV-1/VDV-1 (DWV-B) viruses in honey bee hosts [68,69].

Several viruses discovered in honey bees replicate in other bee species, as evidenced by negative strand detection or amplification over the course of infection including ABPV [22], BQCV [48,70], DWV [24,25,42,48,71], IAPV [29,42,43,72,73], KBV [29], LSVs [44[•],74], and SBPV [73], though the pathogenesis of these viruses in native bees is underexplored. Virus transmission between sympatric pollinator species is mediated by shared floral resources, including pollen [44,71], and is evidenced by a lack of host-species-specific clustering of virus sequences in phylogenetic analyses [25,42,43]. While it is clear that bee viruses replicate within and are transmitted between several bee species, their pathogenesis may be host or virus strain dependent (reviewed in [31^{••}]) and are likely influenced by additional factors including nutritional status [75], host genetic makeup [76], host sex [77], and bee age [78]. In wild bee species viral infection may result in deformity [23], systemic infection [24], reduced reproductive success [29], and/or mortality [22,25]. Second to honey bees, the consequences of bee virus infections have been most investigated in bumble bees. DWV replicates in multiple bumble bee species including *Bombus huntii* [24], *B. impatiens* [24,42], *B. lapidarius* [25], *B. lucorum* [25], *B. monticola* [25], and *B. vagans* [42]), though symptomatic infection has only been described in *B. terrestris* [23–25] and *B. pascorum* [23] (see [31^{••}] for a comprehensive review). *Bombus terrestris* exhibits DWV-associated wing deformities and mortality [23,25]. Similarly, negative consequences of virus infection of *B. terrestris* include reduced fecundity and colony founding associated with KBV infection [29], reduced fecundity due to IAPV infection [29,72], and mortality due to ABPV infection [22]. However, the signs, symptoms, and severity of virus infection may differ across bee species. For example, one study determined that exposure to a mixed inoculum of viruses isolated from honey bees (i.e., IAPV, SBV, and DWV) resulted in lower mortality in two native bee species, *Megachile rotunda* and *Colletes inaequalis*, as compared to honey bees (*Apis mellifera*) [79^{••}]. This result may indicate specific virus–

host adaptation that may result in enhanced, unchanged, or diminished virulence, depending on the specific virus–host combination and directionality of virus transmission. Additional investigation of bee virus pathogenesis and intra-species and inter-species transmission is required to better understand the role of viruses on bee health and their impact on bee losses [25,41[•]] (reviewed in [31^{••}]).

Next generation sequencing and bee virus discovery

Pioneering historic research on bee viruses by Bailey, Ball, and others relied on conventional tools including studies documenting virus-associated disease transmission using filterable agents, electron microscopy, and antibody-mediated virus detection [32,80,81]. Virus genome discovery in bees has been greatly accelerated by next generation sequencing and new assembly tools (Table 1) [34,35^{••},62^{••},63[•],82]. Bee virus discovery efforts have primarily focused on Western honey bee (*Apis mellifera*) and bumble bee (*B. pascorum* and *B. lapidarius*) samples [44[•]], although there have been a few studies in other honey bee-associated species including identification of Moku virus (MV) from *Vespula pensylvanica* wasps which prey on honey bees in Hawaii [63[•]], and Varroa destructor virus 2 (VDV2) and VDV3 from *Varroa destructor* [62^{••}]. Furthermore, recovery of Aphid lethal paralysis virus (ALPV) and Big Sioux River virus (BSRV) genome sequence in aphids [83] and DWV in Argentine ants in New Zealand [84] indicates that ‘bee viruses’, like other insect viruses, likely infect a broad range of insects and arachnids.

The first metagenomic study of honey bees examined the entire RNA profile of honey bee samples obtained from Colony Collapse Disorder (CCD)-affected and healthy colonies [17]. Initially, high prevalence of IAPV and KBV were associated with CCD-affected colonies, but subsequent analyses and additional studies indicate that no single virus is universally associated with CCD or colony losses [15,16,20,32,81]. One of the first studies to utilize next generation (or ultra-high throughput) sequencing for virus discovery in honey bee samples identified four new honey bee associated viruses including two Dicistroviruses (i.e., Aphid-lethal paralysis virus-like virus (ALPV-like 1) and BSRV) and the unique Lake Sinai virus group, including Lake Sinai virus 1 (LSV1) and Lake Sinai virus 2 (LSV2) [82]. Recently, the LSV group has been expanded and phylogenetically resolved into four clades [49,61,82,86,87^{••},88]. The abundance of LSV1 and LSV2 RNA, which respectively peaked at approximately 7.06×10^{10} and 7.16×10^{11} genome copies per bee, and detection of the replicative form of the viral genome using strand-specific PCR, indicated that these viruses replicated in honey bees [82]. Subsequent studies in Belgium, Spain, and the US, and re-evaluation of sequencing data from CCD-affected and non-CCD affected samples, identified several additional LSVs

Table 1**Recently described bee-associated viruses.**

Virus/group	Location(s)	Genome type	Approximate genome size	Family	Associated species	Ref(s)
Aphid lethal paralysis virus	United States, Spain	+ssRNA	4.1 kb	<i>Dicistroviridae</i>	<i>Apis mellifera</i>	[30,82,86,89]
<i>Apis mellifera</i> filamentous virus	Switzerland, United States, Belgium	dsDNA	498.5 kb	Unclassified	<i>Apis mellifera</i> <i>Osmia cornuta</i> <i>Osmia bicornis</i> <i>Andrena vaga</i> <i>Andrena ventralis</i>	[30,37–39]
<i>Apis mellifera</i> bunya virus-1	South Africa	–ssRNA	6 kb	<i>Bunyaviridae</i>	<i>Apis mellifera</i>	[35**]
<i>Apis mellifera</i> bunya virus-2	South Africa	–ssRNA	6.5 kb	<i>Bunyaviridae</i>	<i>Apis mellifera</i>	[35**]
<i>Apis</i> dicistrovirus	Netherlands	+ssRNA	9.1 kb	<i>Dicistroviridae</i>	<i>Apis mellifera</i>	[35**]
<i>Apis mellifera</i> flavivirus	South Africa	+ssRNA	20.4 kb	<i>Flaviviridae</i>	<i>Apis mellifera</i>	[35**]
<i>Apis mellifera</i> nora virus-1	South Africa	+ssRNA	10 kb (partial)	Picorna-like	<i>Apis mellifera</i>	[35**]
<i>Apis mellifera</i> rhabdovirus-1/ bee rhabdovirus-1	United States, Israel, Tonga, Netherlands, South Africa	–ssRNA	14.6 kb	<i>Rhabdoviridae</i>	<i>Apis mellifera</i> <i>Bombus impatiens</i> <i>Varroa destructor</i>	[35**,36]
<i>Apis mellifera</i> rhabdovirus-2	United States, Israel, Tonga, Netherlands, South Africa	–ssRNA	14 kb	<i>Rhabdoviridae</i>	<i>Apis mellifera</i> <i>Varroa destructor</i>	[35**,36]
Big Sioux River virus	United States	+ssRNA	9.6 kb	<i>Dicistroviridae</i>	<i>Apis mellifera</i>	[82]
<i>Halictus scabiosae</i> Adlikon virus	Switzerland	+ssRNA	5.2 kb	Unclassified	<i>Halictus scabiosae</i>	[87**]
Lake Sinai viruses	United States, Europe, Australia, China	+ssRNA	5.9 kb	Unclassified	<i>Apis mellifera</i> <i>Andrena vaga</i> <i>Andrena ventralis</i> <i>Osmia bicornis</i> <i>Osmia cornuta</i> <i>Bombus</i> spp. <i>Messor</i> spp.	[30,44*,82,86,87**,88–90]
Moku virus	United States	+ssRNA	10 kb	<i>Iflaviridae</i>	<i>V. pennsylvanica</i> <i>Apis mellifera</i> <i>Varroa destructor</i>	[63*]
<i>Varroa destructor</i> macula-like virus	United States, Belgium	+ssRNA	6.5 kb	<i>Tymoviridae</i>	<i>Apis mellifera</i> <i>Varroa destructor</i> <i>Osmia cornuta</i> <i>Bombus</i> spp.	[30,34,44*,90]
<i>Varroa destructor</i> virus 2	Israel	+ssRNA	9.6 kb	<i>Iflaviridae</i>	<i>Varroa destructor</i>	[62**]
<i>Varroa destructor</i> virus 3	Israel	+ssRNA	4.2 kb	Unclassified	<i>Varroa destructor</i>	[62**]
<i>Varroa</i> tympo-like virus	United States	+ssRNA	6.2 kb	<i>Tymoviridae</i>	<i>Varroa destructor</i>	[34]

Recent development of next generation sequencing technologies has led to a rapid expansion in the number of viruses in bees and associated taxa (e.g., *Varroa* and *Vespula pennsylvanica*). This table is a list of bee-associated virus genomes that have been published in the last ten years; this list does not include new variants of previously described viruses like Deformed wing virus [110*]. Viruses of note include the first negative-sense single-stranded RNA viruses associated with bees (i.e., ABV-1 and ABV-2, ARV-1 and ABV-2) and the expanded host range of *Apis mellifera* filamentous virus, which includes several solitary bee species.

and indicated that these viruses are globally distributed, abundant, and sometimes associated with poor colony health [16,35**,61,74,89,90]. A recent study identified new viruses from Halictid bees and defined a new virus genus, *Halictivirus*, which is phylogenetically similar to LSVs in the *Sinaiivirus* genus [87**]. ALPV-like virus was also detected in metagenomic sequencing data obtained from honey bee samples from Spain [89]. The negative sense replicative intermediate forms of the APLV-like genome and of *Varroa destructor* macula-like virus (VdMLV), which was renamed Bee macula-like virus (BeeMLV), were detected using a multiplex-ligation

probe dependent amplification based method (i.e., BeeDoctor®) on RNA isolated from honey bees in Belgium [35,90]. Metagenomic sequencing of honey bee samples from Spain also identified two plant viruses (i.e., Turnip ringspot virus and Turnip yellow mosaic virus), which were likely passively associated with honey bees [89]. In contrast, detection of the negative strand of another plant virus, Tomato ringspot virus, indicated that this virus may replicate in honey bees [91].

Recently, short read high-throughput and chain termination sequencing methods were used to assemble the Bee

macula-like virus (BeeMLV) genome from poly-A augmented RNA samples obtained from *Varroa destructor* mites and honey bees [34]. BeeMLV is a polyadenylated + ssRNA virus approximately 6,500 nucleotides in length in the *Tymoviridae* family. BeeMLVs form a new species complex independent of other related viruses (i.e., Tymovirus, Marivirus, and Maculavirus) [34]. The US and European strains of BeeMLV are >70% identical at the nucleotide level and distinct from the related *Varroa* tympo-like virus (VTLV), which was discovered in *Varroa* samples [34]. In addition to the US and France, BeeMLV has been detected in bee and mite samples from Belgium, but was not detected in samples obtained from Sweden, Norway, or the French territory Isle d'Ouessant [34]. Peak BeeMLV prevalence in French apiaries occurred in autumn and coincided with peak mite abundance, although levels of virus abundance in bees and mites were not correlated [34]. Greater relative abundance of BeeMLV subgenomic RNA relative to genomic RNA in honey bee samples is indicative of active viral infection. However, since the ratio of subgenomic to genomic RNA in mites was equivalent to bees in the same colony, BeeMLV in mite samples may be due to virus uptake during mite-feeding, rather than virus replication [34].

Sequences from seven new honey bee-associated viruses were identified in a recent study by Remnant *et al.*, that utilized RNA-sequencing to examine viral diversity in honey bees obtained from colonies that were either bred for or naturally evolved the trait of mite resistance [35^{••}]. Sequencing libraries generated from ribosomal RNA depleted honey bee RNA samples from the Netherlands, South Africa, and Tonga resulted in the identification of the first –ssRNA viruses in both bees and mites. These new viruses are in the family *Rhabdoviridae*, which comprises enveloped –ssRNA viruses that infect a broad range of species, including many arthropods [92]. Sequences derived from *Apis mellifera* rhabdovirus-1 (ARV-1), and *Apis mellifera* rhabdovirus-2 (ARV-2), which are phylogenetically closest to Farmington virus based on 30% aa identity of the RNA-dependent RNA polymerase (RdRp), were detected in all geographic locations. Subsequently, Bee rhabdovirus (BRV) sequences, which shared over 99% homology to ARV-1, were identified in honey bee and bumble bee (i.e., *B. impatiens*) samples from the US, as well as honey bee and *Varroa* samples from Israel [36]. Due to the expanded host range of this virus, Levin *et al.* proposed to rename ARV-1 to BRV-1 [36].

Additional –ssRNA virus the Bunyavirus family (i.e., *Apis mellifera* bunyavirus-1 (ABV-1) and *Apis mellifera* bunyavirus-2 (ABV-2)) were discovered in bee samples obtained from South Africa, but only the largest genome segment, which includes the RdRp, was sequenced [35^{••}]. These viruses may actively infect bees or the

bee-infecting trypanosomatid species, *Crithidia mellifica* and/or *Lotmaria passim* [93,94], since ABV-1 is most similar to Leishbunyavirus (LBV1), which was isolated from the insect trypanosomatid parasite (i.e., *Leptomonas moramango*) [95].

Three additional +ssRNA viruses were identified including the first bee-associated flavivirus, *Apis mellifera* flavivirus (AFV), from a sample obtained from one colony located in South Africa. AFV has a 20,414 nucleotide + ssRNA genome that contains a single open reading frame (ORF) of 6,615 amino acids [35^{••}]. The sequence of the first nora virus, *Apis mellifera* nora virus 1 (ANV-1), was also identified in a bee sample obtained from South Africa. To obtain the full genome of ANV-1, the RNA-Seq-derived contigs were aligned with the *Drosophila pseudoobscura* nora virus. Chain termination sequencing and RT-PCR were utilized to obtain the partial 10,091 nucleotide sequence, including the entire replicase-encoding gene, but not the first ORF [35^{••}]. Finally, *Apis mellifera* dicistrovirus (ADV), isolated from a honey bee sample from the Netherlands, adds to the growing list of identified honey bee dicistroviruses [35^{••}].

Honey bee antiviral defense mechanisms include RNA interference (RNAi) [96–103]. Therefore several recent sequencing efforts have assessed the small inhibitory RNA (siRNA) profiles of naturally and experimentally virus-infected bees and identified the signature 21–22 nucleotide siRNAs produced by Dicer cleavage [35^{••},69,98,104]. Likewise, the siRNA profile of ARV-1 and ARV-2 infected honey bees determined that ARV-1 and ARV-2 siRNAs had characteristics of Dicer processed small RNAs (e.g., 2-nucleotide overhang, 21–22 nucleotides long, and phased from ends of the genome [35^{••}]). Detection of Dicer-processed siRNAs indicates ARV-1 and ARV-2 actively infect honey bees and implicates the involvement of RNAi in honey bee antiviral defense against ARV infections [35^{••}].

Together, these studies illustrate that data from short-read libraries may be used to identify new virus sequences and indicate that many more await discovery, particularly since most studies used similar methods and focused on RNA viruses. Verification of sequence data using longer read methods (e.g., PacBio sequencing) and more accurate chain-termination sequencing is typically carried out to ensure that complete viral genomes are properly assembled and annotated [82,105]. In addition, detection of the replicative intermediate forms of the virus genome by strand-specific amplification (e.g., negative-strand specific tagged PCR), *in situ* hybridization, and/or northern blot analysis provides additional evidence that a recently identified virus is infectious to the host from which it was obtained [44[•],82,106,107]. Complete genome sequences facilitate phylogenetic analyses of these viruses, but more commonly such analyses are performed using nucleotide

and amino acid sequencing of key viral proteins, such as the RdRp and capsid proteins. The outcome of phylogenetic reconstruction may differ depending on the genome regions utilized in the analysis and with the availability of related sequences in the databases (e.g., Lake Sinai virus [35[•],44[•],61,74] and Bee Macula-like virus [34]).

Virus quasispecies

RNA viruses encode and rely on error-prone RdRp for genome replication, resulting in a mutation rate nearly a million-fold higher than eukaryotic host polymerases [108]. This generates a population of related viruses of high variation around one or more ‘master genotypes’ — known as a quasi-species swarm (reviewed in [108,109]). The degree of nucleotide identity that defines a new viral variant or strain varies by virus, and is not clearly defined for many honey bee viruses. For example, Lake Sinai virus sequences in the NCBI database range in sequence identity from 69 to 99% at the nucleotide level [61], whereas proposed DWV master variants range from 79 to 84% identity at the nucleotide level, with up to 98.2% identity among sequences identified as DWV-A [110[•]]. A master variant or master type is the genotype with maximal fitness around which the quasispecies explores sequence space (reviewed in [109]). Recent phylogenetic analysis has suggested the possible existence of three DWV master variants [110[•]].

The Deformed wing virus cluster

Deformed wing virus (DWV) is a picorna-like virus with an approximately 10 kb +ssRNA genome encapsidated by a 30 nm diameter icosahedral capsid (reviewed in [111]). DWV negatively impacts honey bee health and is a major correlate to colony failure, particularly in association with *Varroa destructor* [18,51,53[•],54[•],55,85,112]. *Varroa*-mediated transmission of DWV and mite infestation of DWV-infected honey bee colonies augments DWV abundance and DWV-associated deformities and death [51,54[•],55,56,65,67,113]. Mite-mediated transmission of DWV may also exert a selective bottleneck on DWV at the individual [68,69] and landscape levels [67], perhaps partially explaining the association between *Varroa*-vectored DWV and poor colony health and colony loss [19,67,114]. The observation that Australia, which lacks DWV and *Varroa*, has not reported elevated colony losses supports the hypothesis that the synergistic effect of DWV and *Varroa* drive colony loss [51,54[•],115]. However, several studies indicated that the association of DWV with overwintering colony mortality is sometimes independent of *Varroa* levels [18,19,112,114].

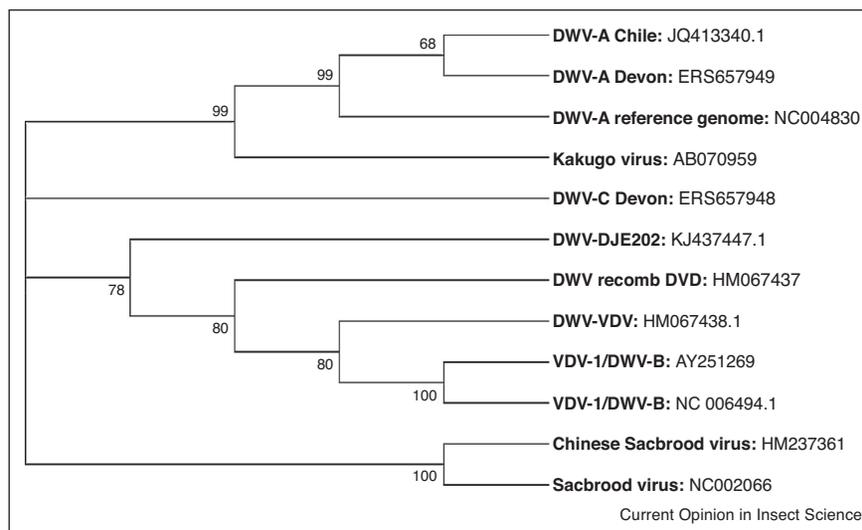
In 2004, *Varroa destructor* virus 1 (VDV1), which shares 84% nucleotide identity with DWV, was isolated from *Varroa* mites [52]. Subsequently, DWV-VDV recombinants were identified as the predominant viral strains in *Varroa*-infested honey bees in the UK [68,69]. These recombinant viruses have since been detected in

geographically widespread regions [116,117]. It has been proposed to designate VDV-1 as DWV ‘master variant’ B (VDV-1 Accession: AY251269) [67,110[•]] and the reference sequence as DWV-A (Accession: NC_004830). In concurrence with this, Mordecai and colleagues recently described DWV-C (Accession: ERS657948) as a third ‘master variant’ of DWV [110[•]]. DWV-A and DWV-B are 84.4% identical [52], and DWV-C shares 79.8% and 79.5% nucleotide identity with DWV-A and DWV-B, respectively (Figure 1) [110[•],118,119–121]. A quantitative polymerase chain reaction assay that distinguishes DWV variants will facilitate future investigation of their relative impacts on bee health [122].

There are some mixed data in the literature concerning the relative virulence of DWV-A and DWV-B. The 2007 introduction and subsequent spread of *Varroa* into honey bee populations on the Hawaiian archipelago increased DWV prevalence and genome copy number by one million fold, and reduced DWV diversity, as indicated by fewer unique RdRp sequences [67]. More recently, Mordecai *et al.* reported that bees resistant to *Varroa* were predominantly infected by DWV-B and exhibited low prevalence and abundance of DWV-A [123]. Since these DWV-B infected colonies were qualitatively assessed to be resistant to *Varroa*, the authors suggest that DWV-B is avirulent and that low DWV-A prevalence was due to superinfection exclusion by DWV-B [123]. However, observed resistance in this study population could also be due to selective breeding for resistance traits in the honey bee [123], while the discrepancies in DWV variant predominance between the Hawaiian and the UK study populations may be due to differences in genetic background of host, pathogen, or parasite. Alternatively, resistance to DWV-A infection could be due to the fact that the populations examined in the latter study had more extensive histories of association with *Varroa* [67,123].

At the colony level, both DWV-B and DWV-A are associated with significant overwintering colony mortality [18,19,112,114,124,125]. In a recent UK-based study, DWV-B genome equivalents and prevalence over time positively correlated with colony mortality, whereas DWV-A was not detected [114]. However, the explanatory power of their statistical models suggest the association between DWV-B and *Varroa* may be more critical for honey bee health than either alone [18,51,54[•],55,112,113,124,125]. This is important because it restates the difficulty of disentangling the close association of DWV with *Varroa* and their contribution to individual bee and colony mortality [19,51,54[•],112,126]. Additionally, while injection of adult bees with DWV-A resulted in greater mortality than that observed in the mock-infected control group, injection with DWV-B or a mixture of the two resulted in higher genome copies and greater mortality as compared to bees infected with

Figure 1



Deformed wing virus phylogenetic relationship inferred from whole genome nucleotide sequence. Maximum Likelihood bootstrap supported consensus tree derived from Maximum Likelihood analysis in Mega v7.0.26 [111] using a whole genome nucleic acid MUSCLE alignment generated in Mega using a neighbor joining clustering method (max iterations = 30, gap open penalty = -400, gap extension penalty = 0) [112]. There were a total of 11,045 positions (nucleotides and gaps) in the final dataset. A mixed-model approach implemented in Mega identified a General Time Reversible model with a discrete Gamma distribution of evolutionary rates among sites (5 categories (+G, parameter = 1.1861)) to be the best fit for these data [110]. The initial tree was obtained using Maximum Parsimony with Subtree-Pruning-Regrafting (SPR level 5) and a heuristic search involving 1000 random addition replicates to determine the optimal tree topology. Maximum Likelihood bootstrap support values (1000 replicates) are reported next to the branches [113]. The bootstrap consensus tree (topology of more than 50% of trees) was visualized and partially edited using Mega v7.0.26 [111]. Sacbrood virus was selected as the out-group since it is a closely related member of the *Iflaviridae* family. Genbank Accession numbers for whole genome sequences are as follows: DWV-A Chile (JQ413340.1), DWV-A Devon (ERS657949), DWV-A reference genome (NC_004830), Kakugo virus (AB070959), DW-C Devon (ERS657948), DWV-DJE202 (KJ437447.1), DWV recombinant DVD (HM067437), DWV-VDV recombinant (HM067438.1), VDV-1/DWV-B (AY251269 and NC_006494.1), Chinese Sacbrood virus (HM237361), Sacbrood virus (NC_002066).

DWV-A alone [26**]. Therefore, laboratory data suggest DWV-B may be more virulent than DWV-A. However, these experiments need to be replicated in bees with different genetic backgrounds from distinct geographical locations to test the generalizability of this conclusion. The development of infectious molecular clones of these viruses will greatly facilitate the study of their relative virulence and fitness. Indeed, an infectious clone of a DWV-A isolate was recently constructed and produced clinical infection when 5×10^6 genome equivalents were injected into pupae [127**]. This infectious molecular clone will facilitate competition assays [26**] and tagged infectious clone experiments required to assess the relative fitness and pathogenesis of these variants [109]. Further development of additional molecular clones will rapidly expand our repertoire of tools that can be used to understand bee-infecting viruses.

Conclusion

Viruses contribute to bee deaths, although their relative role is often difficult to discern among several confounding variables [26**,126,128]. Continued and invigorated efforts to quantitatively track known viruses and discover new virus genomes using next generation sequencing will further our understanding of the role of viruses on bee

health and may facilitate our response to emerging and/or recently identified pathogens [129].

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Arbetman MP, Gleiser G, Morales CL, Williams P, Aizen MA: **Global decline of bumblebees is phylogenetically structured and inversely related to species range size and pathogen incidence.** *Proc R Soc B* 2017, **284**:20170204.
2. Biesmeijer JC, Roberts SP, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas CD *et al.*:

- Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands.** *Science* 2006, **313**:351-354.
3. Burkle LA, Marlin JC, Knight TM: **Plant-pollinator interactions over 120 years: loss of species, co-occurrence and function.** *Science* 2013, **339**:1611-1616.
 4. Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL: **Patterns of widespread decline in North American bumble bees.** *Proc Natl Acad Sci* 2011, **108**:662-667.
 5. Graystock P, Yates K, Darvill B, Goulson D, Hughes WOH: **Emerging dangers: deadly effects of an emergent parasite in a new pollinator host.** *J Invertebr Pathol* 2013, **114**:114-119.
 6. Graystock P, Goulson D, Hughes WOH: **The relationship between managed bees and the prevalence of parasites in bumblebees.** *Peer J* 2014, **2**:e522.
 7. Goulson D, Hanley ME, Darvill B, Ellis JS: **Biotope associations and the decline of bumblebees (*Bombus* spp.).** *J Insect Conserv* 2006, **10**:95-103.
 8. Goulson D, Lye GC, Darvill B: **Decline and conservation of bumble bees.** *Annu Rev Entomol* 2008, **53**:191-208.
 9. Goulson D, Nicholls E, Botías C, Rotheray EL: **Bee declines driven by combined stress from parasites, pesticides, and lack of flowers.** *Science* 2015, **347**:1255-1259.
- This is a concise, pointed review of the factors contributing to honey bee colony losses and wild pollinator declines globally, which includes recommendations to mitigate these threats.
10. Potts SG, Biesmeijer K, Bommarco R, Breeze TD, Carvalho L, Franzén M, González-Varo JP, Holzschuh A, Kleijn D, Klein A *et al.*: **Status and Trends of European Pollinators. Key Findings of the STEP Project.** Pensoft Publishers; 2015.
 11. Woodcock BA, Bullock JM, Shore RF, Heard MS, Pereira MG, Redhead J, Riddling L, Dean H, Sleep D, Henrys P *et al.*: **Country-specific effects of neonicotinoid pesticides on honey bees and wild bees.** *Science* 2017, **356**:1393-1395.
- In this study the authors use a field study with a randomized block design to test the effect of field-realistic applications of common pesticides in oil seed rape on introduced honey bees, bumble bees, and solitary bees. Their results show that while neonicotinoid pesticides may negatively affect pollinator populations by reducing reproductive output (Hungary and the UK), the extent of their actual impact is context dependent.
12. Evans JD, Schwarz RS: **Bees brought to their knees: microbes affecting honey bee health.** *Trends Microbiol* 2011, **19**:614-620.
 13. McMenamin AJ, Brutscher LM, Glenn W, Flenniken ML: **Abiotic and biotic factors affecting the replication and pathogenicity of bee viruses.** *Curr Opin Insect Sci* 2016, **16**:14-21.
 14. McMenamin AJ, Genersch E: **Honey bee colony losses and associated viruses.** *Curr Opin Insect Sci* 2015, **8**:121-129.
 15. Barron AB: **Death of the bee hive: understanding the failure of an insect society.** *Curr Opin Insect Sci* 2015, **10**:45-50.
 16. Cornman RS, Tarpy DR, Chen Y, Jeffreys L, Lopez DJ, Pettis JS, vanEngelsdorp D, Evans JD: **Pathogen webs in collapsing honey bee colonies.** *PLoS One* 2012, **7**:e43562.
 17. Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, Quan P, Briese T, Hornig M, Geiser DM *et al.*: **A metagenomic survey of microbes in honey bee colony collapse disorder.** *Science* 2007, **318**:283-287.
 18. Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, Berg S, Ritter W, Mühlen W, Gisder S *et al.*: **The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies.** *Apidologie* 2010, **41**:332-352.
 19. Highfield AC, Nagar AEI, Mackinder LCM, Noël LMJ, Hall MJ, Martin SJ, Schroeder DC: **Deformed wing virus implicated in overwintering honeybee colony losses.** *Appl Environ Microbiol* 2009, **75**:7212-7220.
 20. Hou C, Rivkin H, Slabezki Y, Chejanovsky N: **Dynamics of the presence of Israeli acute paralysis virus in honey bee colonies with colony collapse disorder.** *Viruses* 2014, **6**:2012-2027.
 21. vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, Frazier M, Frazier J, Cox-Foster D, Chen Y *et al.*: **Colony collapse disorder: a descriptive study.** *PLoS One* 2009, **4**:e6481.
 22. Bailey L, Gibbs AJ: **Acute infection of bees with paralysis virus.** *J Insect Pathol* 1964, **6**:395-407.
 23. Genersch E, Yue C, Fries I, de Miranda JR: **Detection of Deformed wing virus, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities.** *J Invertebr Pathol* 2006, **91**:61-63.
 24. Li J, Peng W, Wu J, Strange JP, Boncristiani H, Chen Y: **Cross-species infection of Deformed wing virus poses a new threat to pollinator conservation.** *J Econ Entomol* 2011, **104**:732-739.
 25. Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJF: **Disease associations between honeybees and bumblebees as a threat to wild pollinators.** *Nature* 2014, **506**:364-366.
 26. McMahon DP, Natsopoulos ME, Doublet V, Fürst MA, Weging S, Brown MJF, Gogol-Döring A, Paxton RJ: **Elevated virulence of an emerging viral genotype as a driver of honeybee loss.** *Proc R Soc B* 2016, **283**:20160811.
- Injection of DWV-A, DWV-B, or a mixed inoculum into adult bees shows that DWV-B is more virulent in adult bees from UK colonies under laboratory conditions. Extensive field surveys show that DWV-B is at a higher prevalence than DWV-A in the UK but also that coinfections are not uncommon and both are widespread. Computer simulations using laboratory data suggest that DWV-B would result in the death of colonies one year earlier than DWV-A alone.
27. Graystock P, Meeus I, Smaghe G, Goulson D, Hughes WOH: **The effects of single and mixed infections of *Apicystis bombi* and Deformed wing virus in *Bombus terrestris*.** *Parasitology* 2016, **143**:358-365.
- This study finds that experimental infection of *Bombus terrestris* with *Apicystis bombi* and Deformed wing virus results in 22% and 50% mortality, respectively, by 15 days after exposure. However, coinfection resulted in 86% mortality by 15 days after exposure. This study is one of the first to experimentally test the effect of coinfection of two widely spread parasites on bumble bee physiology and mortality.
28. Manley R, Boots M, Wilfert L: **Condition-dependent virulence of Slow bee paralysis virus in *Bombus terrestris*: are the impacts of honeybee viruses in wild pollinators underestimated?** *Oecologia* 2017, **184**:305-315.
- In this paper, the authors show that under starvation conditions Slow bee paralysis virus infected bumble bees die faster than uninfected bees as compared to infected bees that are satiated. These results suggest that laboratory assays which provide food *ad libitum* may underestimate the impact of viral infection on wild pollinators since field conditions can often present nutritional stress.
29. Meeus I, de Miranda JR, de Graaf DC, Wäckers F, Smaghe G: **Effect of oral infection with Kashmir bee virus and Israeli acute paralysis virus on bumblebee (*Bombus terrestris*) reproductive success.** *J Invertebr Pathol* 2014, **121**:64-69.
 30. Ravoet J, De Smet L, Meeus I, Smaghe G, Wenseleers T, de Graaf DC: **Widespread occurrence of honey bee pathogens in solitary bees.** *J Invertebr Pathol* 2014, **122**:55-58.
 31. Tehel A, Brown MJF, Paxton RJ: **Impact of managed honey bee viruses on wild bees.** *Curr Opin Virol* 2016, **19**:16-22.
- This is a comprehensive review of the impact of viruses, which were discovered in honey bees, on wild bee species. This review includes an extensive table summarizing bee virus infection.
32. Chen YP, Siede R: **Honey bee viruses.** *Adv Virus Res* 2007, **70**:33-80.
 33. Brutscher LM, McMenamin AJ, Flenniken ML: **The buzz about honey bee viruses.** *PLoS Pathog* 2016, **12**:e1005757.
 34. de Miranda JR, Cornman RS, Evans JD, Semberg E, Haddad NJ, Neumann P, Gauthier L: **Genome characterization, prevalence and distribution of a Macula-like virus from *Apis mellifera* and *Varroa destructor*.** *Viruses* 2015, **7**:3586-3602.
 35. Remnant EJ, Shi M, Buchmann G, Blacquière T, Holmes EC, Beekman M, Ashe A: **A diverse range of novel RNA viruses in geographically distinct honey bee populations.** *J Virol* 2017, **91**:e00158-e217.

Next generation sequencing was utilized to identify seven novel viruses, including the first negative-sense single-stranded RNA viruses in honey bees, obtained from managed colonies located in the Netherlands, South Africa, and the Kingdom of Tonga that were infested by *Varroa* but showed no negative consequences of infestation.

36. Levin S, Galbraith D, Sela N, Erez T, Grozinger C, Chejanovsky N: **Presence of Apis rhabdovirus-1 in populations of pollinators and their parasites from two continents.** *Front Microbiol* 2017, **8**:2482.
 37. Gauthier L, Cornman S, Hartmann U, Cousserans F, Evans JD, de Miranda JR, Neumann P: **The Apis mellifera filamentous virus genome.** *Viruses* 2015, **7**:3798-3815.
 38. Hou C, Li B, Deng S, Chu Y, Diao Q: **Diagnosis and distribution of the Apis mellifera filamentous virus (AmFV) in honey bees (Apis mellifera) in China.** *Insectes Soc* 2017, **64**:597-603.
 39. Hartmann U, Forsgren E, Charrière J-D, Neumann P, Gauthier L: **Dynamics of Apis mellifera filamentous virus (AmFV) infections in honey bees and relationships with other parasites.** *Viruses* 2015, **7**:2654-2667.
 40. Graystock P, Yates K, Evison SE, Darvill B, Goulson D, Hughes WOH: **The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies.** *J Appl Ecol* 2013, **50**:1207-1215.
 41. McMahon DP, Fürst MA, Caspar J, Theodorou P, Brown MJF, Paxton RJ: **A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees.** *J Anim Ecol* 2015, **84**:615-624.
- This is an extensive survey of known bee-infecting viruses in bumble bee species across the UK. Mixed models of their prevalence data for several viruses indicates that while DWV and BQCV are more associated with honey bees while ABPV and SBPV are more associated with bumble bee species. These data highlight the complex ecology of bee-infecting viruses.
42. Levitt AL, Singh R, Cox-Foster DL, Rajotte E, Hoover K, Ostiguy N, Holmes EC: **Cross-species transmission of honey bee viruses in associated arthropods.** *Virus Res* 2013, **176**:232-240.
 43. Singh R, Levitt AL, Rajotte EG, Holmes EC, Ostiguy N, vanEngelsdorp D, Lipkin WI, DePamphilis CW, Toth AL, Cox-Foster DL: **RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-Apis hymenopteran species.** *PLoS One* 2010, **5**:e14357.
 44. Parmentier L, Smaghe G, de Graaf DC, Meeus I: **Varroa destructor Macula-like virus, Lake Sinai virus and other new RNA viruses in wild bumblebee hosts (Bombus pascuorum, Bombus lapidarius and Bombus pratorum).** *J Invertebr Pathol* 2016, **134**:6-11.
- This is the first report of VdMLV and LSVs in European bumble bees and the first evidence by detection of the negative strand that LSVs establish infections in bumble bee species. This paper adds another dimension to the ecology and evolution for LSVs, a globally distributed virus group.
45. Guzman-Novoa E, Hamiduzzaman M, Anguiano-Baez R, Correa-Benitez A, Castañeda-Cervantes E, Arnold NI: **First detection of honey bee viruses in stingless bees in North America.** *J Apic Res* 2016, **54**:93-95.
 46. Ueira-Veira C, Almeida LO, de Almeida FC, Amaral IMR, Brandeburgo MAM, Bonetti AM: **Scientific note on the first molecular detection of the acute bee paralysis virus in Brazilian stingless bees.** *Apidologie* 2015, **46**:628-630.
 47. Lucia M, Reynaldi FJ, Sguazza GH, Abrahamovich AH: **First detection of deformed wing virus in Xylocopa augusti larvae (Hymenoptera: Apidae) in Argentina.** *J Apic Res* 2014, **53**:466-468.
 48. Radzevičiūtė R, Theodorou P, Husemann M, Japoshvili G, Kirkitadze G, Zhusupbaeva A, Paxton RJ: **Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan.** *J Invertebr Pathol* 2017, **146**:14-23.
 49. NCBI Research Coordinators: **Database resources of the National Center for Biotechnology Information.** *Nucleic Acids Res* 2016, **44**:D7-D19.
 50. Bowen-Walker P, Martin S, Gunn A: **The transmission of Deformed wing virus between honeybees (Apis mellifera L.) by the ectoparasitic mite Varroa jacobsoni Oud.** *J Invertebr Pathol* 1999, **73**:101-106.
 51. Nazzi F, Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, Della Vedova G, Cattonaro F, Caprio E, Pennacchio F: **Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies.** *PLoS Pathog* 2012, **8**:e1002735.
 52. Ongus JR, Peters D, Bonmatin J, Bengsch E, Vlak JM, van Oers MM: **Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite Varroa destructor.** *J Gen Virol* 2004, **85**:3747-3755.
 53. Wilfert L, Long G, Legget HC, Schmid-Hempel P, Butlin R, Martin SJ, Boots M: **Deformed wing virus is a recent global epidemic in honeybees driven by Varroa mites.** *Science* 2016, **351**:594-597.
- This paper presents a thorough phylogenetic analysis of global DWV isolates and shows the ecology of this virus is primarily shaped by honey bees and the parasitic mite *Varroa destructor*.
54. Di Prisco G, Annoscia D, Margiotta M, Ferrara R, Varricchio P, Zanni V, Caprio E, Nazzi F, Pennacchio F: **A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health.** *Proc Natl Acad Sci* 2016, **113**:3203-3208.
- Using several measures of immune stimulation this paper demonstrates that higher DWV copy numbers are associated with reduced immunocompetence in 5th instar honey bee larvae and increased fitness of feeding *Varroa* mites. These data suggest a mutualism between DWV and *Varroa*.
55. Gisder S, Aumeier P, Genersch E: **Deformed wing virus: replication and viral load in mites (Varroa destructor).** *J Gen Virol* 2009, **90**:463-467.
 56. Yue C, Genersch E: **RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor).** *J Gen Virol* 2005, **86**:3419-3424.
 57. Chen Y, Pettis JS, Evans JD, Kramer M, Feldlaufer M: **Transmission of Kashmir bee virus by the ectoparasitic mite Varroa destructor.** *Apidologie* 2004, **35**:441-448.
 58. Di Prisco G, Pennacchio F, Caprio E, Boncristiani HF, Evans JD, Chen Y: **Varroa destructor is an effective vector of Israeli acute paralysis virus in the honeybee, Apis mellifera.** *J Gen Virol* 2011, **92**:151-155.
 59. Celle O, Blanchard P, Olivier V, Schurr F, Cougoule N, Faucon JP, Ribière M: **Detection of Chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread.** *Virus Res* 2008, **133**:280-284.
 60. de Miranda JR, Cordoni G, Budge G: **The Acute bee paralysis virus-Kashmir bee virus-Israeli acute paralysis virus complex.** *J Invertebr Pathol* 2010, **103**:S30-S47.
 61. Daughenbaugh KF, Martin M, Brutscher LM, Cavigli I, Garcia E, Lavin M, Flenniken ML: **Honey bee infecting Lake Sinai viruses.** *Viruses* 2015, **7**:3285-3309.
 62. Levin S, Sela N, Chejanovsky N: **Two novel viruses associated with the Apis mellifera pathogenic mite Varroa destructor.** *Sci Rep* 2016, **6**:37710.
- Using next generation sequencing of total RNA and virus-enriched RNA from honey bees and *Varroa* mites identified two novel viruses isolated from *Varroa*. To the best of our knowledge this is the first use of next generation sequencing to identify novel viruses associated with this parasitic mite of honey bees.
63. Mordecai GJ, Brettell LE, Pachori P, Villalobos EM, Martin SJ, Jones IM, Schroeder DC: **Moku virus; a new Iflavirus found in wasps, honey bees and Varroa.** *Sci Rep* 2016, **6**:34983.
- Next generation sequencing of poly-A enriched RNA pools was used to identify Moku virus, a novel virus found in *Vespaula pensylvanica* a social wasp and predator of honey bees. Partial genome sequence was recovered from honey bees and *Varroa*. This is the first potential evidence for a wasp-associated virus that may also infect bees, though this remains to be definitively shown.
64. Chantawannakul P, Ward L, Boonham N, Brown M: **A scientific note on the detection of honeybee viruses using real-time PCR**

- (TaqMan) in Varroa mites collected from a Thai honeybee (*Apis mellifera*) apiary.** *J Invertebr Pathol* 2006, **91**:69-73.
65. Mondet F, de Miranda JR, Kretzschmar A, Le Conte Y, Mercer AR: **On the front line: quantitative virus dynamics in honeybee (*Apis mellifera* L.) colonies along a new expansion front of the parasite *Varroa destructor*.** *PLoS Pathog* 2014, **10**:e1004323.
66. Shen M, Cui L, Ostiguy N, Cox-Foster D: **Intricate transmission routes and interactions between picorna-like viruses (Kashmir bee virus and Sacbrood virus) with the honeybee host and the parasitic *Varroa* mite.** *J Gen Virol* 2005, **86**:2281-2289.
67. Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M, Nikaido S, Schroeder DC: **Global honey bee viral landscape altered by a parasitic mite.** *Science* 2012, **336**:1304-1306.
68. Moore J, Jironkin A, Chandler D, Burroughs N, Evans DJ, Ryabov EV: **Recombinants between Deformed wing virus and *Varroa destructor* virus-1 may prevail in *Varroa destructor*-infested honeybee colonies.** *J Gen Virol* 2011, **92**:156-161.
69. Ryabov EV, Wood GR, Fannon JM, Moore JD, Bull JC, Chandler D, Mead A, Burroughs N, Evans DJ: **A virulent strain of Deformed wing virus (DWV) of honeybees (*Apis mellifera*) prevails after *Varroa destructor*-mediated, or *in vitro*, transmission.** *PLoS Pathog* 2014, **10**:e1004230.
70. Peng W, Li J, Boncristiani H, Strange JP, Hamilton M, Chen Y: **Host range expansion of honey bee Black Queen Cell Virus in the bumble bee, *Bombus huntii*.** *Apidologie* 2011, **42**:650-658.
71. Mazzei M, Carrozza ML, Luisi E, Forzan M, Giusti M, Sagona S, Tolari F, Felicioli A: **Infectivity of DWV associated to flower pollen: experimental evidence of a horizontal transmission route.** *PLoS One* 2014, **9**:e113448.
72. Piot N, Snoeck S, Vanlede M, Smaghe G, Meeus I: **The effect of oral administration of dsRNA on viral replication and mortality in *Bombus terrestris*.** *Viruses* 2015, **7**:3172-3185.
73. Niu J, Smaghe G, De Coninck DIM, Van Nieuwerburgh F, Deforce D, Meeus I: ***In vivo* study of Dicer-2-mediated immune response of the small interfering RNA pathway upon systemic infections of virulent and avirulent viruses in *Bombus terrestris*.** *Insect Biochem Mol Biol* 2016, **70**:127-137.
74. Ravoet J, De Smet L, Wenseleers T, de Graaf DC: **Genome sequence heterogeneity of Lake Sinai virus found in honey bees and Orf1/RdRP-based polymorphisms in a single host.** *Virus Res* 2015, **201**:67-72.
75. DeGrandi-Hoffman G, Chen Y, Huang E, Huang MH: **The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.).** *J Insect Physiol* 2010, **56**:1184-1191.
76. Delaplane KS, Pietravalle S, Brown MA, Budge GE: **Honey bee colonies headed by hyperpolyandrous queens have improved brood rearing efficiency and lower infestation rates of parasitic *Varroa* mites.** *PLoS One* 2015, **10**:e0142985.
77. Retschnig G, Williams GR, Mehlmann MM, Yañez O, de Miranda JR, Neumann P: **Sex-specific differences in pathogen susceptibility in honey bees (*Apis mellifera*).** *PLoS One* 2014, **9**:e85261.
78. Bull JC, Ryabov EV, Prince G, Mead A, Zhang C, Baxter LA, Pell JK, Osborne JL, Chandler D: **A strong immune response in young adult honeybees masks their increased susceptibility to infection compared to older bees.** *PLoS Pathog* 2012, **8**:11-14.
79. Dolezal AG, Hendrix SD, Scavo NA, Carrillo-tripp J, Harris A, ●● Wheelock MJ, Neal MEO, Toth AL: **Honey bee viruses in wild bees: viral prevalence, loads, and experimental inoculation.** *PLoS One* 2016, **11**:e0166190.
- Experimental exposure of solitary bee species (i.e., *Megachile rotunda* and *Colletes inaequalis*) to viruses isolated from honey bees demonstrated less mortality in solitary bees, as compared to honey bees. This paper highlights the importance of understanding the effect these viruses have on particular host species.
80. Bailey L: **Viruses attacking the honey bee.** *Adv Virus Res* 1976, **20**:271-304.
81. Bailey L, Ball BV: *Honey Bee Pathology*. Elsevier Ltd; 1991.
82. Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R, DeRisi JL: **Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, *Nosema*, and *Crithidia*.** *PLoS One* 2011, **6**:e20656.
83. Feng Y, Krueger EN, Liu S, Dorman K, Bonning BC, Miller WA: **Discovery of known and novel viral genomes in soybean aphid by deep sequencing.** *Phytobiomes* 2017, **1**:36-45.
84. Sébastien A, Lester PJ, Hall RJ, Wang J, Moore NE, Gruber MAM: **Invasive ants carry novel viruses in their new range and form reservoirs for a honeybee pathogen.** *Biol Lett* 2015, **11**:20150610.
85. Johnson RM, Evans JD, Robinson GE, Berenbaum MR: **Changes in transcript abundance relating to colony collapse disorder in honey bees (*Apis mellifera*).** *Proc Natl Acad Sci* 2009, **106**:14790-14795.
86. Cepero A, Ravoet J, Gómez-Moracho T, Bernal J, Del Nozal MJ, Bartolomé C, Maside X, Meana A, González-Porto AV, de Graaf DC *et al.*: **Holistic screening of collapsing honey bee colonies in Spain: a case study.** *BMC Res Notes* 2014, **7**:649.
87. Bigot D, Dalmon A, Roy B, Hou C, Germain M, Romary M, Deng S, ●● Diao Q, Weinert LA, Cook JM *et al.*: **The discovery *Halictivirus* resolves the *Sinaivirus* phylogeny.** *J Gen Virol* 2017, **98**:2864-2875.
- This paper uses next generation sequencing to identify the novel virus *Halictus scabiosae* Adlikon virus, belonging to the new virus genus *Halictivirus* in sweat bee samples. Additionally, they find the first evidence of LSVs in ant species (*Messor* spp.) and, using HsAV as an outgroup, resolve the LSV phylogeny into four clades with no apparent geographical pattern.
88. Malfroy SF, Roberts JMK, Perrone S, Maynard G, Chapman N: **A pest and disease survey of the isolated Norfolk Island honey bee (*Apis mellifera*) population.** *J Apic Res* 2016, **55**:202-211.
89. Granberg F, Vicente-Rubiano M, Rubio-Guerri C, Karlsson OE, Kukielka D, Belák S, Sánchez-Vizcaino JM: **Metagenomic detection of viral pathogens in Spanish honeybees: co-infection by Aphid lethal paralysis, Israeli acute paralysis and Lake Sinai viruses.** *PLoS One* 2013, **8**:e57459.
90. Ravoet J, Maharramov J, Meeus I, De Smet L, Wenseleers T, Smaghe G, de Graaf DC: **Comprehensive bee pathogen screening in Belgium reveals *Crithidia mellificae* as a new contributory factor to winter mortality.** *PLoS One* 2013, **8**:e72443.
91. Li JL, Scott Cornman R, Evans JD, Pettis JS, Zhao Y, Murphy C, Peng WJ, Wu J, Hamilton M, Boncristiani HF *et al.*: **Systemic spread and propagation of a plant-pathogenic virus in European honeybees, *Apis mellifera*.** *MBio* 2014, **5**:e00898-e913.
92. Longdon B, Murray GGR, Palmer WJ, Day JP, Parker DJ, Welch JJ, Obbard DJ, Jiggins FM: **The evolution, diversity, and host associations of rhabdoviruses.** *Virus Evol* 2015, **1**:vev014.
93. Schwarz RS, Bauchan GR, Murphy CA, Ravoet J, de Graaf DC, Evans JD: **Characterization of two species of Trypanosomatidae from the honey bee *Apis mellifera*: *Crithidia mellificae* Langridge and McGhee, and *Lotmaria passim* n. gen., n. sp.** *J Eukaryot Microbiol* 2015, **62**:567-583.
94. Runckel C, DeRisi J, Flenniken ML: **A draft genome of the honey bee trypanosomatid parasite *Crithidia mellificae*.** *PLoS One* 2014, **9**:e95057.
95. Akopyants NS, Lye L, Dobson DE, Luke J, Beverley S: **A novel Bunyavirus-like virus of trypanosomatid protist parasites.** *Genome Announc* 2016, **4**:e00715-e716.
96. Brutscher LM, Daughenbaugh KF, Flenniken ML: **Antiviral defense mechanisms in honey bees.** *Curr Opin Insect Sci* 2015, **10**:71-82.
97. Brutscher LM, Flenniken ML: **RNAi and antiviral defense in the honey bee.** *J Immunol Res* 2015, **2015**:941897.
98. Maori E, Paldi N, Shafir S, Kalev H, Tsur E, Glick E, Sela I: **IAPV, a bee-affecting virus associated with colony collapse disorder**

- can be silenced by dsRNA ingestion. *Insect Mol Biol* 2009, **18**:55-60.
99. Desai SD, Eu YJ, Whyard S, Currie RW: **Reduction in Deformed wing virus infection in larval and adult honey bees (*Apis mellifera* L.) by double-stranded RNA ingestion.** *Insect Mol Biol* 2012, **21**:446-455.
 100. Brutscher LM, Daughenbaugh KF, Flenniken ML: **Virus and dsRNA-triggered transcriptional responses reveal key components of honey bee antiviral defense.** *Sci Rep* 2017, **7**:6448.
 101. Hunter W, Ellis J, vanEngelsdorp D, Hayes J, Westervelt D, Glick E, Williams M, Sela I, Maori E, Pettis J *et al.*: **Large-scale field application of RNAi technology reducing Israeli acute paralysis virus disease in honey bees (*Apis mellifera*, Hymenoptera: Apidae).** *PLoS Pathog* 2010, **6**:e1001160.
 102. Liu X, Zhang Y, Yan X, Han R: **Prevention of Chinese Sacbrood virus infection in *Apis cerana* using RNA interference.** *Curr Microbiol* 2010, **61**:422-428.
 103. Flenniken ML, Andino R: **Non-specific dsRNA-mediated antiviral response in the honey bee.** *PLoS One* 2013, **8**:e77263.
 104. Chejanovsky N, Ophir R, Schwager MS, Slabezki Y, Grossman S, Cox-Foster D: **Characterization of viral siRNA populations in honey bee colony collapse disorder.** *Virology* 2014, **454-455**:176-183.
 105. Goodwin S, McPherson JD, McCombie WR: **Coming of age: ten years of next-generation sequencing technologies.** *Nat Rev Genet* 2016, **17**:333-351.
 106. Craggs JK, Ball JK, Thomson BJ, Irving WL, Grabowska AM: **Development of a strand-specific RT-PCR based assay to detect the replicative form of hepatitis C virus RNA.** *J Virol Methods* 2001, **94**:111-120.
 107. Plaskon NE, Adelman ZN, Myles KM: **Accurate strand-specific quantification of viral RNA.** *PLoS One* 2009, **4**:e7468.
 108. Andino R, Domingo E: **Viral quasispecies.** *Virology* 2015, **479-480**:46-51.
 109. Lauring AS, Andino R: **Quasispecies theory and the behavior of RNA viruses.** *PLoS Pathog* 2010, **6**:e1001005.
 110. Mordecai GJ, Wilfert L, Martin SJ, Jones IM, Schroeder DC:
 - **Diversity in a honey bee pathogen: first report of a third master variant of the Deformed wing virus quasispecies.** *ISME J* 2016, **10**:1264-1273.
 This paper defines three DWV master variants and provides the first data on the existence of DWV-C.
 111. de Miranda JR, Genersch E: **Deformed wing virus.** *J Invertebr Pathol* 2010, **103**:S48-S61.
 112. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P: **Dead or alive: Deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees.** *Appl Environ Microbiol* 2012, **78**:981-987.
 113. Yang X, Cox-Foster DL: **Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification.** *Proc Natl Acad Sci* 2005, **102**:7470-7475.
 114. Natsopoulou ME, McMahon DP, Doublet V, Frey E, Rosenkranz P, Paxton RJ: **The virulent, emerging genotype B of Deformed wing virus is closely linked to overwinter honeybee worker loss.** *Sci Rep* 2017, **7**:5242.
 115. Roberts JMK, Anderson DL, Durr PA: **Absence of Deformed wing virus and *Varroa destructor* in Australia provides unique perspectives on honeybee viral landscapes and colony losses.** *Sci Rep* 2017, **7**:6925.
 116. Cornman RS: **Relative abundance of Deformed wing virus, *Varroa destructor* virus 1, and their recombinants in honey bees (*Apis mellifera*) assessed by kmer analysis of public RNA-Seq data.** *J Invertebr Pathol* 2017, **149**:44-50.
 117. Dalmon A, Desbiez C, Coulon M, Thomasson M, Le Conte Y, Alaux C, Vallon J, Moury B: **Evidence for positive selection and recombination hotspots in Deformed wing virus (DWV).** *Sci Rep* 2017, **7**:41045.
 118. Nei M, Kumar S: *Molecular Evolution and Phylogenetics*. Oxford University Press; 2000.
 119. Kumar S, Stecher G, Tamura K: **MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets.** *Mol Biol Evol* 2016, **33**:1870-1874.
 120. Edgar RC: **MUSCLE: multiple sequence alignment with high accuracy and high throughput.** *Nucleic Acids Res* 2004, **32**:1792-1797.
 121. Felsenstein J: **Confidence limits on phylogenies: an approach using the bootstrap.** *Evolution (NY)* 1985, **39**:783-791.
 122. Kevill JL, Highfield A, Mordecai GJ, Martin SJ, Schroeder DC: **ABC assay: method development and application to quantify the role of three DWV master variants in overwinter colony losses of European honey bees.** *Viruses* 2017, **9**:314.
 123. Mordecai GJ, Brettell LE, Martin SJ, Dixon D, Jones IM, Schroeder DC: **Superinfection exclusion and the long-term survival of honey bees in *Varroa*-infested colonies.** *ISME J* 2016, **10**:1182-1191.
 124. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P: **Predictive markers of honey bee colony collapse.** *PLoS One* 2012, **7**: e32151.
 125. Dainat B, Neumann P: **Clinical signs of Deformed wing virus infection are predictive markers for honey bee colony losses.** *J Invertebr Pathol* 2013, **112**:278-280.
 126. Nazzi F, Pennacchio F: **Disentangling multiple interactions in the hive ecosystem.** *Trends Parasitol* 2014, **30**:556-561.
 127. Lamp B, Url A, Seitz K, Eichhorn J, Riedel C, Sinn LJ, Indik S,
 - **Köglberger H, Rumenapf T: Construction and rescue of a molecular clone of Deformed wing virus (DWV).** *PLoS One* 2016, **11**:e0164639.
 This work presents the first published infectious molecular clone of a honey bee-infecting virus. The development of infectious molecular clones will greatly improve our ability to dissect the biology of these viruses and understand how they contribute to poor pollinator health.
 128. Manley R, Boots M, Wilfert L: **Emerging viral disease risk to pollinating insects: ecological, evolutionary and anthropogenic factors.** *J Appl Ecol* 2015, **52**:331-340.
 129. Marston HD, Folkers GK, Morens DM, Fauci AS: **Emerging viral diseases: confronting threats with new technologies.** *Sci Transl Med* 2014, **6**:253ps10.