ANNUAL REVIEWS

Annual Review of Virology

Deformed Wing Virus in Honeybees and Other Insects

Stephen J. Martin[1] and Laura E. Brettell[2]

[1]School of Environment and Life Sciences, University of Salford, Manchester M5 4WT, United Kingdom; email: s.j.martin@salford.ac.uk

[2]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, New South Wales 2751, Australia; email: L.Brettell@westernsydney.edu.au

Annu. Rev. Virol. 2019. 6:49–69

First published as a Review in Advance on June 11, 2019

The *Annual Review of Virology* is online at virology.annualreviews.org

https://doi.org/10.1146/annurev-virology-092818-015700

Keywords

honeybees, pollinators, RNA viruses, colony collapse, structure, prevalence

Abstract

Deformed wing virus (DWV) has become the most well-known, widespread, and intensively studied insect pathogen in the world. Although DWV was previously present in honeybee populations, the arrival and global spread of a new vector, the ectoparasitic mite *Varroa destructor*, has dramatically altered DWV epidemiology. DWV is now the most prevalent virus in honeybees, with a minimum average of 55% of colonies/apiaries infected across 32 countries. Additionally, DWV has been detected in 65 arthropod species spanning eight insect orders and three orders of Arachnida. Here, we describe the significant progress that has been made in elucidating the capsid structure of the virus, understanding its ever-expanding host range, and tracking the constantly evolving DWV genome and formation of recombinants. The construction of molecular clones, working with DWV in cell lines, and the development of immunohistochemistry methods will all help the community to move forward. Identifying the tissues in which DWV variants are replicating and understanding the impact of DWV in non-honeybee hosts are major new goals.

GENERAL INTRODUCTION

Deformed wing virus (DWV) has transformed from a largely unknown, minor pathogen of honeybees to the most well-known, widespread, and intensively studied insect pathogen in the world. This dramatic rise in DWV's prevalence and infamy is solely down to its association with the *Varroa* mite (*Varroa destructor*), an ectoparasite and also, devastatingly, an efficient virus vector between honeybees (*Apis mellifera*) (1–6). This introduction of a new DWV transmission route—i.e., inoculation directly into the hemolymph during mite feeding—has been closely associated with the death of millions of honeybee colonies and has changed the entire viral landscape of honeybees (7–9) and other associated insects (10, 11). This three-way association between the honeybee (host), mite (vector), and DWV (pathogen) has generated thousands of studies, created numerous research teams, and even turned many beekeepers into pseudo-virologists. Due to honeybees' both importance and ease of manipulation, this tripartite system continues to develop into an excellent insect-host-viral pathogen model system. This review focuses predominantly on the major advances made since 2010, since studies prior to this are well covered by the reviews of de Miranda & Genersch (12) and Genersch & Aubert (13). The key advances covered in this review are in DWV's worldwide prevalence, virion structure, genetic variants and recombination, transmission, host range, and impact on host, as well as future directions. For recent reviews on other bee-associated viruses, see (14); for reviews on the *Varroa* mite and host immunity against DWV, see (15) and (16), respectively, since all these topics lie beyond the scope of this review.

BRIEF HISTORY OF DEFORMED WING VIRUS DISCOVERY AND CURRENT PREVALENCE

DWV was the last virus to be isolated from honeybees by the legendary Bill Bailey and Brenda Ball at the Rothamsted Research Centre in the United Kingdom. Between them, they identified the majority of honeybee-associated viral pathogens and laid a strong foundation for all subsequent work. In 1982 they discovered a virus isolated from dead Japanese honeybees that was serologically related to Egypt bee virus (EBV). This Japanese isolate of EBV was later named DWV, owing to the deformed wings of the bee from which the virus was isolated (17) (**Figure 1**). Subsequently, three more colonies from Belize, the United Kingdom, and South Africa all died suddenly with high loads of DWV (18); it therefore appeared that DWV was a rare pathogen that was very occasionally associated with the death of a honeybee colony. It was another 10 years before DWV was detected again, this time in dead *Varroa*-infested colonies in the United Kingdom (19). Initially people were skeptical about the role of DWV in the death of *Varroa*-infested colonies, since the well-established dogma was that deformed bees and colony death were caused by hemolymph extraction by the feeding mites. As the availability of molecular technologies increased, many countries around the world conducted prevalence surveys (**Table 1**) of the main honeybee-associated viruses, including DWV, facilitated by a standardized set of methods and primers for studying all bee-associated viruses (20). Many studies indicated that DWV was consistently (20 out of 24 studies) the most prevalent viral pathogen detected in honeybees (**Table 1**), with a worldwide prevalence (based on data from 32 countries) of approximately 55% of colonies/apiaries infected, although this figure may be higher, as prevalence levels vary with the detection sensitivity (8).

In every location presented in **Table 1**, the *Varroa* mite had already become well established within honeybee populations, and in the vast majority of countries colonies died if beekeepers did not control their mite populations (21). However, in a small number of locations such as Uganda (22), Australia (23), and the Canadian island of Newfoundland (24), the *Varroa* mite has not yet

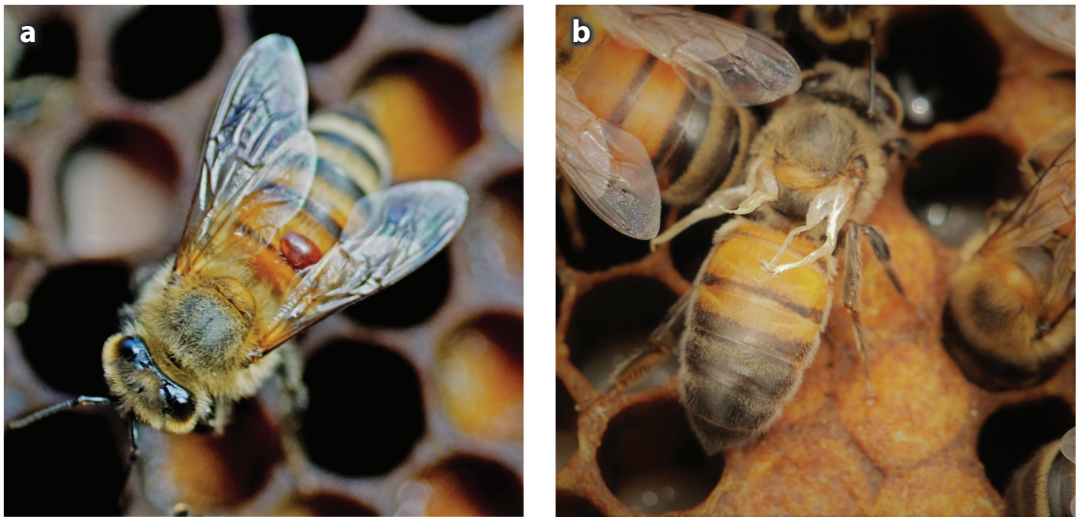


Figure 1

(*a*) A normal-looking asymptomatic honeybee that hosts an adult female *Varroa destructor* mite, the oval, brown object seen on her abdomen between her wings. (*b*) A newly emerged bee showing the deformed wings typically associated with symptomatic deformed wing virus. Photos courtesy of Ethel Villalobos.

become established. Correspondingly, DWV either has not been detected or is seen only at low levels at the threshold of molecular detection capability.

This close association between DWV and the *Varroa* mite has long been understood in general terms (25), but recent research by Wilfert et al. (9), who used molecular evolutionary tools to track the spread of DWV around the world, elucidated the tight link between the global spread of the *Varroa* mite (26) and the corresponding spread of DWV. Decreasing molecular costs now enable researchers to analyze increasing sample sizes, and some are extracting RNA from only the heads of bees, as this helps cut down processing times. Although Boncristiani et al. (27) found that chemicals within the large compound eyes of bees can inhibit polymerase chain reactions (PCRs) and false negatives can be generated if noncolumn-based RNA extraction methods are used, this can easily be avoided by using column-based extraction kits or adding a column-based purification step (27).

THE STRUCTURE OF DEFORMED WING VIRUS

DWV is a member of the *Iflaviridae* family within the *Picornavirales* order. Like all Iflaviruses, DWV exists as a nonenveloped icosahedral virion about 30 nm in diameter, which contains one copy of a single-stranded positive-sensed RNA genome. The ~10-kb genome contains a single open reading frame, which encodes a 2,893-amino acid polyprotein (28) that is post-translationally processed, and an extended 1.1-kb 5′-proximal UTR containing an internal ribosome entry site. The 3′ end terminates with a poly-A tail (29). The structural proteins (VP1–VP4) are located at the N-terminal (5′) end, while the nonstructural proteins, involved in replication and polyprotein processing, are present at the C-terminal (3′) region. This protein order is strictly conserved within the Iflaviruses (30) (**Figure 2**).

Since de Miranda & Genersch's review (12), the major advance in DWV research has been in our understanding of the capsid structure. Sánchez-Eugenia et al. (31) showed using the Triatoma

Table 1 The prevalence (%) of DWV in colonies (or apiaries if the data were pooled) from 32 countries, followed by the sample size (n), whether DWV was the most prevalent RNA virus detected (where more than one virus had been studied), and the source reference

Country	Prevalence	Sample size (n)	Was DWV the most prevalent RNA virus detected?	Reference(s)
Europe				
Austria	91%	131	Y	25
Belgium	69%	164	Y	126
Bulgaria	10%	50	Y	127
Croatia	95%	82	Y	128
France	84%	90	Y	129
	97%	36	Y	130
Germany	20%	1,104	Y	66
Poland	76%	1,000	Y	131
Serbia	76%	55	Y	132
	74%	150	Y	133
Spain	15%	456	N/A	134
	10%	10	N	135
	19%	438	N	136
Switzerland	74%	337	Y	137
United Kingdom	100%	250	N/A	8
	36%	34	N/A	10
	100%	34		138
Asia				
China	94%	34	Y	139
	90%	17	Y	140
	74%	46	N/A	141
Japan	84%	65	Y	90
Vietnam	75%	1	N	141
Americas				
Chile	42%	60	Y	142
Mexico	80%	5	Y	143
United States	100%	Unknown	Y	144
	75–95%	79–170	Y	68
Middle East				
Iraq	27%	30	N/A	145
Israel	23%	71	Y	146
Jordan	45%	60	N/A	145
Lebanon	68%	13	N/A	145
Palestine	37%	25	N/A	145
Syria	77%	35	N/A	145
Yemen	38%	16	N/A	145
Africa				
Algeria	40%	40	N/A	147
Egypt	20%	20	N/A	145
Kenya	50%	32	Y	148
Libya	20%	15	N/A	145

(*Continued*)

Table 1 (*Continued*)

Country	Prevalence	Sample size (n)	Was DWV the most prevalent RNA virus detected?	Reference(s)
Morocco	45%	21	N/A	145
South Africa	13%	31	N	149
Sudan	0%	4	N/A	145
Tunisia	34%	56	N/A	150
	17%	32	N/A	145
Average	**~55%**			

Abbreviations: DWV, deformed wing virus; N, no; N/A, not applicable; NY, yes.

virus, a virus within the same order as DWV (*Picornavirales*), that during cell entry the capsid undergoes a conformational change, and the VP4 region may function by being inserted directly into cell membranes prior to viral insertion. Further insight into DWV structure was then provided by two independent teams, Škubník et al. (32) and Organtini et al. (33), who used cryo-electron microscopy and X-ray crystallography to determine the structure of the DWV virion down to a resolution of 3–4 Å. They confirmed that the DWV virion is constructed from the VP1, VP2, and VP3 subunits and arranged into a capsid with a pseudo-T3 icosahedral symmetry. They also showed how this three-dimensional structure changed under different pH conditions, particularly the p-domains, which are protruding globular extensions of the capsid proteins that may act as catalytic sites to enable viral entry into the host cell. Organtini et al. (33) showed that the procapsid (immature capsid prior to RNA genome insertion) and RNA-containing capsid both shared the same conformation, whereas putative entry intermediates, or A particles, and empty capsids that remain after genome release had undergone a conformational transformation. Both studies showed that these changes occurred in the p-domains, but the order of events remains controversial (**Figure 3**).

A further finding from these studies was that the viral genome interacts with the VP3 protein close to the fivefold icosahedral axis, an interaction that could contribute to stability or assembly of the mature virion, and additionally this fivefold vertex may be the location of genome escape. Although the study by Škubník et al. (32) showed that variations in pH could trigger changes in the p-domain structure, the levels used were not physiologically realistic; thus, it remains unknown which factor or combinations of factors, such as heat, receptor interactions, or pH, may trigger genome release. The only major recent advance in the understanding of the genome sequence, which was already annotated by 2010, was by Lamp et al. (34), who constructed molecular clones and showed that the UTR at the 5′ end was longer than previously thought, with 21 nucleotides missing compared to the typical DWV genome sequence.

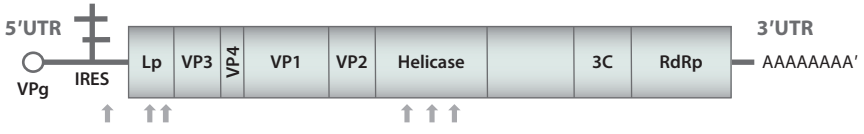


Figure 2

The basic structure of the DWV genome. The IRES in the 5′ untranslated region may initiate translation of the polyprotein. Arrows indicate some of the key recombination regions (52, 53). Abbreviations: DWV, deformed wing virus; IRES, internal ribosome entry site; Lp, leader protein; RdRp, RNA-dependent RNA polymerase; VPg, viral protein genome-linked. Figure adapted from Reference 34.

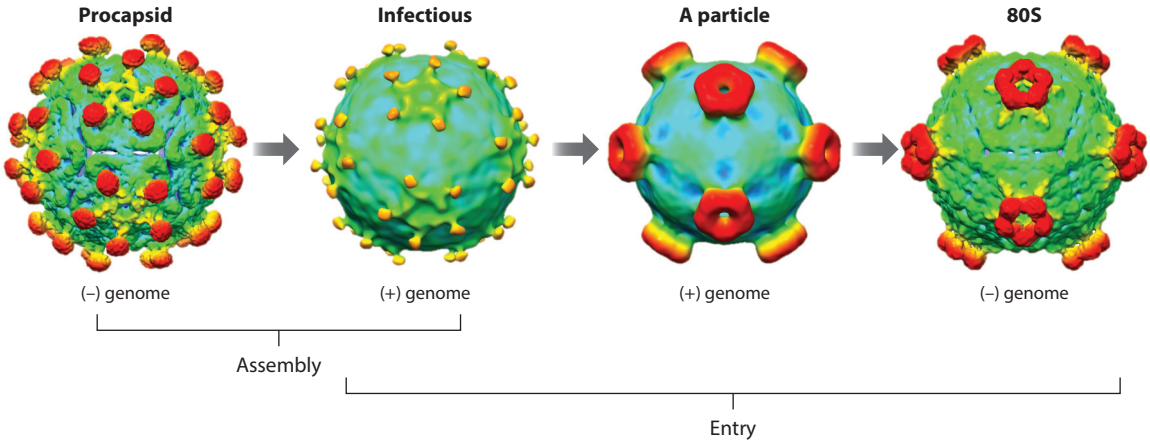


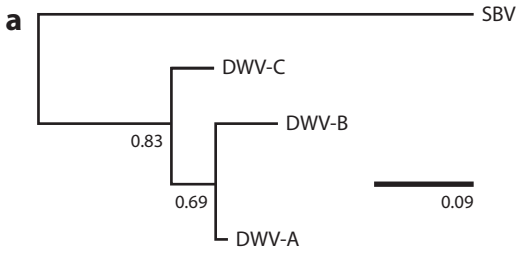
Figure 3

Images showing the proposed conformational change that occurs during genome release according the work published in Reference 33 showing that the genome packages into an open structure to produce the infectious virus. After interacting with host cells, the open form changes to the closed conformation during genome release. However, the structural changes involving genome packaging and release are controversial, since the model in Reference 32 suggested that the closed form of the capsid is used for packaging the genome and the virus changes to an open form for RNA release. Figure reproduced courtesy of Susan Hafenstein and Lindsey Organtini.

THE DEFORMED WING VIRUS COMPLEX

One of the key advances in the field of DWV research in the last decade is the discovery made by two independent teams, Martin et al. (7) and Ryabov et al. (8), who, by using a part of the RNA-dependent RNA polymerase gene, identified that DWV persists naturally as a covert swarm of DWV variants. However, when these diverse strains were transmitted horizontally by the *Varroa* mite, the diversity was vastly decreased to a near-clonal DWV population, the A strain (7). At the colony level, this occurred over a period of 2–3 years and corresponded with an increase in virus load and eventual death of the honeybee colony (7). Ryabov et al. (8) later demonstrated that this loss of diversity did not occur in the mite but in the developing bee into which DWV had been injected and occurred within days in an individual bee pupa. Ryabov et al. (8) also showed that oral acquisition of DWV did not affect the low viral load or diverse nature of DWV. As the loss of strain diversity occurred in the bee pupae and not in the mites, this suggests that the mites are just transmitting the reduced diversity rather than generating it. Amazingly, a parallel loss of strain diversity occurred in DWV detected in the social wasp *Vespula pensylvanica* from Hawaii (35). Although wasps are not parasitized by *Varroa*, they are an active honeybee predator; thus, the variant sharing between *Vespula* and *Apis* suggests that these wasps can acquire DWV directly or indirectly from honeybees.

The existence of multiple strains and the capability to infect several species are common features of viral infections and influence many viral processes, including replication, transmission, and the induction of disease. DWV is no different, although it may be many years until the full impact and evolution of strain diversity in DWV are realized. Ever since Lanzi et al. (28) first sequenced the full DWV genome, the amount of available sequence data has increased rapidly. These data have revealed that DWV is a viral complex comprising at least three distinct genotypes or master variants—types A, B, and C (7, 36, 37)—with significant variation often being present around each genotype (**Figure 4**). The original DWV genotype (28) is now known as the DWV type-A master variant, which includes most previous DWV sequences and Kakugo virus



	DWV-A	DWV-B	DWV-C
DWV-A		95.2	89.5
DWV-B	**84.4**		89.1
DWV-C	**79.1**	**78.9**	

Figure 4

(*a*) Bayesian phylogeny using a conserved amino acid sequence encoding for the RdRp region for the three DWV master variants (A, B, and C) along with SBV, another member of the *Iflaviridae* family. Bayesian support values are shown on the nodes with bars indicating the number of nucleotide substitutions per site. (*b*) Percentage identity matrix of DWV variant amino acid (*upper right*) and nucleotide alignments (*bold lower left*) between the three master variants (A, B, and C) based on de novo assembly of whole genomes. Abbreviations: DWV, deformed wing virus; RdRp, RNA-dependent RNA polymerase; SBV, Sacbrood virus. Figure adapted with permission from Reference 36.

(38). Owing to the phylogenetic clustering of the virus sequences and the frequently occurring homologous recombination, the DWV species complex has now been extended to include *Varroa* destructor virus-1 (VDV-1) (39), which is now taxonomically assigned as part of the type-B clade and the most recently discovered type-C master variant (36). The situation regarding nomenclature and VDV-1 has been further complicated by the discovery of three new RNA viruses detected in the *Varroa* mite, which have been named VDV-2, VDV-3 (40), and VDV-4 (41). These three newly described viruses both lie well outside the DWV clade and as far as we know are restricted to *Varroa*, in contrast to VDV-1, which lies within the DWV group and is commonly found in both honeybees and *Varroa* mites. The differences and phylogenic relationship between the three types of DWV are given in **Figure 4**.

The majority of virologists in the field now accept this nomenclature, and Kevill et al. (42) have designed an assay with specific primers to distinguish between these three variants. Using molecular clock estimations, Mordecai et al. (36) predicted that type C diverged from the other master variants ~319 years ago, whereas types A and B disassociated from each other more recently around 181 years ago (**Figure 4**).

Furthermore, Lamp et al. (34) showed using molecular clones that most of the 5′-UTR was highly conserved between all DWV strains. However, when comparing the 5′-terminal stretch in their DWV-A 1414 clone to other DWV master sequences, they found 10 nucleotides missing from Kakugo virus (DWV-A) and at least 21 nucleotides missing from both DWV-A and DWV-B genomes, while the DWV-C was 14 nucleotides shorter compared with the DWV-A 1414 clone. Currently, the significance of these differences remains unclear.

We are now discovering that competition is occurring within the host between the three master variants, whose dynamic evolution continues to change the viral landscape of entire countries. For example, in 2010 a DWV survey of honeybees across the United States found DWV type B in only 3% of colonies, whereas in 2016 it had increased to 65% of colonies (43). When type B and type A are coinjected into adult bees, type B outcompetes type A (44); this could be linked to the landscape-scale changes that are being observed (45). Since its discovery, DWV type C remains elusive, only occasionally being detected in honeybees (42); however, a recent study by De Souza et al. (46) found type C to be present and often the dominant variant in all sampled colonies of the stingless bee *Melipona subnitida* from Brazil, suggesting different host species may vary in their susceptibility to the different DWV variants.

One of the key challenges for future research programs is to continue to track the evolving DWV landscape and understand the impacts that each of these and any new emerging master variants have on the various insect hosts.

RECOMBINATION

As individual hosts are commonly coinfected with multiple viral strains (47), recombination is expected to occur. Recombination in DWV was first studied by both Moore et al. (48) and Zioni et al. (49) in bees in the United Kingdom and Israel, respectively. Both groups found a similar recombinant comprising the DWV type-B structural region with the type-A nonstructural region, but a range of recombinant forms containing a number of recombination breakpoints have now been discovered (**Figure 2**). In a later study of the same UK population, Ryabov et al. (8) also found the B/A recombinant to dominate. This recombinant has a recombination junction within the helicase gene and consisted of a DWV-A, 5′ UTR, and nonstructural region with the DWV-B capsid-encoding region and 3′ UTR. Wang et al. (50) found a similar recombinant (5′-DWV-A/DWV-B/-DWV-A-3′) along with the full-length DWV-B strain to be the most prevalent forms of DWV in their study in Oxford, United Kingdom. Natsopoulou et al. (51) and Cornman et al. (52) both independently confirmed the presence of DWV recombination hotspots in the 5′ UTR (many) and within the conserved helicase gene (few), as previously discovered (48, 50). Dalmon et al. (53) went on to identify nine recombination breakpoints (**Figure 2**), identifying four positions that showed evidence of positive selection. Three of these were in the putative leader protein, and one was in the Helicase gene, suggesting these regions are important in the evolution of DWV. However, even though we now know recombinants to be widespread, it appears from a recent study by Chejanovsky et al. (54), who found only full-length DWV-A in Florida, that full-length types A and B may more commonly dominate samples rather than recombinants.

ACTIVATORS AND LOCATION OF DEFORMED WING VIRUS REPLICATION IN HONEYBEES AND MITES

Honeybees

Under natural (*Varroa* mite–free) conditions, DWV infections are rare, and when present, viral loads are often at the limit of detection capability. As such, there are no available data concerning tissue localization since the majority of studies using these mite-free populations use whole individuals or pooled bees to maximize the chances of detecting viruses (e.g., 23, 55). Furthermore, we do not have any idea what changes a covert-persistent infection into an overt infection that goes on to kill a honeybee colony, since these events are very infrequent and have been recorded in only a few studies (18, 7, 24). Understanding how and why DWV changes from a covert- to a persistent- or acute-overt infection under natural conditions will be a major challenge for the future, especially since honeybee populations that are infected with DWV but remain free from *Varroa* mites are a scarce resource.

To mimic the effects of mite feeding, Annoscia et al. (56) experimentally removed increasing volumes of hemolymph from well-developed honeybee pupae and found this resulted in increasing DWV viral loads. In addition, Zhang & Han (57) suggested that an injection of a toxic protein by the *Varroa* mite into developing pupae during feeding caused DWV loads to increase and subsequently cause the development of deformed wings in the adults. However, both of these attractive ideas cannot explain why *Varroa* mites that have been feeding on honeybees covertly infected with DWV on the remote Brazilian island of Fernando de Noronha have not caused DWV to become

an overt infection during the past 30 years (55), since the mites are both removing hemolymph and potentially injecting toxic proteins. Furthermore, the idea of mite feeding activating DWV has been a popular one (16), although clear empirical support has remained scarce. This may be because it is difficult to obtain truly noninfected mites and bees to study, and it is not possible to test for viral loads of live bees and mites prior to exposure to one another. It is, however, much more feasible to investigate interactions when bees are already infected with overt-persistent DWV. As such, when infection is already present, many factors have been associated with increasing the DWV loads in the honeybee population, such as cold stress and colony strength (58); diet, with higher DWV levels in sugar-fed bees relative to pollen-fed bees (59); and exposure to neonicotinoid pesticides, which negatively modulates NF-κB activation and helps promote DWV proliferation (60).

In general, DWV studies and surveys have used RNA extracted from either whole bee or mite samples or, more recently, just the bee heads. The various strains (i.e., DWV-A and DWV-B, and recombinants thereof) can be detected in all parts of the bees' body (27, 38, 49, 61–63). Using immunohistochemistry, Lamp et al. (34) showed DWV-A replicated in the head, thorax, and abdomen of bee pupae. Using the DWV-VP1 antigen, they found DWV-A in the ocular cells, central nervous system, and glandular systems in the head; in the connective tissue cells and glands of the thorax; but not in the muscles of the thorax or hemocytes (34), whereas Gisder et al. (64) found both DWV-A and DWV-B within the thorax and heads of artificially injected adult bees, although the proportion of the DWV-A in the head was significantly lower than that of DWV-B. Then using fluorescence in situ hybridization (FISH), they confirmed DWV-B in the brain tissue but not DWV-A. Yue et al. (65) found that vertically transmitted DWV was typically lacking from the heads of the bees but present in the thorax and abdomen. Genersch et al. (66) found that when DWV diagnosis was restricted to using the presence of DWV in the honeybees' head, colony losses in Germany could be significantly related to DWV infection, which makes sense given what we know about *Varroa*-transmitted DWV being the most important for bee and colony health. Studies are now starting to look at tissue tropism in bees (34, 64) using methods such as FISH, but these methods now need to be applied to mites.

Mites

Although it is well established that there is a strong correlation between the DWV load in mites and the corresponding honeybee pupa they feed on (67–69), there still is a lack of compelling evidence regarding whether DWV replication occurs in mites. This is important from an evolutionary standpoint since mites and honeybees belong to two very different branches of the arthropods, so any virus able to successfully maintain fitness in such diverse hosts must harbor a range of generalist traits. Gisder et al. (70) used negative-strand reverse transcription polymerase chain reaction (RT-PCR) to suggest that DWV replicated in mites collected from deformed bees but could not detect any negative-strand DWV RNA from mites collected from asymptomatic (overt-persistent) bees. Campbell et al. (71) then constructed a cDNA library from the brain (synganglia) of female *Varroa* mites and detected contigs coding for the DWV-B strain and a B-A DWV recombinant; however, they subsequently failed to detect any negative-strand DWV in any of the mite tissues assayed. Santillán-Galicia et al. (72), however, found DWV to be present only in the mite's gut, in structures assumed to be fecal pellets. Erban et al. (73) subsequently demonstrated the absence of nonstructural proteins and a high abundance of structural proteins in *Varroa*, suggesting that DWV proteins accumulated in the gut after feeding and not because of viral replication within the mite.

The confusion lies in the fact that when *Varroa* start to feed on the developing pupa, they normally have very low DWV loads relative to those leaving with the fully developed honeybee

12 days later (74). This increase in DWV load could be a result of viral replication within the mite or instead simply arise from the mite obtaining increasingly higher doses of DWV from the pupa, which the mites feed upon regularly. Current methods to detect negative strands of DWV can generate false positives through either self-priming of positive-strand RNA during reverse transcription or random priming by, for example, tRNAs. To try to overcome this nonspecific binding, newer tagged RT-PCR methods use biotinylated primers combined with streptavidin-coated beads (75). However, concerns remain when using whole individuals regarding potential contamination from the contents of the gut. We urgently need methods such as FISH probes, immunohistochemical techniques, or use of small interfering RNAs that detect an antiviral response (76) to separate true infections from those present in the gut contents.

TRANSMISSION ROUTES

DWV can be naturally transmitted via drones during mating by infecting the queen's ovaries and spermatheca (77, 78), consequently enhancing the vertical transmission route of DWV via the queens' eggs (65). Amiri et al. (78) have shown that DWV appears to adhere to the surface of eggs (transovum) rather than transmit within the eggs (intracellular). This may suggest that the queens' role in DWV vectoring may not be as high as is theoretically possible (79). Furthermore, within the bee colony DWV can be transmitted to the larvae via the food that is produced by workers (70); however, Mockel et al. (62) demonstrated that when 1×10^7 or greater DWV genome equivalents were fed to adult bees, only covert infections developed, whereas an injected dosage of only 80 DWV equivalents was sufficient to induce an overt infection and caused damaged wings in 30% of the emerging pupa (62). This supports the well-established fact that the major horizontal transmission route of DWV is by the *Varroa* mite (1, 72), although uncertainty remains regarding whether viral transmission is a passive mechanical process that occurs during feeding or is helped by possible viral replication within the mites' glands or tissues (see the section titled Mites). In addition, since the *Varroa* mites' host range is restricted to only a small number of honeybee species, the finding by Mazzei et al. (63) that DWV extracted from pollen remains viable has opened up another horizontal transmission route via flowers (80). Although this route is expected to be relatively minor in terms of impact to honeybees, it has helped to explain how DWV could be transmitted to other species with which bees share floral resources.

HOST RANGE OF DEFORMED WING VIRUS

For many invertebrates, infection by multiple RNA viruses is likely to be the norm rather than the exception (81, 82), as their evolutionary history is characterized by both host switching and codivergence. Genetic relatedness and the geographical proximity of host species likely play important roles in the host range of viruses. This has been demonstrated for DWV by phylogenetic analyses carried out by Zhang et al. (83), who suggested that DWV might have moved from *A. mellifera* to *Apis florea* and *Apis dorsata*. In 2006, Genersch et al. (84) discovered DWV in bumblebees (*Bombus terrestris* and *Bombus pascuorum*), and since then numerous surveys have brought to light the generalist nature of DWV. To date DWV has been detected in at least 64 species spanning eight orders, extending outside insects to encompass the Arachnida, resulting in a very wide host range for a single viral pathogen (**Table 2**; **Supplemental Table 1**). Furthermore, it appears that the prevalence of DWV in arthropods associated with a honeybee hive can be worryingly high; Levitt et al. (85) found DWV was the most prevalent viral pathogen among the 29 arthropod species associated with honeybee hives, with 59% of individuals testing positive. Further studies (45, 86, 87) also found DWV to be the most prevalent honeybee-associated viral pathogen among the

Supplemental Material >

Table 2 The range and number of species in which DWV has been detected. See Supplemental Table 1 for more details on individual species and the Supplemental References

Supplemental Material >

ORDER or Common name (family/genus)
HYMENOPTERA 48 spp.
Honeybees (*Apis*) 4 spp.
Bumblebees (*Bombus*) 11 spp.
Solitary bees (Apidae) 21 spp.
Stingless bees (Apidae) 3 spp.
Ants (Formicidae) 2 spp.
Social wasps (Vespidae) 7 spp.
Solitary wasps (Vespidae) 1 sp.
HEMIPTERA (True Bugs) 1 sp.
COLEOPTERA (Beetles) 3 spp.
DIPTERA (Flies) 3 spp.
LEPIDOPTERA (Butterflies and Moths) 3 spp.
DERMAPTERA (Earwigs) 1 sp.
BLATTODEA (Cockroach) 1 sp.
ARACHNIDA (Spiders and Harvestman) 4 spp.

Abbreviation: DWV, deformed wing virus.

arthropod community. However, reports on prevalence within the broader insect community are inconsistent. For instance, Evison et al. (88) found DWV in the bumblebee species *B. terristris* and *B. pascuorum* and the wasp species *Vespula vulgaris* but not in another 13 species (125 individuals) of bumblebees or wasps tested. Studies using larger sample sizes are needed to better understand DWV prevalence in different hosts. Since the majority of these studies have detected DWV using RT-PCR with primers originally designed using DWV variants infecting honeybee hosts, there may be genetically diverse DWV variants infecting non-*Apis* hosts that are being missed. Any unrecognized genetic diversity will emerge as more next-generation sequencing (NGS) studies are performed. Detection of DWV replicative intermediates using negative-strand PCR is used to indicate active replication in many species; false positives could be an issue here (see the section titled Mites), although negative strands of other honeybee-associated RNA viruses are rarely detected in non-honeybee hosts.

IMPACTS ON THEIR HONEYBEE HOSTS

Due to honeybees' global importance, the association between DWV and the *Varroa* mite—and its resulting impact on honeybees—has been the main focus of recent research in the field. Relative to other RNA viral pathogens that infect honeybees (89), DWV can be classified as having low virulence, since overt-acute infections are rare and now overt-chronic infections are common. This low virulence increases the probability of transmission since the adult bees become long-term reservoirs of the pathogen. As previously stated, when *Varroa* is absent, DWV persists at low prevalence and load and only very rarely causes the death of a honeybee colony. This also appears to be the situation in *Varroa*'s original host, the eastern honeybee *Apis cerana*, where DWV loads are consistently low (41, 90–92), as are the *Varroa* levels.

Unlike other honeybee-associated RNA viral pathogens such as slow paralysis virus (SPV) that can kill infected pupae within 3–4 days (72), if DWV is injected either artificially or via *Varroa*, its main effect on the host is to reduce the life span of adults. This occurs in both adults that become

infected by *Varroa* as pupae (93) and adults that become infected after emergence (94, 95). The avirulence of DWV toward honeybee pupae has long been known (88) and is supported by new studies that artificially injected either DWV-A (96) or DWV-A and/or DWV-B (97) and found almost 0% or 18% pupae mortality, respectively, despite high DWV loads being present in the surviving pupae. However, Gisder et al. (64) found the mortality of injected pupae after 10 days was ~65% for DWV-B and ~35% for DWV-A. Khongphinitbunjong et al. (98) showed that another ectoparasitic mite (*Tropilaelaps mercedesae*), whose natural host is the giant honeybee (*A. dorsata*), can also transmit DWV and likewise reduces the infected adults' longevity and emergence weight. This reduction in honeybee life span on its own is sufficient to cause a colony to die during the overwintering period in temperate zones (93, 99). This prediction has since been supported by several studies; for example, Budge et al. (100) found that only DWV was able to negatively affect colony strength in a predictable manner; colonies testing positive for DWV were likely to have fewer combs of bees or brood. Gauthier et al. (101) conducted a large survey of honeybee queens and found that DWV infections had little impact on the health and functional status of the queen, although DWV-infected queens with the deformed wing phenotype do exist (102). This may be linked to the finding that *Varroa* mites rarely invade brood cells containing developing queen pupae (103), so they can become infected by DWV only via an oral route or during feeding by a phoretic mite.

The other challenging issue is the link between DWV and the development of deformed wings. The appearance of honeybees with deformed wings has long been used by beekeepers as an indicator of DWV in their colony; this rule of thumb was supported by Dainat & Neumann (104), who found that the number of deformed bees in the colony was a predictive marker of winter colony losses. It must be noted, however, that on rare occasions deformed wings are caused by other, nonpathogen factors, such as the pupa receiving insufficient fluids during development (1). Bees that develop deformed wings die within a few days after emergence and contribute nothing to the colony. When pupae were injected with DWV at various doses from 1×10^2 to 1×10^7 genome copies, very high proportions (83–100%) developed deformed wings (62, 97), suggesting that DWV is the causative agent of the deformed-wing syndrome. Zhang & Han (57) on the other hand, showed that the injection of a toxic protein by *Varroa* into the pupae also caused the development of deformed wings. Nevertheless, the vast majority of *Varroa*-infested, DWV-infected pupae do not go on to develop deformed wings (105). Furthermore, deformed wings can occur in apparently healthy *Varroa*-free colonies (1, 106). Finally, a dead *Varroa*-free colony containing high loads of DWV (i.e., detectable by ELISA, so greater than $\sim 1 \times 10^7$ particles) was observed with no deformed bees present (S.J. Martin, unpublished observations). Using NGS data, Brettell et al. (105) found a greater difference in DWV sequences between colonies than between overt-acute (deformed) and overt-persistent (nondeformed) bees from the same colony, ruling out the idea that a particular DWV strain or variant was linked with wing deformity. The same conclusion was made by a study that artificially injected either DWV-A or DWV-B into developing pupae (97). A common feature of deformed bees is that they typically have a higher viral load than asymptomatic (overt-persistent) bees, even when these overt-persistently infected bees were parasitized by *Varroa* (105). This led to the idea that deformed wings are caused by only the subset of *Varroa* mites in which DWV is replicating (70), although this idea remains to be supported by direct evidence (see the section titled Mites) and similarly high DWV titers have been detected in both asymptomatic bees and those with deformed wings (97).

While honeybees are frequently coinfected with a number of microbial pathogens (107), synergistic or antagonistic effects between these are largely not well understood. Any such effects may be related to several factors, including competition for host resources or the weakening of host immunity by a primary pathogen resulting in increased susceptibility to a secondary pathogen.

Nosema is a gut parasite that causes damage to the mid-gut epithelial ventricular cells and actively suppresses the honeybees' immune response; both of these effects could increase the virulence of viral pathogens within the bee. Despite this, several studies have found either no or weakly negative correlations between DWV and *Nosema* (108–110). However, Zheng et al. (111) identified a synergy between the two pathogens that was affected by both dose and nutrition, showing the relationship between *Nosema* and DWV to be more complex than initially thought.

The effects of DWV infection on honeybee behavior were first shown by Fujiyuki et al. (38), who linked the presence of DWV (Kakugo virus) in their brains to increased aggression, yet a subsequent study (112) found DWV was not related to aggressiveness in honeybees. More recently, Gisder et al (64) showed using artificial inoculation that cognitive capacity (learning performance and memory retention) was reduced when injected with DWV-B but not when injected with DWV-A. Baracchi et al. (113) found DWV affected the putative recognition compounds (cuticular hydrocarbons) on the surface of newly emerged workers that had been infested by *Varroa* as pupae that could potentially be used by other bees to identify and remove them from the colony, although this behavior has yet to be demonstrated. Finally, Benaets et al. (95) found DWV caused workers to forage prematurely.

IMPACT ON NON-HONEYBEE HOSTS

One of the key questions concerning DWV is what is its impact on other species, especially pollinators that may be particularly vulnerable due to their sharing of foraging networks with honeybees (114). Due to the nature of beekeeping operations, both *A. mellifera* and *Bombus* sp. bees are often kept in higher densities than would naturally be expected, leading to increased contact with other arthropods in the environment. It has been suggested that this can lead to increased cross-species transmission of honeybee-associated pathogens into wild insect populations (115–117). Also, worryingly, it is becoming clear that the presence of *Varroa* is having an indirect effect on DWV prevalence in other species of bees and wasps (11). While much research into non-*Apis* DWV hosts infers cross-species transmission, it has also been demonstrated experimentally from honeybees to both bumblebees (*B. terrestris*) (10) and the more phylogenetically distant small hive beetle (*Aethina tumida*) (118). Thus far, information regarding the degree to which DWV can be pathogenic to non-honeybee hosts is severely lacking, with studies until now having been limited to *Bombus* spp. Genersch et al. (84) identified DWV in *B. terrestris* and *B. pascuorum* with deformed wings, suggesting that this particular pathology is not limited to honeybee hosts, although this is yet to be experimentally tested. Additionally, studies by Fürst et al. (10) and Graystock et al. (119) found that DWV leads to reduced longevity in *B. terrestris* individuals, similar to in honeybees (93), with the additional finding that DWV causes bumblebees' sugar sensitivity to decrease (119).

It is known that a number of generalist RNA viruses originally described as honeybee viruses are also able to cause pathogenicity in other insect species, so the original hosts may not have been honeybees (120). Manley et al. (121) showed that another bee-associated RNA virus, SPV, reduced the longevity of the bumblebee *B. terrestris* under starvation conditions; Meeus et al. (122) demonstrated that another two honeybee-associated viral pathogens, Kashmir bee virus and Israeli acute paralysis virus, both affect offspring production in *B. terrestris* colonies. Furthermore, in fire ants (*Solenopsis invicta*), Hsu et al. (123) showed that Solenopsis invicta virus 1 affected foraging efficiency and altered the food preferences of the ants.

FUTURE DIRECTIONS AND UNANSWERED QUESTIONS

While DWV research has come a long way in the last decade, the virus remains a considerable problem for honeybees, with a number of questions still unanswered. For example, why does the

effect of DWV on longevity remain proportional to the expected longevity for that time of the year, as first shown by Martin (93)? Further gaps remain in our understanding of the evolution of the DWV complex and intracellular competition. The use of cell lines is now beginning. In combination with the development of molecular clones (e.g., 34), this will undoubtedly provide an important tool for understanding how DWV infects cells and how competition between genotypes and recombinants occurs in both natural and laboratory systems. These techniques and reagents will also open up the possibility of developing possible antiviral agents. The ubiquitous nature of DWV has resulted in contamination and subsequent persistence in cell lines, which is a major problem that needs to be overcome (124).

Given what we are learning about the vast host range, there is much research needed into whether and how DWV (and other honeybee-associated viruses) may be affecting non-*Apis* arthropods (125). It is of particular importance to determine the limits of host susceptibility, understand the frequency and mechanisms behind inter- and intraspecies transmission between non-*Apis* arthropods, and identify potential pathogenicity, as any effects on nonmanaged insects would likely not become apparent in all but extreme cases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This article is dedicated to Leslie "Bill" Bailey, PhD, ScD, 1922–2017, the father of honeybee virology.

LITERATURE CITED

1. Bowen-Walker PL, Martin SJ, Gunn A. 1999. The transmission of deformed wing virus between honeybees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni* Oud. *J. Invertebr. Pathol.* 73:101–6
2. Highfield AC, El Nagar A, Mackinder LCM, Laure M-L, Hall MJ, et al. 2009. Deformed wing virus implicated in overwintering honeybee colony losses. *Appl. Environ. Microbiol.* 75:7212–20
3. Schroeder DC, Martin SJ. 2012. Deformed wing virus: the main suspect in unexplained honeybee deaths worldwide. *Virulence* 3:589–91
4. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. 2012. Predictive markers of honey bee colony collapse. *PLOS ONE* 7:e32151
5. Francis RM, Nielsen SL, Kryger P. 2013. Varroa-virus interaction in collapsing honey bee colonies. *PLOS ONE* 8:e57540
6. Mondet F, de Miranda JR, Kretzschmar A, Le Conte Y, Mercer AR. 2014. 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.* 10:e1004323
7. Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, et al. 2012. Global honey bee viral landscape altered by a parasitic mite. *Science* 336:1304–6
8. Ryabov EV, Wood GR, Fannon JM, Moore JD, Bull JC, et al. 2014. A virulent strain of deformed wing virus (DWV) of honeybees (*Apis mellifera*) prevails after *Varroa destructor*-mediated, or *in vitro*, transmission. *PLOS Path.* 10:e1004230
9. Wilfert L, Long G, Leggett HC, Schmid-Hempel P, Butlin R, et al. 2016. Deformed wing virus is a recent global epidemic in honeybees driven by *Varroa* mites. *Science* 351:594–97
10. Fürst MA, McMahon DP, Osborne JL, Paxton RJ, Brown MJF. 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators. *Nature* 506(7488):364–66

11. Santamaria J, Villalobos EM, Brettell LE, Nikaido S, Graham JR, Martin SJ. 2017. Evidence of *Varroa*-mediated deformed wing virus spillover in Hawaii. *J. Invertebr. Pathol.* 151:126–30
12. de Miranda JR, Genersch E. 2010. Deformed wing virus. *J. Invertebr. Pathol.* 103:S48–61
13. Genersch E, Aubert M. 2010. Emerging and re-emerging viruses of the honey bee (*Apis mellifera* L.). *Vet. Res.* 41:54
14. Grozinger CM, Flenniken ML. 2019. Bee viruses: ecology, pathogenicity, and impacts. *Annu. Rev. Entomol.* 64:205–26
15. Nazzi F, Conte YL. 2016. Ecology of *Varroa destructor*, the major ectoparasite of the Western honey bee, *Apis mellifera*. *Annu. Rev. Entomol.* 61:417–32
16. Nazzi F, Pennacchio F. 2018. Honey bee antiviral immune barriers as affected by multiple stress factors: a novel paradigm to interpret colony health decline and collapse. *Viruses* 10:159
17. Bailey L, Ball BV. 1991. *Honey Bee Pathology*. London: Academic. 2nd ed.
18. Allen M, Ball B. 1996. The incidence and world distribution of honey bee viruses. *Bee World* 77:141–62
19. Carreck NL, Ball BV, Martin SJ. 2010. Honey bee colony collapse and changes in viral prevalence associated with *Varroa destructor*. *J. Apic. Res.* 49:93–94
20. de Miranda JR, Bailey L, Ball BV, Blanchard P, Budge GE, et al. 2013. Standard methods for virus research in *Apis mellifera*. *J. Apic. Res.* 52(4):1–56
21. Martin SJ, Ball BV, Carreck NL. 2010. Prevalence and persistence of deformed wing virus (DWV) in untreated or acaricide-treated *Varroa destructor* infested honey bee (*Apis mellifera* L.) colonies. *J. Apic. Res.* 49:72–79
22. Kajobe R, Marris G, Budge G, Laurenson L, Cordoni G, et al. 2010. First molecular detection of a viral pathogen in Ugandan honey bees. *J. Invertebr. Pathol.* 104:153–56
23. Roberts JM, Anderson DL, Durr PA. 2017. Absence of deformed wing virus and *Varroa destructor* in Australia provides unique perspectives on honeybee viral landscapes and colony losses. *Sci. Rep.* 7:6925
24. Shutler D, Head K, Burgher-MacLellan KL, Colwell MJ, Levitt AL, et al. 2014. Honey bee *Apis mellifera* parasites in the absence of *Nosema cerana* fungi and *Varroa destructor* mites. *PLOS ONE* 9:e98599
25. Berényi O, Bakonyi T, Derakhshifar I, Köglberger H, Nowotny N. 2006. Occurrence of six honeybee viruses in diseased Austrian apiaries. *Appl. Environ. Microbiol.* 72:2414–20
26. Griffiths DA, Bowman CE. 1981. World distribution of the mite *Varroa jacobsoni*, a parasite of honeybees. *Bee World* 62:154–63
27. Boncristiani H, Li J, Evans JD, Pettis J, Chen Y. 2011. Scientific note on PCR inhibitors in the compound eyes of honey bees, *Apis mellifera*. *Apidologie* 42:457–60
28. Lanzi G, de Miranda JR, Boniotti MB, Cameron CE, Lavazza A, et al. 2006. Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). *J. Virol.* 80:4998–5009
29. Ongus JR, Roode EC, Pleij CWA, Vlak JM, van Oers MM. 2006. The 5′ non-translated region of *Varroa destructor* virus-1 (genus *Iflavirus*): structure prediction and IRES activity in *Lymantria dispar* cells. *J. Gen. Virol.* 87:3397–407
30. van Oers MM. 2010. Genomics and biology of *Iflaviruses*. In *Insect Virology*, ed. S Asgari, K Johnson, pp. 231–50. Norfolk, UK: Caister Academic
31. Sánchez-Eugenia R, Goikolea J, Gil-Cartón D, Sánchez-Magraner L, Guérin DMA. 2015. Triatoma virus recombinant VP4 protein induces membrane permeability through dynamic pores. *J. Virol.* 89:4645–54
32. Škubník K, Nováček J, Füzik T, Přidal A, Paxton RJ, Plevka P. 2017. Structure of deformed wing virus, a major honey bee pathogen. *PNAS* 114:3210–15
33. Organtini LJ, Shingler KL, Ashley RE, Capaldi EA, Durrani K, et al. 2017. Honey bee deformed wing virus structures reveal that conformational changes accompany genome release. *J. Virol.* 91:e01795
34. Lamp B, Url A, Seitz K, Eichhorn J, Riedel C, et al. 2016. Construction and rescue of a molecular clone of deformed wing virus (DWV). *PLOS ONE* 11:e0164639
35. Loope KJ, Baty JW, Lester PJ, Wilson Rankin EE. 2019. Pathogen shifts in a honeybee predator following the arrival of the *Varroa* mite. *Proc. R. Soc. B* 286:20182499
36. Mordecai GJ, Wilfert L, Martin SJ, Jones IM, Schroeder DC. 2016. Diversity in a honey bee pathogen: first report of a third master variant of the Deformed Wing Virus quasispecies. *ISME J.* 10:1264–73

37. Mordecai GJ, Brettell L, Martin SJ, Dixon D, Jones IM, Schroeder DC. 2016. Superinfection exclusion and the long-term survival of honey bees in Varroa-infested colonies. *ISEM J.* 10:1182–91
38. Fujiyuki T, Takeuchi H, Ono M, Ohka S, Sasaki T, et al. 2004. RNA-like virus identified in the brains of aggressive worker honeybees. *J. Virol.* 78:1093–100
39. Ongus JR, Peters D, Bonmatin JM, Bengsch E, Vlak JM, van Oers MM. 2004. Complete sequence of a picorna-like virus of the genus *Iflavirus* replicating in the mite *Varroa destructor*. *J. Gen. Virol.* 85:3747–55
40. Levin S, Sela N, Chejanovsky N. 2016. Two novel viruses associated with the *Apis mellifera* pathogenic mite *Varroa destructor*. *Sci. Rep.* 6:37710
41. Levin S, Sela N, Erez T, Nestel D, Pettis J, et al. 2019. New viruses from the ectoparasite mite *Varroa destructor* infesting *Apis mellifera* and *Apis cerana*. *Viruses* 11:94
42. Kevill JL, Highfield A, Mordecai GJ, Martin SJ, Schroeder DC. 2017. ABC assay: method development and application to quantify the role of three DWV master variants in overwinter colony losses of European honey bees. *Viruses* 9:314
43. Ryabov EV, Childers AK, Chen Y, Madella S, Nessa A, Evans JD. 2017. Recent spread of Varroa destructor virus-1, a honey bee pathogen, in the United States. *Sci. Rep.* 7:17447
44. McMahon DP, Natsopoulou ME, Doublet V, Furst M, Weging S, et al. 2016. Elevated virulence of an emerging viral genotype as a driver of honeybee loss. *Proc. R. Soc. B* 283:20160811
45. Radzevičiūtė R, Theodorou P, Husemann M, Japoshvili G, Kirkitadze G, et al. 2017. Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan. *J. Invertebr. Pathol.* 146:14–23
46. De Souza FS, Kevill JL, Correia-Oliveira ME, de Carvalho CAL, Martin SJM. 2019. Occurrence of deformed wing virus variants in the stingless *Melipona subnitida* and honey bee *Apis mellifera* populations in Brazil. *Gen. J. Viol.* 100:289–94
47. Jamnikar-Ciglenecki U, Ocepek MP, Toplak I. 2018. Genetic diversity of deformed wing virus from *Apis mellifera carnica* (Hymenoptera: Apidae) and varroa mite (Mesostigmata: Varroidae). *J. Econ. Entomol.* 312:11–19
48. Moore J, Jironkin A, Chandler D, Burroughs N, Evans DJ, et al. 2011. Recombinants between *Deformed wing virus* and *Varroa destructor virus-1* may prevail in *Varroa destructor*-infested honeybee colonies. *J. Gen. Virol.* 92:156–61
49. Zioni N, Soroker V, Chejanovsky N. 2011. Replication of *Varroa destructor* virus 1 (VDV-1) and a *Varroa destructor* virus 1–deformed wing virus recombinant (VDV-1–DWV) in the head of the honey bee. *Virology* 417:106–12
50. Wang H, Xie J, Shreeve TG, Ma J, Pallett DW, et al. 2013. Sequence recombination and conservation of *Varroa destructor virus-1* and *Deformed wing virus* in field collected honey bees (*Apis mellifera*). *PLOS ONE* 8:e74508
51. Natsopoulou ME, McMahon DP, Doublet V, Frey E, Rosenkranz P, Paxton RJ. 2017. The virulent, emerging genotype B of *Deformed wing virus* is closely linked to overwinter honeybee worker loss. *Sci. Rep.* 7:41045
52. Cornman RS. 2017. 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.* 149:44–50
53. Dalmon A, Desbiez C, Coulon M, Thomasson M, Le Conte Y, et al. 2017. Evidence for positive selection and recombination hotspots in *Deformed wing virus* (DWV). *Sci. Rep.* 7:41045
54. Chejanovsky N, Ophir R, Schwager MS, Slabezki Y, Grossman S, Cox-Foster D. 2014. Characterization of viral siRNA populations in honey bee colony collapse disorder. *Virology* 454:176–83
55. Brettell LE, Martin SJ. 2017. Oldest *Varroa* tolerant honey bee population provides insight into the origins of the global decline of honey bees. *Sci. Rep.* 7:45953
56. Annoscia D, Brown S, Di Prisco G, De Paoli G, Fabbro SD, et al. 2018. Haemolymph removal by the parasite *Varroa destructor* can trigger the proliferation of the Deformed Wing Virus in mite infested bees (*Apis mellifera*), contributing to enhanced pathogen virulence. bioRxiv 257667. **https://doi.org/10.1101/257667**

57. Zhang Y, Han R. 2018. A saliva protein of *Varroa* mites contributes to the toxicity toward *Apis cerana* and the DWV elevation in *A. mellifera*. *Sci. Rep*. 8:3387
58. Di Prisco G, Zhang X, Pennacchio F, Caprio E, Li J, et al. 2011. Dynamics of persistent and acute deformed wing virus infections in honey bees (*Apis mellifera*). *Viruses* 3:2425–41
59. DeGrandi-Hoffman G, Chen Y, Huang E, Huang MH. 2010. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). *J. Insect Physiol.* 56:1184–91
60. Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, et al. 2013. Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *PNAS* 110:18466–71
61. Shah KS, Evans EC, Pizzorno MC. 2009. Localization of deformed wing virus (DWV) in the brains of the honeybee, *Apis mellifera* Linnaeus. *Virol. J.* 6:182
62. Mockel N, Gisder S, Genersch E. 2011. Horizontal transmission of deformed wing virus: pathological consequences in adult bees (*Apis mellifera*) depend on the transmission route. *J. Gen. Virol.* 92:370–77
63. Mazzei M, Carrozza ML, Luisi E, Forzan M, Giusti M, et al. 2014. Infectivity of DWV associated to flower pollen: experimental evidence of a horizontal transmission route. *PLOS ONE* 9:e113448
64. Gisder S, Möckel N, Eisenhardt D, Genersch E. 2018. *In vivo* evolution of viral virulence: switching of deformed wing virus between hosts results in virulence changes and sequence shifts. *Environ. Microbiol.* 20:4612–28
65. Yue C, Schröder M, Gisder S, Genersch E. 2007. Vertical transmission routes for deformed wing virus of honeybees (*Apis mellifera*). *J. Gen. Virol.* 88:2329–36
66. Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, et al. 2010. The German bee monitoring project: a long-term study to understand periodically high winter losses of honey bee colonies. *Apidologie* 41:332–52
67. Nordstrom S. 2003. Distribution of deformed wing virus within honey bee (*Apis mellifera*) brood cells infested with the ectoparasitic mite *Varroa destructor*. *Exp. Appl. Acarol.* 29:293–302
68. Kielmanowicz MG, Inberg A, Lerner IM, Golani Y, Brown N, et al. 2015. Prospective large-scale field study generates predictive model identifying major contributors to colony losses. *PLOS Pathog.* 11:e1004816
69. Wu Y, Dong X, Kadowaki T. 2017. Characterization of the copy number and variants of Deformed Wing Virus (DWV) in the pairs of honey bee pupa and infesting *Varroa destructor* or *Tropilaelaps mercedesae*. *Front. Microbiol.* 8:1558
70. Gisder S, Aumeier P, Genersch E. 2009. Deformed wing virus: replication and viral load in mites (*Varroa destructor*). *J. Gen. Virol.* 90:463–67
71. Campbell EM, Budge GE, Watkins M, Bowman AS. 2016. Transcriptome analysis of the synganglion from the honey bee mite, *Varroa destructor* and RNAi knockdown of neural peptide targets. *Insect Biochem. Mol. Biol.* 70:116–26
72. Santillán-Galicia MT, Ball BV, Clark SJ, Alderson PG. 2010. Transmission of deformed wing virus and slow paralysis virus to adult bees (*Apis mellifera* L.) by *Varroa destructor*. *J. Apic. Res.* 49:141–48
73. Erban T, Harant K, Hubalek M, Vitamvas P, Kamler M, et al. 2015. In-depth proteomic analysis of *Varroa destructor*: detection of DWV-complex, ABPV, VdMLV and honeybee proteins in the mite. *Sci. Rep.* 5:13907
74. Martin SJ, Carreck NL, Ball BV. 2013. The role of deformed wing virus in the initial collapse of varroa infested honey bee colonies in the UK. *J. Apic. Res.* 52:251–58
75. Boncristiani HF, Di Prisco G, Pettis JS, Hamilton M, Chen YP. 2009. Molecular approaches to the analysis of deformed wing virus replication and pathogenesis in the honey bee, *Apis mellifera*. *Virol. J.* 6:221
76. Remnant EJ, Shi M, Buchmann G, Blacquière T, Holmes EC, et al. 2017. A diverse range of novel RNA viruses in geographically distinct honey bee populations. *J. Virol.* 91:e00158-17
77. de Miranda JR, Fries I. 2008. Venereal and vertical transmission of deformed wing virus in honeybees (*Apis mellifera* L.). *J. Invertebr. Pathol.* 98:184–89

78. Amiri E, Kryger P, Meixner MD, Strand MK, Tarpy DR, Rueppell O. 2018. Quantitative patterns of vertical transmission of deformed wing virus in honey bees. *PLOS ONE* 13:e0195283
79. Amiri E, Meixner MD, Kryger P. 2016. Deformed wing virus can be transmitted during natural mating in honey bees and infect the queens. *Sci. Rep.* 6:33065
80. Singh R, Levitt AL, Rajotte EG, Holmes EC, Ostiguy N, et al. 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-*Apis* hymenopteran species. *PLOS ONE* 5:e14357
81. Ryabov EV. 2016. Invertebrate RNA virus diversity from a taxonomic point of view. *J. Invertebr. Pathol.* 147:37–50
82. Shi M, Lin X-D, Tian J-H, Chen L-J, Chen X, et al. 2016. Redefining the invertebrate RNA virosphere. *Nature* 540:539–43
83. Zhang X, He SY, Evans JD, Pettis JS, Yin GF, Chen YP. 2012. New evidence that deformed wing virus and black queen cell virus are multi-host pathogens. *J. Invertebr. Pathol.* 109:156–59
84. Genersch E, Yue C, Fries I, de Miranda JR. 2006. Detection of deformed wing virus, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. *J. Invertebr. Pathol.* 91:61–63
85. Levitt AL, Singh R, Cox-Foster DL, Rajotte E, Hoover K, et al. 2013. Cross-species transmission of honey bee viruses in associated arthropods. *Virus Res.* 176:232–40
86. Tehel A, Brown MJ, Paxton RJ. 2016. Impact of managed honey bee viruses on wild bees. *Curr. Opin. Virol.* 19:16–22
87. Dolezal AG, Hendrix SD, Scavo NA, Carrillo-Tripp J, Harris MA, et al. 2016. Honey bee viruses in wild bees: viral prevalence, loads, and experimental inoculation. *PLOS ONE* 11:e0166190
88. Evison SEF, Roberts KE, Laurenson L, Pietravalle S, Hui J, et al. 2012. Pervasiveness of parasites in pollinators. *PLOS ONE* 7:e30641
89. McMahon DP, Wilfert L, Paxton RJ, Brown MJF. 2018. Emerging viruses in bees: from molecules to ecology. *Adv. Virus Res.* 101:251–91
90. Kojima Y, Toki T, Morimoto T, Yoshiyama M, Kimura K, Kadowaki T. 2011. Infestation of Japanese native honey bees by tracheal mite and virus from non-native European honey bees in Japan. *Microb. Ecol.* 62:895–906
91. Li J, Qin H, Wu J, Sadd BM, Wang X, et al. 2012. The prevalence of parasites and pathogens in Asian honeybees *Apis cerana* in China. *PLOS ONE* 7:e47955
92. Choe SE, Nguyen LTK, Noh JH, Koh HB, Jean YH, et al. 2012. Prevalence and distribution of six bee viruses in Korean *Apis cerana* populations. *J. Invertebr. Pathol.* 109:330–33
93. Martin SJ. 2001. The role of *Varroa* and viral pathogens in the collapse of honey bee colonies: a modelling approach. *J. Appl. Ecol.* 38:1082–93
94. Dainat B, Evans JD, Chen YP, Gauthier L, Neumann P. 2011. Dead or alive: deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. *J. Appl. Environ. Microbiol.* 78:981–87
95. Benaets K, Van Geystelen A, Cardoen D, De Smet L, de Graaf DC, et al. 2017. Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival. *Proc. R. Soc. B* 284:20162149
96. Remnant EJ, Mather N, Gillard TL, Yagound B, Beekman M. 2019. Direct transmission by injection affects competition among RNA viruses in honeybees. *Proc. R. Soc. B* 286:20182452
97. Tehel A, Vu Q, Bigot D, Gogol-Döring A, Koch P, et al. 2019. The two prevalent genotypes of an emerging infectious disease, *Deformed wing virus*, cause equally low pupal mortality and equally high wing deformities in host honey bees. *Viruses* 11:114
98. Khongphinitbunjong K, Neumann P, Chantawannakul P, Williams GR. 2016. The ectoparasitic mite *Tropilaelaps mercedesae* reduces western honey bee, *Apis mellifera*, longevity and emergence weight, and promotes Deformed wing virus infections. *J. Invertebr. Pathol.* 137:38–42
99. Sumpter D, Martin SJ. 2004. The dynamics of virus epidemics in *Varroa*-infested honeybee colonies. *J. Anim. Ecol.* 73:51–63
100. Budge GE, Pietravalle S, Brown M, Laurenson L, Jones B, et al. 2015. Pathogens as predictors of honey bee colony strength in England and Wales. *PLOS ONE* 10:e0133228

101. Gauthier L, Ravallec M, Tournaire M, Cousserans F, Bergoin M, et al. 2011. Viruses associated with ovarian degeneration in *Apis mellifera* L. queens. *PLOS ONE* 6:e16217
102. Williams GR, Rogers RE, Kalkstein AL, Taylor BA, Shutler D, Ostiguy N. 2009. Deformed wing virus in western honey bees (*Apis mellifera*) from Atlantic Canada and the first description of an overtly-infected emerging queen. *J. Invertebr. Pathol.* 101:77–79
103. Calderone NW, Lin S, Kuenen LPS. 2002. Differential infestation of honey bee *Apis mellifera* worker and queen brood by the parasitic mite *Varroa destructor*. *Apidologie* 33:389–98
104. Dainat B, Neumann P. 2013. Clinical signs of deformed wing virus infection are predictive markers for honey bee colony losses. *J. Invertebr. Pathol.* 112:278–80
105. Brettell LE, Mordecai GJ, Schroeder DC, Jones IM, da Silva JR, et al. 2017. A comparison of deformed wing virus in deformed and asymptomatic honey bees. *Insects* 8:28
106. Forsgren E, Fries I, de Miranda JR. 2012. Adult honey bees (*Apis mellifera*) with deformed wings discovered in confirmed varroa-free colonies. *J. Apic. Res.* 51:136–38
107. McMahon DP, Fürst MA, Caspar J, Theodorou P, Brown MJF, Paxton RJ. 2015. A sting in the spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. *J. Anim. Ecol.* 84:615–24
108. Martin SJ, Hardy J, Villalobos E, Martin-Hernandez R, Nikaido S, Higes M. 2013. Do the honeybee pathogens *Nosema ceranae* and deformed wing virus act synergistically? *Environ. Microbiol. Rep.* 5:506–10
109. Costa C, Tanner G, Lodesani M, Maistrello L, Neumann P. 2011. Negative correlation between *Nosema ceranae* spore loads and deformed wing virus infection levels in adult honey bee workers. *J. Invertebr. Pathol.* 108:224–25
110. Doublet V, Natsopoulou ME, Zschiesche L, Paxton R. 2015. Within-host competition among the honey bee pathogens *Nosema ceranae* and Deformed wing virus is asymmetric and to the disadvantage of the virus. *J. Invertebr. Pathol.* 124:31–34
111. Zheng H-Q, Gong H-R, Huang S-K, Sohr A, Hu F-L, Chen YP. 2015. Evidence of the synergistic interaction of honey bee pathogens *Nosema ceranae* and Deformed wing virus. *Vet. Microbiol.* 177:1–6
112. Rortais A, Tentcheva D, Papachristoforou A, Gauthier L, Arnold G, et al. 2006. Deformed wing virus is not related to honey bees' aggressiveness. *Virol. J.* 3:61
113. Baracchi D, Fadda A, Turillazzi S. 2012. Evidence for antiseptic behaviour towards sick adult bees in honey bee colonies. *J. Insect Physiol.* 58:1589–96
114. Li J, Peng W, Wu J, Strange JP, Boncristiani H, Chen Y, 2011. Cross-species infection of deformed wing virus poses a new threat to pollinator conservation. *J. Econ. Entomol.* 104:732–39
115. Colla SR, Otterstatter MC, Gegear RJ, Thomson JD. 2006. Plight of the bumble bee: pathogen spillover from commercial to wild populations. *Biol. Conserve.* 129:461–67
116. Graystock P, Yates K, Evison SE, Darvill B, Goulson D, Hughes WO. 2013. The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. *J. Appl. Ecol.* 50:1207–15
117. Murray TE, Coffey MF, Kehoe E, Horgan FG. 2013. Pathogen prevalence in commercially reared bumble bees and evidence of spillover in conspecific populations. *Biol. Conserv.* 159:269–76
118. Eyer M, Chen YP, Schäfer MO, Pettis J, Neumann P. 2009. Small hive beetle, *Aethina tumida*, as a potential biological vector of honeybee viruses. *Apidologie* 40:419–28
119. Graystock P, Meeus I, Smagghe G, Goulson D, Hughes WO. 2016. The effects of single and mixed infections of *Apicystis bombi* and deformed wing virus in *Bombus terrestris*. *Parasitol.* 143:358–65
120. Gisder S, Genersch E. 2017. Viruses of commercialized insect pollinators. *J. Invertebr. Pathol.* 147:51–59
121. Manley R, Boots B, Wilfert L. 2017. Condition-dependent virulence of slow bee paralysis virus in *Bombus terrestris*: Are the impacts of honeybee viruses in wild pollinators underestimated? *Oecologia* 184:305–15
122. Meeus I, de Miranda JR, de Graaf DC, Wäckers F, Smagghe G. 2014. Effect of oral infection with Kashmir bee virus and Israeli acute paralysis virus on bumblebee (*Bombus terrestris*) reproductive success. *J. Invertebr. Pathol.* 121:64–69
123. Hsu H-W, Chiu M-C, Shoemaker D, Yang C-CS. 2018. Viral infections in fire ants lead to reduced foraging activity and dietary changes. *Sci. Rep.* 8:13498

124. Carrillo-Tripp J, Dolezal AG, Goblirsch MJ, Miller WA, Toth AL, et al. 2016. *In vivo* and *in vitro* infection dynamics of honey bee viruses. *Sci. Rep.* 6:22265
125. McMenamin AJ, Flenniken ML. 2018. Recently identified bee viruses and their impact on three bee pollinators. *Curr. Opin. Insect Sci.* 26:120–29
126. De Smet L, Ravoet J, de Miranda JR, Wenseleers T, Mueller MY, et al. 2012. BeeDoctor, a versatile MLPA-based diagnostic tool for screening bee viruses. *PLOS ONE* 7:e47953
127. Shumkova R, Neov B, Sirakova D, Georgieva A, Gadjev D, et al. 2018. Molecular detection and phylogenetic assessment of six honeybee viruses in *Apis mellifera* L. colonies in Bulgaria. *PeerJ* 6:e5077
128. Gajger IT, Kolodziejek J, Bakonyi T, Nowotny N. 2014. Prevalence and distribution patterns of seven different honeybee viruses in diseased colonies: a case study from Croatia. *Apidologie* 45:701–6
129. Mouret C, Lambert O, Piroux M, Beaudeau F, Provost B, et al. 2013. Prevalence of 12 infectious agents in field colonies of 18 apiaries in western France. *Revue Méd. Vét.* 164:577–82
130. Tencheva D, Gauthier L, Zappulla N, Dainat B, Cousserans F, et al. 2004. Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. *Appl. Environ. Microbiol.* 70:7185–91
131. Pohorecka K, Bober A, Skubida M, Zdanska D. 2011. Epizootic status of apiaries with massive losses of bee colonies (2008–2009). *J. Apic. Sci.* 55:137–48
132. Simeunović P, Stevanović J, Vidanović D, Nišavić J, Radović D, et al. 2014. A survey of deformed wing virus and acute bee paralysis virus in honey bee colonies from Serbia using real-time RT-PCR. *Acta Vet.* 64:81–92
133. Cirkovic D, Stevanovic J, Glavinic U, Aleksic N, Djuric S, et al. 2018. Honey bee viruses in Serbian colonies of different strength. *PeerJ* 6:e5887
134. Antúnez K, Anido M, Garrido-Bailón E, Botías C, Zunino P, et al. 2012. Low prevalence of honeybee viruses in Spain during 2006 and 2007. *Res. Vet. Sci.* 93:1441–45
135. Cepero A, Ravoet J, Gómez-Moracho T, Bernal J, Del Nozal M, et al. 2014. Holistic screening of collapsing honey bee colonies in Spain: a case study. *BMC Res. Notes* 7:649
136. Buendía M, Martín-Hernández R, Ornosa C, Barrios L, Bartolomé C. Higes M. 2018. Epidemiological study of honeybee pathogens in Europe: the results of Castilla-La Mancha (Spain). *Span. J. Agric. Res.* 16:e0502
137. Berthoud H, Imdorf A, Haueter M, Radloff S, Neumann P. 2010. Virus infections and winter losses of honey bee colonies (*Apis mellifera*). *J. Apic. Res.* 49:60–65
138. Thompson CE, Biesmeijer JC, Allnutt TR, Pietravalle S, Budge GE. 2014. Parasite pressures on feral honey bees (*Apis mellifera sp.*). *PLOS ONE* 9:e105164
139. Ai H, Yan X, Han R. 2012. Occurrence and prevalence of seven bee viruses in *Apis mellifera* and *Apis cerana* apiaries in China. *J. Invertebr. Pathol.* 109:160–64
140. Yang B, Peng G, Li T, Kadowaki T. 2013. Molecular and phylogenetic characterization of honey bee viruses, *Nosema* microsporidia, protozoan parasites, and parasitic mites in China. *Ecol. Evol.* 3:298–311
141. Forsgren E, Wei S, Guiling D, Zhiguang L, Van Tran T, et al. 2014. Preliminary observations on possible pathogen spill-over from *Apis mellifera* to *Apis cerana*. *Apidologie* 46:265–75
142. Rodríguez M, Vargas M, Antúnez K, Gerding M, Ovídio CF, Zapata N. 2014. Prevalence and phylogenetic analysis of honey bee viruses in the Biobío region of Chile and their association with other honey bee pathogens. *Chil. J. Agric. Res.* 74:170–77
143. Guzman-Novoa E, Hamiduzzaman MM, Espinosa-Montaño LG, Correa-Benítez A, Anguiano-Baez R, Ponce-Vázquez R. 2012. First detection of four viruses in honey bee (*Apis mellifera*) workers with and without deformed wings and *Varroa destructor* in Mexico. *J. Apic. Res.* 51:342–46
144. Chen YP, Siede R. 2007. Honey bee viruses. *Adv. Virus Res.* 70:33–80
145. Haddad N, Al-Gharaibeh M, Nasher A, Anaswah E, Alammari Y, Horth L. 2018. Scientific note: molecular detection of pathogens in unhealthy colonies of *Apis mellifera jemenitica*. *Apidologie* 49:84–88
146. Soroker V, Hetzroni A, Yakobson B, David D, David A, et al. 2011. Evaluation of colony losses in Israel in relation to the incidence of pathogens and pests. *Apidologie* 42:192–99
147. Loucif-Ayad W, Chefrour A, Algharibeh M, Haddad N. 2013. First detection of *Deformed wing virus* of honeybees in Algeria. *Phytoparasitica* 41:445–47

148. Ongus JR, Fombong AT, Irungu J, Masiga D, Raina S. 2017. Prevalence of common honey bee pathogens at selected apiaries in Kenya, 2013–2014. *Int. J. Trop. Insect Sci.* 38:58–70
149. Strauss U, Human H, Gauthier L, Crewe RM, Dietemann V, Pirk CWW. 2013. Seasonal prevalence of pathogens and parasites in the savannah honeybee (*Apis mellifera scutellata*). *J. Invertebr. Pathol.* 114:45–52
150. Abdi K, Belguith K, Hamdi C, Souissi Y, Essanaa J, et al. 2018. Parasites-*Iflavirus* association and emergence of three master variants of DWV affecting *Apis mellifera intermissa* in Tunisian apiaries. *Bull. Insectol.* 71:273–82