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 pathogeneses 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 pathogeneses 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	*Dicistroviridae*	*Apis mellifera*	[30,82,86,89]
Apis mellifera filamentous virus	Switzerland, United States, Belgium	dsDNA	498.5 kb	Unclassified	*Apis mellifera* *Osmia cornuta* *Osmia bicornis* *Andrena vaga* *Andrena ventralis*	[30,37–39]
Apis mellifera bunya virus-1	South Africa	−ssRNA	6 kb	*Bunyaviridae*	*Apis mellifera*	[35••]
Apis mellifera bunya virus-2	South Africa	−ssRNA	6.5 kb	*Bunyaviridae*	*Apis mellifera*	[35••]
Apis dicistrovirus	Netherlands	+ssRNA	9.1 kb	*Dicistroviridae*	*Apis mellifera*	[35••]
Apis mellifera flavivirus	South Africa	+ssRNA	20.4 kb	*Flaviviridae*	*Apis mellifera*	[35••]
Apid mellifera nora virus-1	South Africa	+ssRNA	10 kb (partial)	Picorna-like	*Apis mellifera*	[35••]
Apis mellifera rhabdovirus-1/ bee rhabdovirus-1	United States, Israel Tonga Netherlands South Africa	−ssRNA	14.6 kb	*Rhabdoviridae*	*Apis mellifera* *Bombus impatiens* *Varroa destructor*	[35••,36]
Apis mellifera rhabdovirus-2	United States, Israel Tonga Netherlands South Africa	−ssRNA	14 kb	*Rhabdoviridae*	*Apis mellifera* *Varroa destructor*	[35••,36]
Big Sioux River virus	United States	+ssRNA	9.6 kb	Dicistroviridae	*Apis mellifera*	[82]
Halictus scabiosae Adlikon virus	Switzerland	+ssRNA	5.2 kb	Unclassified	*Halictus scabiosae*	[87••]
Lake Sinai viruses	United States Europe Australia China	+ssRNA	5.9 kb	Unclassified	*Apis mellifera* *Andrena vaga* *Andrena ventralis* *Osmia bicornis* *Osmia cornuta* *Bombus* spp. *Messor* spp.	[30,44••,82,86,87••,88–90]
Moku virus	United States	+ssRNA	10 kb	*Iflaviridae*	*V. pensylvanica* *Apis mellifera* *Varroa destructor*	[63•]
Varroa destructor macula-like virus	United States Belgium	+ssRNA	6.5 kb	*Tymoviridae*	*Apis mellifera* *Varroa destructor* *Osmia cornuta* *Bombus* spp.	[30,34,44•,90]
Varroa destructor virus 2	Israel	+ssRNA	9.6 kb	*Iflaviridae*	*Varroa destructor*	[62••]
Varroa destructor virus 3	Israel	+ssRNA	4.2 kb	Unclassified	*Varroa destructor*	[62••]
Varroa tymo-like virus	United States	+ssRNA	6.2 kb	*Tymoviridae*	*Varroa destructor*	[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 pensylvanica*). 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 *Sinaivirus* 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, Marifivirus, and Maculavirus) [34]. The US and European strains of BeeMLV are >70% identical at the nucleotide level and distinct from the related Varroa tymo-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 mellificae* 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
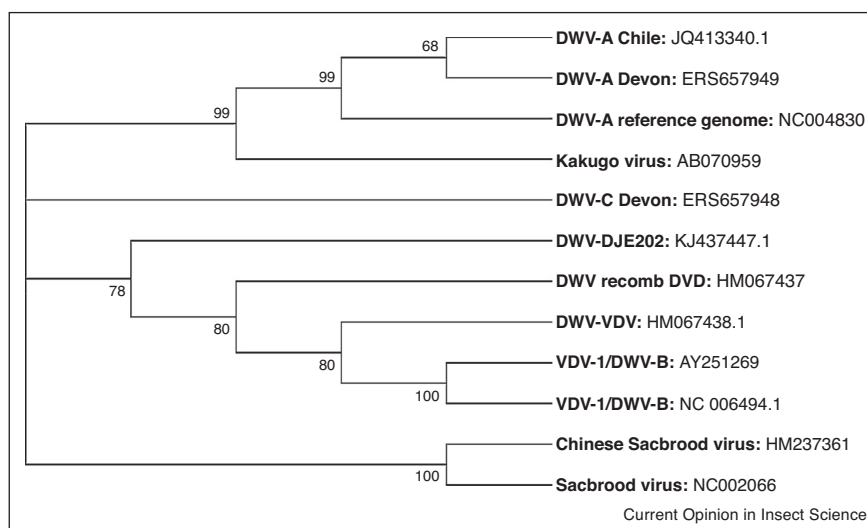
Deformed wing virus phylogenetic relationship inferred from whole genome nucleotide sequence. Maximum Likelihood bootstrap supported consensus tree derived from Maximum Likelihood analysis in Mega v7.0.26 [111] using a whole genome nucleic acid MUSCLE alignment generated in Mega using a neighbor joining 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 pathogeneses 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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