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Deformed wing virus

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ABSTRACT

Deformed wing virus (DWV; Iflaviridae) is one of many viruses infecting honeybees and one of the most heavily investigated due to its close association with honeybee colony collapse induced by *Varroa destructor*. In the absence of *V. destructor* DWV infection does not result in visible symptoms or any apparent negative impact on host fitness. However, for reasons that are still not fully understood, the transmission of DWV by *V. destructor* to the developing pupae causes clinical symptoms, including pupal death and adult bees emerging with deformed wings, a bloated, shortened abdomen and discolouration. These bees are not viable and die soon after emergence. In this review we will summarize the historical and recent data on DWV and its relatives, covering the genetics, pathobiology, and transmission of this important viral honeybee pathogen, and discuss these within the wider theoretical concepts relating to the genetic variability and population structure of RNA viruses, the evolution of virulence and the development of disease symptoms.

1. Introduction

Wing deformities in honeybees (*Apis mellifera* L.) (Fig. 1) have long been associated with a virus, appropriately named deformed wing virus (DWV), transmitted by the ectoparasitic mite *Varroa destructor* (*V. destructor*). These deformities are symptomatic of the final stages of colony collapse due to uncontrolled mite infestation and as a result DWV is now one of the main viruses associated with the collapse of honeybee colonies due to infestation with *V. destructor* (Ball, 1983; Ball and Allen, 1988; Bowen-Walker et al., 1999; Nordström et al., 1999; Ribière et al., 2008; Sumpter and Martin, 2004; Tentcheva et al., 2004b). Examination of the first samples of deformed bees in the early 1980s revealed very high titers of a rather unstable icosahedral virus with a single strand RNA genome (Bailey and Ball, 1991). Subsequent studies using a variety of techniques confirmed a nearly 100% association of the wing deformities with highly elevated titers of the virus and reduced titers in phenotypically normal bees from the same colonies (Bowen-Walker et al., 1999; Chen et al., 2005a; Nordström, 2000, 2003; Tentcheva et al., 2006., 2004a; Yue and Genersch, 2005). Nevertheless, a direct causal link between the virus and the symptoms has been difficult to establish, mostly due to the difficulty of excluding other pathogens from contributing to the symptoms.

In the absence of *V. destructor* DWV normally persists at low levels within the bee colony with no detrimental effect, and can be found in all life stages, from egg to adult bee as well as in the glandular secretions used to feed larvae and the queen (Chen et al., 2005a, 2006b; Yue and Genersch, 2005). DWV is transmitted between bees by both horizontal (faecal–cannibal–oral) and vertical (parent–offspring) transmission (Chen et al., 2006b, 2005b; Yue and Genersch, 2005; Yue et al., 2006, 2007; de Miranda and Fries, 2008).

In this review, we will briefly describe the history and distribution of DWV. Second, we will discuss the genetics of DWV and its close relatives, Varroa destructor virus-1 (VaDV-1) (Ongus et al., 2004) and Kakugo virus (KV) (Fujiyuki et al., 2004) with special emphasis on the quasispecies concept (Eigen, 1993, 1996) which may help to explain the sequence diversity found within the DWV/VaDV-1/KV group. Third, we will introduce the terms *covert infection* and *overt infection* and discuss how these terms can be applied to better understand the pathology of DWV. Finally, we will describe the transmission routes of DWV and the distinction between horizontal and vertical transmission, which is important for moulding pathogen virulence both at the individual bee level and at the colony level (Chen et al., 2006a; Fries and Camazine, 2001).

2. History and distribution of DWV

The history, distribution and pathology of DWV have been reviewed in great detail recently (Ribière et al., 2008). DWV was first

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Fig. 1. Adult bee with deformed wings; one of the mites which parasitized the pupa is still clinging to the bee.

known as Egypt bee virus (EBV), which was isolated from asymptomatic adult bees collected in Egypt in 1977 (Bailey et al., 1979). Subsequently, a virus isolated from deformed adult bees collected in Japan in 1982 was found to be distantly related to EBV by serology, and briefly named the Japanese isolate of EBV, before being renamed deformed wing virus after the symptoms with which it was closely associated (Bailey and Ball, 1991; Ribière et al., 2008). Nothing further is known of EBV, while DWV has since become synonymous world-wide with colony collapse due to varroa infestation (Ribière et al., 2008). Due to this close association with varroa infestation, DWV has currently a global distribution, following in the wake of the dispersal of *V. destructor* during the 1970s and 1980s (Allen and Ball, 1996; Ellis and Munn, 2005). However, given its current prominence it is difficult to imagine that this virus was completely unknown prior to the spread of varroa and was essentially the last virus to be discovered by Bailey, Ball, and colleagues at Rothamsted Research in Harpenden, England (Bailey and Ball, 1991; Ball, 1983). This raises the question of whether DWV, like varroa, has its origins in South East Asia, and perhaps in the Asian honeybee *Apis cerana*, the original host of varroa, or whether it is an original virus of *A. mellifera*. Partial answers to this question come from a case of clinical DWV in Britain in the mid-1980s (Ball, 1989), prior to the first record of varroa there (Paxton, 1992), and from records of a low incidence of DWV in Northern Scandinavia, beyond the expansion front of varroa (de Miranda and Fries, 2008; Yue and Genersch, 2005). However, these observations come from regions dominated by managed beekeeping, where DWV may have arrived through trade in bees and queens from infected areas. Ultimately the question of whether DWV existed in *A. mellifera* prior to varroa will be best answered through analysis of historical samples, or from island populations known to be isolated from before the arrival of varroa. Due to the dominant role of varroa in DWV epidemiology, the seasonal distribution of DWV closely follows that of the mite, growing in prevalence and titer as the bee season progresses (Gauthier et al., 2007; Nordström et al., 1999; Tentcheva et al., 2004b) and receding to lower levels with effective mite control (Sumpter and Martin, 2004).

As is the case for honeybee viruses, with the exception of chronic bee paralysis virus (CBPV) (Celle et al., 2008), there has been very little specific research into the host distribution of DWV. DWV has been detected in commercial and wild bumble bees (*Bombus terrestris*, *Bombus pascorum*) displaying wing deformities, with infected honeybees almost certainly the source of the infection in both cases (Genersch et al., 2006), as well as by serology in *A. cerana* and the dwarf bee *A. florea* (Allen and Ball, 1996; Ellis and Munn, 2005). Not surprisingly, given their parasitic lifestyle and key role in virus transmission, DWV is also detected in *V. destructor* (Bowen-Walker et al., 1999; Chen et al., 2005a; Gauthier et al., 2007; Nordström et al., 1999; Tentcheva et al., 2004a; Yue and Genersch, 2005) and in *Tropilaelaps mercedesae*, a similar hemolymph-feeding ectoparasite (Dainat et al., 2009; Forsgren et al., 2009). DWV has also been detected in the small hive beetle *Aethina tumida*, a scavenger in the hive rather than a parasite of honeybees (Eyer et al., 2008). The evidence that detection of DWV in these bees, pests, and parasites represents an active infection, and not simply passive acquisition, is mixed. Indirect evidence includes the regular observation of higher virus titers in varroa mites than in the corresponding bee host (Bowen-Walker et al., 1999; Nordström, 2000). Better evidence for replication is the localization of the virus within the tissues and cells of the host, rather than just the body cavity, especially if associated with crystalline arrays typical of active virus replication. In *V. destructor* this has been shown for VaDV-1 (Zhang et al., 2007), which is closely related to DWV (Ongus et al., 2004), but it has still to be shown for DWV (Santillan-Galicia et al., 2008). However, the best evidence of true infection is the detection of virus proteins or nucleic acid species that are only produced during virus replication, such as the non-structural proteins or the replicative, negative-strand RNA. The latter approach has become particularly popular recently, through the use of strand-specific RT-PCR assays (Gisder et al., 2009; Ongus et al., 2004; Yue and Genersch, 2005; Dainat et al., 2009; Eyer et al., 2008). These studies clearly show that DWV replication occurs in some varroa mites, indicating an active infection, while the majority of mites passively acquire and mechanically transmit the virus (Gisder et al., 2009; Yue and Genersch, 2005, see below). It is probable that a similar situation exists for *Tropilaelaps* mites and the small hive beetle.

3. Genetics of DWV

DWV produces a 30 nm icosahedral particle consisting of a single, positive strand RNA genome and three major structural proteins (Bailey and Ball, 1991; Lanzi et al., 2006; Ongus et al., 2004), characteristics that are common to many picorna-like insect viruses (Moore and Eley, 1991). The genome organization of DWV and VaDV-1 (Fig. 2) is typical of the iflaviruses, a genus of the recently formalized picorna-like family Iflaviridae, and consists of a single open reading frame (ORF) flanked by a long 5′ untranslated region (5′ UTR) and a short, highly conserved 3′ UTR and is terminated with a 3′ poly-A tail. Both untranslated regions are involved in regulating the replication and translation of the genome (Belsham, 2009; Gromeier et al., 1999; Nakashima and Uchiumi, 2009; Roberts and Groppelli, 2009), and as a result have considerable interaction with numerous host factors required for these processes. Within the 5′ UTR lies an Internal Ribosome Entry Site (IRES) which is active *in vivo* in several insect cell lines (VaDV-1; Ongus et al., 2006) and in mammalian, insect and plant cell-free translation systems (DWV; Roberts and Groppelli, 2009). Although the structure has not yet been elucidated, the essential components of the IRES have been mapped to the 300 nucleotides immediately prior to the start of the ORF (Roberts and Groppelli, 2009). IRESs have also been identified in the 5′ UTR of other iflaviruses (Isawa et al., 1998; Lu et al., 2006; Wu et al., 2007), picornaviruses (Belsham, 2009; Fernández-Miragall et al., 2009) and dicistroviruses (Jan, 2006). They are thought to be a means for the virus to avoid, and possibly disrupt, the host's CAP-dependent mRNA translation mechanism (Belsham, 2009; Carter and Genersch, 2008), because IRES-mediated translation requires far fewer host factors than CAP-dependent translation (Pestova and Hellen, 2006; Pestova et al., 2004).

By analogy with related viruses (Belsham, 2009; Fernández-Miragall et al., 2009), the open reading frame is most likely initi-

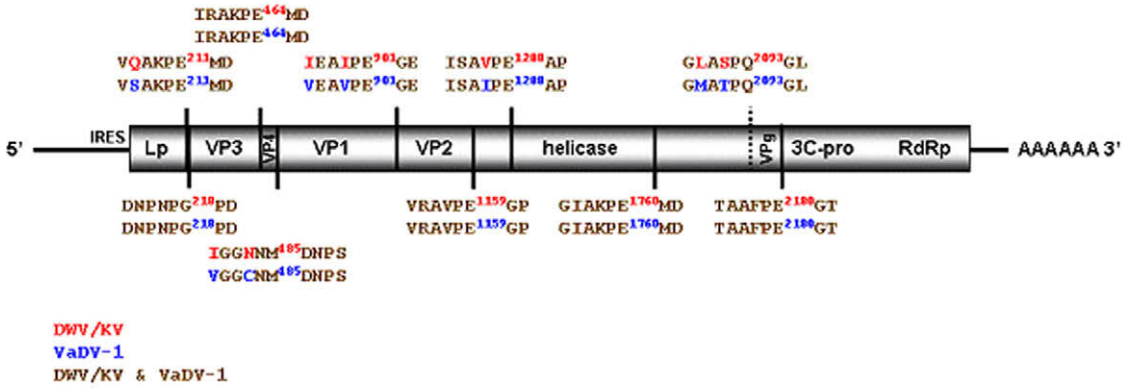

Fig. 2. Organization of the DWV–KV–VaDV-1 genome. The identified functional domains are for the four capsid proteins (VP1–VP4), the helicase, the genome-linked viral protein (VPG), the 3C-protease (3C-pro), and the RNA-dependent RNA polymerase (RdRp). An IRES is expected in the 5′ untranslated region (5′ UTR; Ongus et al., 2006). The order of the capsid proteins is according to the current convention for iflaviruses (Roberts and Groppelli, 2009). The genomic RNA is naturally polyadenylated. Also shown are the experimentally determined and inferred proteolytic processing sites, with the position of the splicing site in the peptide sequence indicated for each virus.

ated with a methionine, although this is not necessarily a requirement for IRES-mediated translation (Jan, 2006). The order of the viral proteins in the polyprotein (Fig. 2) is typical of the iflaviruses, and similar to that of mammalian picornaviruses (Gromeier et al., 1999) with the structural proteins in the N-terminal end and the non-structural proteins in the C-terminal end of the polyprotein. Several well-known functional domains can be identified within the polyprotein, such as a helicase, a 3C-protease (3C-pro), an RNA-dependent RNA polymerase (RdRp) and two capsid protein domains (Fujiyuki et al., 2004; Lanzi et al., 2006; Ongus et al., 2004), as well as a putative genome-linked viral protein (VPg) and a leader polypeptide (Lp; L-protein) immediately prior to the structural proteins (Lanzi et al., 2006). The DWV helicase domains A, B, and C include the perfectly conserved NTP binding residues ^{1472}GxxGxGKS^{1479} in domain-A and ^{1518}Qx$_5$DD^{1525} in domain-B (Koonin and Dolja, 1993), while domain-C (^{1561}KKx$_4$Px$_5$NTN^{1575}) is slightly different from the consensus sequence KGx$_4$Sx$_5$STN (Gorbalenya and Koonin, 1989). The 3C-pro domains include the cysteine protease motif ^{2305}GxCG^{2308} and the putative substrate binding residues ^{2322}GxHxxG^{2327} (Gorbalenya et al., 1989). C^{2307} is the third residue of the protease catalytic triad that also involves a histidine residue and either an aspartate or glutamate residue (Ryan and Flint, 1997), with H^{2190} and D^{2225} the most likely candidates (Lanzi et al., 2006). All eight recognized RdRp domains are also found, including the "core" domains-IV, -V, and -VI thought to be involved in catalysis and NTP binding (Koonin and Dolja, 1993), as well as an additional, highly conserved domain (^{2495}TSxGxP^{2500}) located immediately prior to RdRp domain-I.

The VPg is a small protein common to most positive strand RNA viruses that binds covalently to the 5′ end of the genome and is involved in RNA stability, genome replication, translation and movement (Hébrard et al., 2009). VPgs are a highly heterogenous class of proteins (Hébrard et al., 2009). In picornaviruses the VPg is about 23 amino acids long with an early tyrosine (Y) residue as the physical link to the RNA (Weitz et al., 1986), and is located immediately prior to the 3C-protease domains. In a number of dicistroviruses, a sister family of the iflaviruses, multiple VPg sequences were identified between the helicase and 3C-protease (Nakashima and Nakamura, 2008; Nakashima and Shibuya, 2006). For DWV, only a weak VPg motif between amino acids 2093 and 2118 that includes a Y residue at position 2097 was identified (Lanzi et al., 2006).

The Lp, located immediately before VP3 (Fig. 2), is also found in other iflaviruses (Isawa et al., 1998; Wu et al., 2002) and a number of cardio- and aphtho-picornaviruses (Gromeier et al., 1999; Palmenberg, 1990). Like the VPg, the Lp may have multiple functions, with protease activity a common feature (Gorbalenya et al., 1991; Guarné et al., 1998; Hinton et al., 2002; Koonin and Dolja, 1993). There are also several weak protease motifs in the DWV Lp sequence (Lanzi et al., 2006). L-proteins are often highly variable at the amino acid level, which is also true for the DWV Lp (Lanzi et al., 2006) and are implicated in disease pathology through the inhibition of host CAP-dependent mRNA translation (Glaser et al., 2001) and stimulation of viral IRES activity (Hinton et al., 2002).

The polyprotein is processed by protease digestion to produce the functional proteins, with the virus-encoded 3C-protease providing most of the protease activity (Gromeier et al., 1999; van Munster et al., 2002). Fig. 2 shows the predicted protease sites for DWV and VaDV-1, based on the N-terminal sequences of the VP1 and VP2 proteins (Lanzi et al., 2006; Ongus et al., 2004). The amino acids at each site that are unique to DWV are colored red, those unique to VaDV-1 are blue, and those common to both are brown. Generally speaking, there is very little difference between DWV and VaDV-1 at each site, despite the considerable genetic difference between DWV and VaDV-1 (84% nucleotide identity). This contrasts sharply with the much greater variation found for the protease sites of the viruses in the acute bee paralysis virus (ABPV)–Kashmir bee virus (KBV)–Isreali acute paralysis virus (IAPV) species complex (de Miranda et al., 2010). All sites are suitably located for the separation of functional viral proteins from the polyprotein, with excellent agreement between the estimated and predicted molecular weights for the structural proteins (Lanzi et al., 2006). There are three types of protease activity found within the DWV genome. The most common proteolytic sites are for the 3C-protease, and between all sites the consensus 3C-protease recognition sequence is 'AxPE', followed by either 'G', 'M' or 'A'. This conforms to the classic pattern for viral 3C-proteases which cut after either glutamine (Q) or glutamic acid (E) (Gromeier et al., 1999; Palmenberg, 1990). The flanking amino acids are usually also conserved for individual 3C-proteases (Gromeier et al., 1999; Isawa et al., 1998) with the glycine (G), proline (P), and alanine (A) residues common in the shown positions (Blair and Semler, 1991; Palmenberg, 1990). There is one further site of interest, at position 2093, that has 'Q' rather than 'E' as the canonical site and could release the VPg from the polyprotein. The differences between the 3C-protease sites indicate a certain amount of flexibility in site recognition by the protease, a property which may be beneficial for viruses in certain circumstances (de Miranda et al., 2010). Site 218 concerns a possible 2A-type protease activity (Luke et al., 2008), based on the NPG^{218}P catalytic site. This site is very close to a highly probable 3C-protease site at position 211, and may

not be essential for functional processing of the polyprotein. In common with other picorna-like viruses, the junction between VP4 and VP1 (site 485) is most likely processed autocatalytically (i.e. without a protease) in the final stages of particle maturation, concurrent with the packaging of the viral RNA within the virion (Liljas et al., 2002; Nakashima and Uchiumi, 2009). For this site the 'D' and 'P' amino acids are relatively conserved between different iflaviruses (Lanzi et al., 2006).

The differences between DWV, KV, and VaDV-1 are heavily concentrated in the 5′ end of the genome, particularly the 5′ UTR and (at amino acid level especially) the Lp region (Lanzi et al., 2006). Other regions with elevated levels of variability have also been identified in the genome (Fujiyuki et al., 2006; Lanzi et al., 2006). Genetic variability is a defining characteristic of virus existence, and individual variants should not be considered so much as separate entities, but rather as elements in a constantly changing swarm of variants, each contributing to the success of the swarm as a whole (Biebricher and Eigen, 2005; Domingo and Holland, 1997; Stich et al., 2007; Vignuzzi et al., 2006). The concept of defining a virus by its variability has been explored theoretically and experimentally within the quasispecies framework (Biebricher and Eigen, 2005; Domingo and Holland, 1997; Eigen, 1971, 2002; Elena and Sanjuan, 2007; Stich et al., 2007), with obvious parallels to population genetics in higher organisms (Domingo, 2002; Holmes and Moya, 2002; Wilke, 2005). Most of the variation within a quasispecies is generated by the RNA polymerase, primarily through faulty incorporation of nucleotides and secondarily through recombination with other viruses (or even host RNAs), by switching templates during replication (Domingo and Holland, 1997; Roossinck, 1997). The vast majority (~80%) of nucleotide substitutions are transversions, i.e. A–G or C–U changes, which are caused by the ability of uracyl (U) to form a stable, mutating base-pair with guanine (G), as well as with its legitimate partner adenine (A) in RNA molecules (Roossinck, 1997). The variability of a viral quasispecies is dependent on the capacity of the viral genome to generate and absorb variation, which is different for different viruses (Schneider and Roossinck, 2001). Increasing the variation within the quasispecies beyond the natural limits for the virus results in severely compromised fitness (Biebricher and Eigen, 2005; Crotty et al., 2001; Sierra et al., 2000), and is in fact one of the molecular mechanisms of several anti-viral treatments (Eigen, 2002; Vignuzzi et al., 2005). Similarly, decreasing the variation within a quasispecies can also compromise fitness (Biebricher and Eigen, 2005; Codoñer et al., 2006; Comas et al., 2005). As a result, RNA viruses maintain an optimum mutation rate that balances positive selection via occasionally more successful variants with the generally deleterious load of random mutation (Biebricher and Eigen, 2005; Eigen, 2002; Vignuzzi et al., 2005). Above this optimum rate the quasispecies becomes overburdened by excessively non-functional genomes while below this rate it loses the ability to adapt. Both the composition and the amount of variation within this swarm are influenced by the host, with certain hosts allowing greater variability than others, or favouring a particular composition (Schneider and Roossinck, 2001). This modulating ability can even vary between sampling times or different tissues within a host (Briones et al., 2003; Casado et al., 2001) and constitutes part of a perpetual symbiotic interplay between a virus and its host that can range from pathogenic to mutualistic, depending on the circumstances and perspective (Elena and Sanjuan, 2007; Roossinck, 2003, 2005). Sometimes several major variants can be detected within a stable quasispecies, each with its own swarm of mutants, as well as recombinants between the variants (Palacios et al., 2008), whereas in other seemingly identical cases a single homogenous quasispecies is present (Casado et al., 2001). The structure and composition of different quasispecies within the DWV–VaDV complex have also been studied. One study, based on RdRp sequences, described a rather homogenous quasispecies, with no consistent change in composition between different bee castes, organs, or between varroa mites and the bees they parasitize (Fujiyuki et al., 2006). Only different colonies and/or years of isolation had distinct identities. Another study, based on the Lp region, found several clear instances of a quasispecies with at least two major variants, in pupae infested with the parasitic mite, *T. mercedesae* (Forsgren et al., 2009). Although there were no quasispecies differences between the pupae and their infesting mites, there was considerable difference between different cells, with one variant common to all cells and a separate variant unique to each cell. This means that the variability of the DWV quasispecies is best accessed by individual samples and that the engine for generating variation is the individual brood cell, with only a minor shift in the polymorphism between bees and mites in each cell. By compartmentalizing the generation of variation to individual cells, coupled with transmission by mites, both the universal and unique polymorphic sequences can be effectively maintained within the DWV quasi-species (Forsgren et al., 2009). The large degree of variation found in this study, based on a few samples from a single hive in South East China, contrasts sharply with the much more homogenous sequences found in a worldwide geographic survey of DWV. This homogeneity was attributed to recent radiation of DWV from a rather homogenous source, facilitated by the worldwide trade in bees and bee products (Berenyi et al., 2007). However, these different impressions of DWV heterogeneity are strongly affected by the type of sample assayed and the genomic region investigated (Forsgren et al., 2009). Other studies have also found that natural DWV populations can be quite variable, with different variants predominating in individual bees or colonies (Yañez, Forsgren, Paxton, Fries, and de Miranda, unpublished). Usually two or three major variants are present, constituting a sequence polymorphism in the DWV quasispecies. Fig. 3 highlights a number of such polymorphic variants from Sweden (in blue text) and Ireland (in red text), as well as from the *Tropilaelaps* study (in green text; Forsgren et al., 2009), based on the DWV Lp gene. In each case there is great internal consistency within each variant, shown by the high bootstrap value associated with each terminal group, but a far less clear relationship between the different variants. In Sweden, the Gotland variants appear so far to be unique to Gotland, an island in the Baltic sea, while the Ultuna variant is present in both Gotland and the Swedish mainland. On Gotland, the variants are equally distributed in the overall quasispecies. By contrast, in the bee population studied in Ireland, the Kilkenny variant is predominant relative to the Tipperary variant, although both are part of a single polymorphic quasispecies, while the DWV quasispecies found in Antrim (in black text) is homogenous. These polymorphisms are very useful for studying competition between naturally occurring virus strains during various phases of the bee life cycle, such as infection of different tissues, life stages, adult castes, and virus transmission routes, to investigate how such sequence polymorphisms are maintained in the DWV quasispecies.

The remaining isolates in Fig. 3 include, in purple text, the published complete sequences of DWV from the USA and Italy (Lanzi et al., 2006), Kakugo virus from Japan (Fujiyuki et al., 2004) and VaDV-1 from the Netherlands (Ongus et al., 2004) and a number of historic–geographic isolates from the Rothamsted reference collection (in black text), including the original 1982 DWV isolate from Japan. These isolates reflect the contrasting reports of the variability of DWV, in that there is both a large and homogenous group of isolates covering a wide geographic and historic range (across Europe and the USA; 1988–2007), reflecting the findings of Berenyi et al. (2007), together with evidence of polymorphism and considerable genetic heterogeneity when analysing natural populations in greater detail. The reasons for this disparity are not immediately obvious. Although some of the homogeneity in

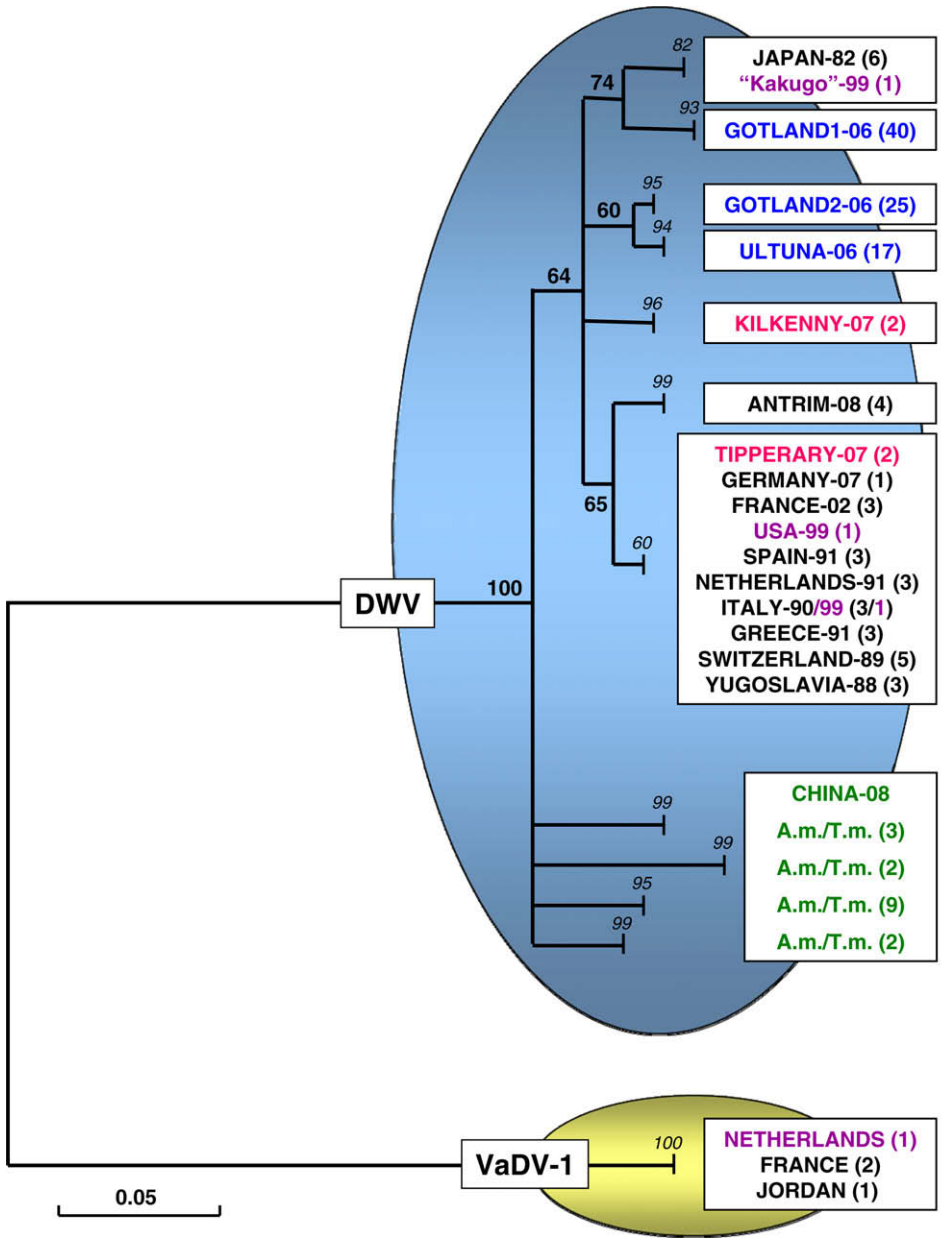

Fig. 3. Phylogram depicting the relationships between different DWV isolates, as inferred from the Lp region of the DWV genome (Lanzi et al., 2006), using Varroa destructor virus-1 (VaDV-1) as outgroup. The isolates were classified according to their geographic origin and year of isolation. The published sequences of DWV (Lanzi et al., 2006), KV (Fujiyuki et al., 2004), and VaDV-1 (Ongus et al., 2004) are shown in purple text. Isolates that are polymorphic within the same bee populations are colored blue (Sweden), red (Ireland) and green (China, from *Tropilaelaps*-infested colonies; Forsgren et al., 2009). The original phylogram was constructed by MEGA-4 (Tamura et al., 2007), using Minimum Evolution criteria. The statistical strength of the nodes is shown as the percentage of correct partitions in a 1000 replicate bootstrap analysis, and is shown in **bold** for each internal branch, and in *italic* for the terminal node leading to each taxonomic genogroup. Branches with less than 60% bootstrap support were collapsed. After de Miranda, Yañez, Paxton, Fries, and Ball (unpublished).

the European group of isolates will be because these isolates were purified from bulk samples of bees, which would obscure the presence of unique variants found when analysing individual bees (Forsgren et al., 2009), the majority will be a genuine reflection of the genetic state of DWV during that period. Because this is also the period when the varroa front expanded through Europe, it is very well possible that the homogeneity of this DWV genetic group is due to its epidemic spread by varroa, and may represent a strain of DWV particularly well-adapted to transmission by varroa that displaced earlier, pre-varroa strains of DWV, if these existed. For this reason too, the discovery of minor, polymorphic, regional DWV variants is of great interest, since they may reflect remnants of historic DWV distributions and variability, hidden within the current DWV quasispecies as a type of molecular memory (Briones et al., 2006; Wilke and Novella, 2003).

Another item of interest concerns the high degree of similarity of DWV and Kakugo virus, both isolated in Japan nearly 17 years apart. The original 1982 DWV isolate from Japan was definitely associated with wing deformities, because it was this isolate that gave DWV its name (Ball, 1983). Kakugo virus was identified while screening for genes associated with aggression in bees (Fujiyuki et al., 2004) by its elevated expression in the brains of aggressive guard bees, hence its name ("Kakugo" means "ready to attack" in Japanese). No wing deformities have been associated with this virus, although wing deformities are only observed with high levels of varroa infestation, and no varroa was observed in these stud-

ies (Fujiyuki et al., 2006). Similarly, no elevated levels of DWV were found in naturally aggressive races of bees (Rortais et al., 2006) or in guard bees of DWV-symptomatic colonies (Lanzi et al., 2006). However, these symptoms need not be mutually exclusive. Several honeybee viruses have both behavioral and pathological characteristics, i.e. chronic bee paralysis virus (Ribière, 2010) and sacbrood virus (Anderson and Giacon, 1992; Bailey and Fernando, 1972) relating to different requirements for transmission and exploitation, and this may also be true for DWV and Kakugo virus (Iqbal and Mueller, 2007; Lanzi et al., 2006).

The final item of interest is the contrast between the close relationship within each terminal group of isolates, each of which has a unique and distinct genetic character, and the uncertain relationship between the different groups. This discrepancy is common for many viruses and is due to the influence of recombination, convergence, parallel evolution, and complementation in shaping the variation within a quasispecies. These forms of homoplasy severely disrupt the reconstruction of the phylogenetic relationships between the viruses, i.e. the history of their descent and origins. When rare, or limited to close relatives, their effect on phylogenetic reconstruction will be buffered by more conventional patterns of evolution elsewhere in the genome. However, if frequent, it may be the main explanation for the often difficult and unstable phylogenetic reconstructions between virus strains. This uncertainty in identifying the relationships between different viruses is also seen on a wider phylogenetic scale in Fig. 4, which describes the position of DWV and VaDV-1 within the Iflavirus genus and their further relationship to the cripaviruses; the sole genus of the Dicistroviridae. By deleting certain taxa, the affinities between the remaining taxa can sometimes emerge, only to disappear again with further additions or deletions of taxa, all reflecting the uncertainties of the overall relationships covering the entire genome. As a result, it is clear that a phylogenetic approach to analysing viral population variation has severe limitations.

4. Pathology of DWV

Honeybee pathologists use various terms to describe the outcome of viral infections in honeybees. We surveyed the literature and found consensus that most honeybee viruses normally cause infections without any clinical symptoms. However, when describing this type of infection various terms such as asymptomatic infection, inapparent infection (Bailey and Woods, 1974; Sumpter and Martin, 2004), persistent latent infection (Chen et al., 2005a), persistent benign infection (Martin, 2001), and persistent inapparent infection (Shen et al., 2005a) were used. Alternatively, it was stated that these virus infections are 'probably latent, persistent, and kept under control by host immunity' (Shen et al., 2005b) or that DWV has a 'chronic nature' (Martin, 2001). The results of our search demonstrated that in the field of bee virology the terms latent, persistent, chronic, benign, and inapparent are used as synonyms although they actually define different forms of viral infections. In modern virology the categories: (i) lytic infections, (ii) persistent infections (Oldstone, 2006), (iii) latent infections (Efstathiou and Preston, 2005; Klein, 1982), and (iv) infections leading to immortalization (Klein, 1972; Rapp and Westmoreland, 1976; Varmus, 1988; Wyke, 1981) are used depending on the effect virus infections have on the host cell. The classification into these categories is mainly based on cellular and molecular data obtained from *in vivo*- and *in vitro*-models.

Unfortunately, for bee viruses neither cell culture models nor sufficient cellular and molecular data exist. In addition, no clinical or laboratory diagnosis in its classical (vertebrate) sense is available for bees and, hence, physiological and serological parameters defining disease symptoms cannot be obtained. Therefore, the above mentioned categories are not easily applicable to bee virus infections. This obviously resulted in a random use of otherwise defined technical terms in the field of bee virology. Given that effective scientific communication is impossible without terminology that is clearly defined and properly used, random terminology or lack of defined terms are likely to lead to confusion. This problem can be avoided by using the more descriptive terms *overt* and *covert* infections when describing the type of infection caused by bee viruses. These terms proved useful in invertebrate and insect virology and are, therefore, widely used. Applying the same terminology to honeybee viruses will result in more consistency and clarity and will allow for effective communication between bee and other invertebrate virologists. Therefore, we will start this section on DWV pathology by introducing and defining the terms *overt infection* and *covert infection* aiming to establish this terminology in bee virology. Furthermore, we will describe latent and persistent infections as categories of covert infections (for a more detailed review on infection strategies of insect viruses see also Hails et al., 2008).

Overt infections are characterized by obvious disease symptoms in the host as a result of the infection (causal relationship) and a high level of virus particle production. The infections have a clearly defined end-point as the insect either succumbs to a fatal infection or the infection is cleared and the host survives in the absence of further virus production. Overt infections are usually transmitted horizontally although additional transmission routes may exist.

Overt infections can be sub-divided further into acute and chronic infections. Acute infections are characterized by a short lived, highly productive infection manifesting clear symptoms of varying severity or death. Examples of overt acute infections caused by bee viruses include the fatal infections of adult bees by chronic bee paralysis virus (CBPV) (Bailey, 1968, 1976; Ball and Bailey, 1997; Ribiere et al., 2002; Rinderer and Green, 1976), and sacbrood virus (SBV) infections of honey bee larvae (Bailey and Ball, 1991; Ball and Bailey, 1997). A special case of overt acute infections are the inapparent infections which are very similar to overt acute infections, in that they are short term infections with high levels of virus production, but, unlike acute infections, there are no signs or symptoms of disease (Dimmock and Primrose, 1987).

Chronic infections are overt infections characterized by long-term production of virus particles over the lifetime of the host, or in the case of most insect viruses, the duration of the infected life stage, in the presence of clear disease symptoms. There are no examples of proven chronic infections by bee viruses although evidence is accumulating that DWV may cause chronic infections under certain circumstances (see below).

To summarize, overt infections, both acute and chronic, are primarily horizontally transmitted either directly between infected and susceptible individuals, or via infectious particles that can persist in the environment external to the host. Overtly infected hosts exhibit visible symptoms to varying degrees, and the disease causing pathogen has a significant impact on host fitness. The only difference between overt acute and overt chronic infection is the duration of the infection. Comparing this definition of overt infections with the categories of modern virology reveals that it is consistent with the definition of lytic infections. Lytic infections in modern virology are characterized by large scale virus production, destruction of the host cell, and cell death and which may be chronic or acute depending on the duration of the disease.

Covert infections are defined as conditions characterized by (i) an absence of overt disease symptoms (although there may still be an unknown cost to the host) in the presence of viral particles or nucleic acids; (ii) persistence of the viruses beyond the current life stage due to vertical transmission possibly over many generations (Burden et al., 2002; Kukan, 1999); and (iii) fully competent

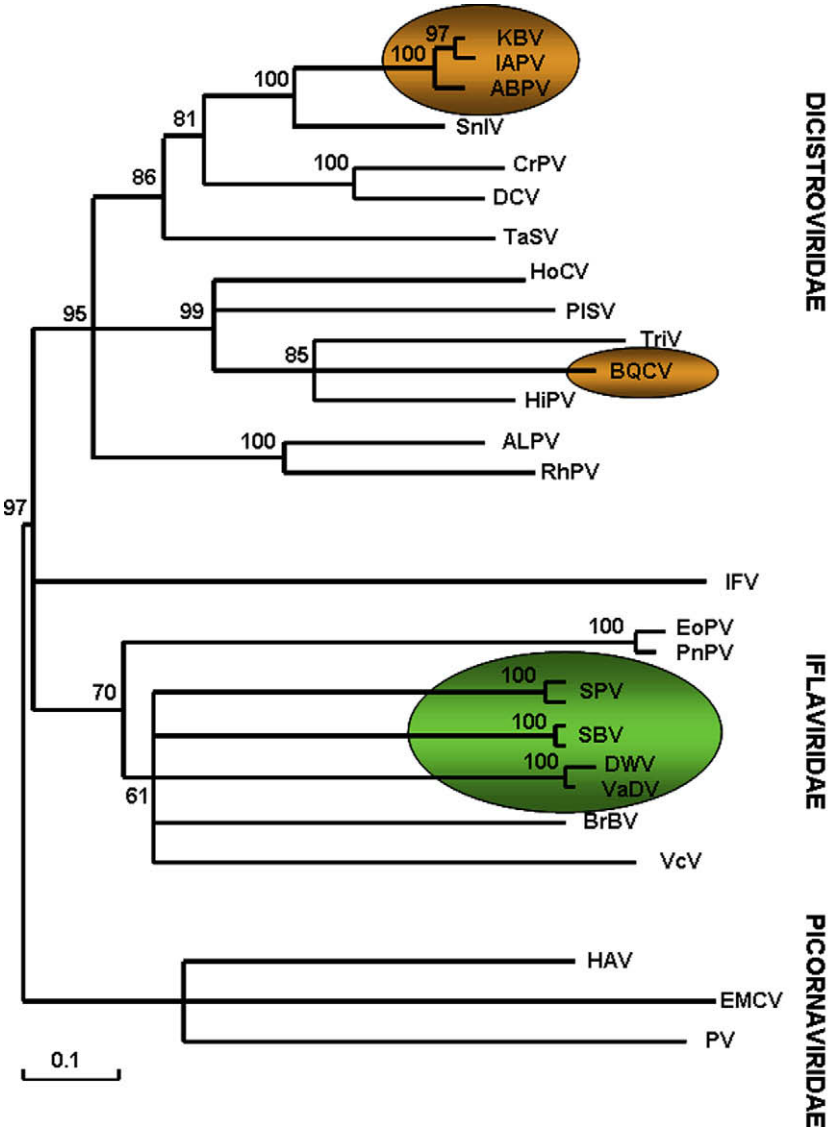

Fig. 4. Phylogram relating DWV/KV and VaDV-1 to other iflaviruses, dicistroviruses, and picornaviruses, based on conserved amino acid domains in the capsid proteins, helicase, 3C-protease and RNA-dependent RNA polymerase. The virus abbreviations are: ABPV (acute bee paralysis virus), KBV (Kashmir bee virus), IAPV (Israeli acute paralysis virus), SnIV (Solenopsis invicta virus), CrPV (cricket paralysis virus), DCV (Drosophila C virus), TaSV (Taura syndrome virus), HoCV (Homalodisca coagulata virus), PISV (Plautia stali intestine virus), TriV (triatoma virus), BQCV (black queen cell virus), HiPV (Himetobi P virus), ALPV (aphid lethal paralysis virus), RhPV (Rhopalosiphum padi virus), IFV (infectious flacheri virus), EoPV (Ectropis obliqua picorna-like virus), PnPV (Perina nuda picorna-like virus); SPV (slow paralysis virus), SBV (sacbrood virus), DWV (deformed wing virus), VaDV (Varroa destructor virus), BrBV (Brevicoryne brassicae picorna-like virus), VcV (Venturia canescens virus), HAV (hepatitis A virus), EMCV (Encephalomyocarditis virus), PV (polio virus). The phylogram was constructed by MEGA-4 (Tamura et al., 2007), using Minimum Evolution criteria. The statistical strength of the nodes is shown as the percentage of correct partitions in a 1000 replicate bootstrap analysis. Branches with less than 60% bootstrap support were collapsed. Viruses infecting bees are highlighted in orange (Dicistroviridae), and green (Iflaviridae). After de Miranda, Dainat, Stoltz, Neumann, and Ball (unpublished).

viruses which can re-emerge to cause overt infections (Burden et al., 2003); these occasional outbreaks of overt disease from covert infections may be influenced by a variety of environmental and other factors. This definition of a covert infection gives a clear distinction from an inapparent infection which is also characterized by the absence of disease symptoms. But unlike covert infections, inapparent viral infections are defined as short term infections with high levels of virus production in the absence of visible symptoms and which are transmitted horizontally (Dimmock and Primrose, 1987).

In modern virology there are two categories of covert infections: persistent infections and latent infections (Dimmock and Primrose, 1987). A latent virus infection is characterized by the absence of viral particle production. Instead, the viral genome is either integrated into the host cell genome or maintained as an extrachromosomal episome. Viral gene transcription is reduced to only a very limited number of latency associated transcripts and proteins (Dimmock and Primrose, 1987).

A persistent virus infection is characterized by a constant low level production of virus particles in an infected cell. There are three prerequisites for viral persistence: (i) the viruses evade the host's immunological surveillance system; (ii) the viruses regulate expression of both their own genes and host genes in a way allowing for replication and residence in a non-lytic state within the infected cells; (iii) persisting viruses can infect differentiated or specialized host cells virtually over the life time of the host. The infected cell survives or cell death is limited and counterbalanced by the production of new cells, and no net loss of cells occurs (Dimmock and Primrose, 1987). Persistent infections, therefore, represent a balance between the host and the virus and persistent

viral replication, although causing disease, does not destroy the infected host cell. Nevertheless, the normal homeostasis of the host is disturbed resulting in a negative effect on host fitness.

For honey bee viruses, no true latency (i.e. integration of the virus RNA into the host's genome or existing as an episome) has been demonstrated so far. The only viruses with RNA genomes known to integrate a DNA version of their genome into host chromosomal DNA are the retroviruses. Retroviruses use a virally encoded reverse transcriptase to generate a DNA-copy of their genome which is inserted into the host DNA by a virus-encoded integrase; these reactions are required for normal replication (Flint et al., 2004; Goff, 1992; Hu and Temin, 1990). For plus-stranded ssRNA viruses that replicate without a DNA intermediate, the transfer of genetic material from virus to host genome is far less likely, requiring a series of fortuitous interactions, including the cooperation of endogenous retro-transposons (Geuking et al., 2009; Tanne and Sela, 2005). These are essentially evolutionary accidents (Zhdanov, 1975) involving only small fragments of the genome (Klenerman et al., 1997; Crochu et al., 2004) that may subsequently be adapted for use in the RNAi anti-viral defense mechanism (Grassmann and Jang, 2008; Shi et al., 2008; van den Berg et al., 2008; Obbard et al., 2009). Sequences of a honeybee virus, Israeli acute paralysis virus (IAPV), were also demonstrated to persist in DNA form integrated into the genome of honeybees (Maori et al., 2007a,b; see de Miranda et al., 2010) indicating the possibility of genetic transfer from honeybee RNA viruses to honeybee cells. Although the integration of small viral RNA genome fragments into host DNA is not comparable with the integration of the whole viral genome, as required for latency, we cannot rule out that such cases might exist.

For honey bee viruses, true persistence has thus far also not been demonstrated. However, covert infections were demonstrated for black queen cell virus (BQCV), DWV, ABPV, chronic bee paralysis virus (CBPV), KBV, and sacbrood virus (SBV). True latency as one type of covert infection is not conceivable for these RNA viruses (see above), therefore, the above mentioned covert honey bee viruses can only fall into the category of persistent viruses. We will now review the literature to see how DWV infections fit into these categories. DWV was initially isolated as 'Japanese strain of Egypt bee virus' (EBV) from adult honeybees originating from *V. destructor* infested colonies in Japan (Ball, 1983) and it was usually, although not invariably, found in mite-infested colonies (Allen and Ball, 1996). The causal relationship between wing deformity and DWV infection was initially not evident, because deformed wings were originally attributed solely to the feeding activities of *V. destructor* during pupal development (Akratanakul and Burgett, 1975; De Jong et al., 1982; Koch and Ritter, 1991; Marcangeli et al., 1992). There is only one report suggesting that DWV has been experimentally confirmed as the aetiological agent of the symptom 'deformed wings' (Allen and Ball, 1996) but no experimental details are given. Nevertheless, nowadays it is the accepted view that DWV is directly or indirectly responsible for the malformed appendages of emerging bees. Therefore, DWV causes overt infections which are characterized by wing deformity. These symptoms only became evident after varroa became established as an ectoparasite in the population of *A. mellifera* indicating the strong association between the mite and overt DWV disease outbreaks. Even in mite-infested colonies, the majority of DWV infected bees do not exhibit any visible symptoms indicating that DWV causes asymptomatic (i.e. possibly covert) infections.

As outlined above, true covert infections are characterized by (i) the presence of viral particles or sequences in the absence of diseases symptoms, (ii) vertical transmission, and (iii) occasional overt outbreaks as proof of maintained virulence. The first characteristic 'presence of DWV in the absence of disease symptoms' has been proven at the individual-insect-level for all three adult castes, i.e. workers, drones and queens and all earlier life stages via RT-PCR methods and *in situ*-hybridization (Chen et al., 2005a,b; Fievet et al., 2006; Gauthier et al., 2007; Williams et al., 2009; Yue and Genersch, 2005). 'Presence of DWV in the absence of obvious disease symptoms' at the colony level is implicated in several epidemiological studies revealing that DWV is the most prevalent virus in mite-infested colonies regardless of whether the colonies are strong, weak or even about to collapse (Berenyi et al., 2006; Forgach et al., 2008; Nielsen et al., 2008; Sanpa and Chantawannakul, 2009; Tentcheva et al., 2004b). Furthermore, DWV viral RNA could be detected in asymptomatic bees from a region of northern Sweden from where *V. destructor* infestation had not been reported at that time (Yue and Genersch, 2005) suggesting that, despite the close association of the mite with overt outbreaks of disease, the phenomenon 'presence of DWV in the absence of visible symptoms' exists independent of *V. destructor*.

The second characteristic of covert infections, 'vertical transmission of DWV', is a more complex topic. There are several routes of transmission pathogens can use which can be classified broadly into the two categories 'horizontal transmission' and 'vertical transmission'. In horizontal transmission, pathogens are transmitted between individuals of the same generation, with generation being defined as 'living at the same time'. Hence, transmission among worker bees or between the queen and her worker offspring through air-borne or food-borne transmission is horizontal transmission. In vertical transmission, pathogens are transmitted from parent to offspring during reproduction via sperm and/or eggs, either on the surface of the eggs (transovum transmission) or within the egg (transovarial transmission). The special case with bees is that reproduction not only takes place at the individual-insect level but also at the colony level. Bees reproduce at the individual level through the reproductive activity of the queens laying unfertilized (drones) or fertilized (workers and new queens) eggs. At the colony level, bees reproduce by reproductive swarming, i.e. the production of two or more new colonies through division of a strong colony. Therefore, the evaluation of vertical and horizontal transmission in honeybees always has to be considered at both levels, the individual-insect level resulting predominantly in within-colony transmission and the colony level resulting in between-colony transmission.

For DWV, the question of whether vertical colony level transmission via reproductive swarming exists can easily be answered. The above mentioned epidemiological studies demonstrated that DWV is widely distributed and prevalent in colonies infested by *V. destructor*. Numerous studies showed that DWV is present in nearly all colonies and bees negative for DWV are rarely found regardless of whether the colonies are classified as diseased or strong or whether the bees have deformed wings or are asymptomatic (Berenyi et al., 2006; Tentcheva et al., 2004b; Yue and Genersch, 2005). All of these colonies still produce new colonies by reproductive swarming as can be deduced from the fact that the global population of managed honey-bee hives has increased ~45% during the last half century (Aizen et al., 2008; Aizen and Harder, 2009). Because the DWV infection status of bees and colonies obviously does not affect colony-level reproduction it is save to assume that DWV is vertically transmitted at the colony level.

Vertical transmission of DWV from queen to offspring was suggested by the detection of viral sequences in ovaries and the spermatheca of queens and in the drone reproductive tract as well as in eggs and in semen (Chen et al., 2006b, 2005b; Fievet et al., 2006; Yue et al., 2006). In addition, direct experimental evidence was provided showing that DWV is transmitted vertically at an individual-insect level. Virgin queens produced in mite-infested colonies were shown to lay either DWV-negative or DWV-positive unfertilized eggs. Artificial insemination of virgin queens laying DWV-negative eggs with DWV-positive sperm resulted in 100% DWV-

positive fertilized eggs. DWV-positive unfertilized eggs developed into DWV-infected drones and DWV-positive fertilized eggs developed into DWV-infected workers neither of which exhibited any clinical symptoms. Therefore, DWV indeed is vertically transmitted through drones and queens (Yue et al., 2007). These results were confirmed and in addition it was shown that drones can venereally (i.e. sexually via a horizontal route) infect a queen's ovaries and spermatheca followed by vertical transmission of DWV to the progeny via the infected queen reproductive tissues, further supporting the existence of vertical transmission routes for DWV (de Miranda and Fries, 2008).

Covert infections are furthermore characterized by viruses which remain fully competent in the covert state to re-emerge causing overt infections (Burden et al., 2003). In the absence of *V. destructor* occasional outbreaks are not well documented for DWV although some reports point in this direction. Queen and colony mortality were attributed to DWV infections in Britain and South Africa before the mite became established there although these outbreaks were not accompanied by any noticeable pathology other than death (Ball, 1989). In the presence of mite infestation the occurrence of bees with deformed wings qualifies as overt outbreaks of an otherwise covert infection residing in the colony.

In summary, all three characteristics of true covert infections could be demonstrated for DWV suggesting that DWV indeed causes true covert infections (Table 1). The sub-type of infection most likely is a persistent infection (Table 1) although final experimental proof for this is still lacking. The absence of pathological effects of covert DWV infections, even though the viral titers in asymptomatic individuals are often very high (Chen et al., 2005a), suggests that DWV is unusually well adapted to the honeybee host and the impact of covert infections on individual bee and colony fitness are low (Tables 1 and 2).

Overt DWV infections with the manifestation of clear disease symptoms (malformed appendages, shortened and bloated abdomens, miscolouring) are closely associated with the vectorial transmission of DWV by *V. destructor*, i.e., transmission by "injecting" the virus into pupae. Although consensus exists in the literature that transmission of DWV to pupae through parasitizing mites is the prerequisite for the development of deformed wings (Ball and Allen, 1988; Bowen-Walker et al., 1999; Shen et al., 2005b; Yue and Genersch, 2005) the exact mechanism behind this clinical symptom remains elusive.

One line of evidence suggests that *V. destructor* acts as a mechanical virus vector but more importantly induces immunosuppression in the parasitized pupa, thereby activating covert virus infections including DWV up to a yet-to-be-defined 'clinical threshold' (Shen et al., 2005b; Yang and Cox-Foster, 2007, 2005). In the case of DWV, such activated virus infections then result in overt outbreaks of disease recognizable by the characteristic clinical symptoms in emerging bees (predominantly deformed wings) and a life expectancy of <67 h (Yang and Cox-Foster, 2007). The mite infestation level of a single pupa was shown to be positively correlated with the probability and, especially, severity of wing deformations as categorized by a wing deformity ranking scale (Bowen-Walker et al., 1999; Yang and Cox-Foster, 2005) further supporting the hypothesis that the mite induced immunosuppression is the key factor for overt disease outbreaks. Mechanistically, it is not clear how the bee immune response is impacted by *Varroa*. One study found somewhat lower transcript levels for genes encoding antimicrobial peptides (Gregory et al., 2005), but only for pupae with low mite abundances. Heavily parasitized pupae actually showed higher levels of these immune effectors. Similarly, a recent survey of honey bee immune-gene activity using microarrays, did not show a systematic change in the activity of predicted immune pathways (Navajas et al., 2008).

Another line of evidence started from the finding that the transmission of DWV to pupae is necessary but not sufficient for the development of deformed wings because even in highly infested colonies with 100% DWV-transmitting mites a high proportion of infested bees still emerge asymptomatic (Yue and Genersch, 2005). These authors linked overt outbreaks of DWV disease with the ability of the mite to not only act as a mechanical vector of DWV but to have also the potential to act as a biological vector supporting DWV replication prior to transmission. The correlation between DWV replication in mites and the development of wing deformity (and likewise the correlation between the absence of replication in mites and the absence of clinical symptoms in bees) suggested that, although DWV transmission to pupae by *V. destructor* is a prerequisite for the development of deformed wings, a crucial factor is the replication of DWV in the mite prior to transmission (Yue and Genersch, 2005). Furthermore, it was shown that a high viral titer in the mites (10^{10}–10^{12} genome equivalents per mite), which only occurred in mites supporting viral replication, also correlated with the development of deformed wings. This suggests that DWV replication in mites resulting in a virus titer above a certain threshold followed by vectorial transmission is necessary and sufficient for truly infected bees to emerge with deformed wings (Gisder et al., 2009). Contradictory to what was shown by Yang and Cox-Foster (2005), in the study by Gisder and co-workers (2009) no significant difference in mite infestation level could be demonstrated between pupae emerging as adult bees with deformed or with normal wings.

Table 1
Possible outcomes of DWV infections in honeybees (adapted from Hails et al., 2008).

Infection type	Sub-type	Obvious disease symptoms	Impact on host fitness	Vertical transmission	Horizontal transmission (direct)	Horizontal transmission (vectorial)
Overt	Acute	Crippled wings death	High	No	No	Yes
	Chronic	Learning deficits reduced longevity	Medium	?	Yes	Yes
Covert	Persistent	None	Low	Yes	Yes	Yes

Table 2
Possible outcomes of DWV transmission in honeybees.

Transmission type	Infection type	Infection sub-type	Impact on host fitness	Duration
Vertical	Covert	Persistent	Low	Long
Horizontal (direct)				
Horizontal (vectorial)	Overt	Chronic	Medium	Long
		Acute	High	Short

Note: Vertical transmission through sperm and eggs; horizontal (direct) transmission through larval food, trophallaxis, and, possibly faeces; horizontal (vectorial) transmission through *V. destructor* to pupae (acute and chronic) and adult bees (chronic).

The pathological signs or visible clinical symptoms in individual bees associated with overt DWV infections are: death in the pupal stage or adult bees dying shortly (<67 h) after emergence with deformed wings and sometimes associated with shortened and bloated abdomens and miscolouring. These overt infections are clearly acute infections, because they are short lived with a high level of production of virus particles (Chen et al., 2005a) and manifesting obvious disease symptoms as a result of infection (Table 1). Such acute infections were considered inevitably associated with virus transmission to pupae through *V. destructor*. Recently it was shown, that another ectoparasitic mite of *A. mellifera*, *T. mercedesae*, also transmits DWV to the pupal stages of honeybees resulting in overt infections characterized by wing deformity (Dainat et al., 2009; Forsgren et al., 2009). These results suggest that it is the transmission route (virus injection into the hemolymph through an arthropod vector) rather than the mite species which determines the outcome of the infection.

No overt infections can be attributed to viral transmission to adult bees if only morphological symptoms are used for diagnosis. However, recently it was shown that these diagnostic criteria are too narrow for the complex pathology of DWV infections. Using controlled artificial infection of forager bees it was demonstrated that DWV infection resulted in specific impairment of sensory responsiveness and associative olfactory learning, while non-associative forms of learning like sensitization and habituation were not affected (Iqbal and Mueller, 2007). For successful establishment of such a symptomatic DWV infection, injection bioassays were performed mimicking DWV transmission to adult bees by phoretic *V. destructor*. No difference in the survival rates of infected bees and control bees were observed although the infected bees showed a slight decrease in motor activity (e.g., slow extension of the proboscis) after sucrose stimulation. No infection could be established in adult bees via the oral route, i.e. by feeding DWV-contaminated sucrose. These results suggest that under certain circumstances vectorial DWV transmission to adult bees may lead to overt chronic infections (Table 1). Such chronic infections in the honeybee brain that are characterized by learning deficits will almost certainly go unnoticed under natural conditions and only become noticeable once the weak performance of the infected foragers affect colony performance. Whether or not viral infections in the brain also cause behavioral changes like increased aggressiveness needs further evaluation (Fujiyuki et al., 2004; Rortais et al., 2006).

Bees emerging with deformed wings will soon succumb to this fatal DWV infection (Yang and Cox-Foster, 2007). The premature loss of worker bees and their contribution to colony performance, as well as the energy expended by raising these bees result in a clear negative impact on colony fitness when too many of these non-viable bees are produced (Table 1). In addition, chronic infections due to vectorial DWV transmission leading to learning deficits will also affect colony fitness (Table 1). Accordingly, DWV vectored by *V. destructor* plays an important role in the 'parasitic mite syndrome' and varroa-induced colony collapse as evident from field observations and supported by a modeling approach (Hung et al., 1996, 1995; Martin, 2001; Martin et al., 1998). This 'parasitic mite syndrome' describes the clinical symptoms of an overt DWV outbreak observed at the colony level. When the colony is building up (i.e. in spring) bee losses due to overt DWV infections of individuals can be compensated, but when brood rearing slows down and mite levels peak (i.e. in autumn), the virus epidemic accelerates and the excessive loss of working bees causes the colony to dwindle rapidly, and ultimately die. Such collapse is usually indicated by masses of dead and dying bees in and around the hive, many with shrivelled wings (symptoms of an overt DWV infection), spotty and neglected brood and pupae failing to emerge. The irony is that it is the less virulent viruses, such as DWV, that are usually associated with varroa-induced collapse because the more virulent viruses kill the pupae (and hence the mite) before the mite can complete its reproduction, during the bee pupal phase (Martin, 2001; Sumpter and Martin, 2004). These observations suggest that colony collapse is due to a complex interplay between mite and bee population dynamics, virus transmission (by mites and among bees) and virus virulence.

5. Transmission and virulence

The topic of transmission and virulence of DWV cannot be addressed without first defining the term virulence and the related term pathogenicity since especially in invertebrate pathology different definitions for these terms exist (Shapiro-Ilan et al., 2005; Thomas and Elkinton, 2004). For a given host and pathogen, pathogenicity is absolute whereas virulence is variable, e.g., due to strain or environmental effects. Pathogenicity is a qualitative term. An organism is either pathogenic to a host or it is not. By contrast, virulence is a quantitative term and, therefore, virulence is the measurable capacity of a pathogen to cause disease. Virulence is assumed to be positively correlated with the pathogen's reproduction rate within the host and negatively correlated with host fitness. These definitions of pathogenicity and virulence are highly consistent with those used in other disciplines, particularly in medicine and microbiology (Casadevall and Pirofski, 1999; Sacristan and Garcia-Arenal, 2008; Shaner et al., 1992).

Virulence evolution in pathogens is constrained by a trade-off between pathogen transmission and pathogen virulence (trade-off model; Ebert and Herre, 1996; Ewald, 1983; Read, 1994; Read and Harvey, 1994). If the living host is needed for pathogen reproduction and transmission then a healthy host is beneficial for long-term pathogen survival in the host population. However, reduction in host fitness is an unavoidable consequence of pathogen reproduction in most host–pathogen relationships and reduction in host fitness directly relates to pathogen virulence. Therefore, it is in the interest of the pathogen to achieve a balance between virulence and transmission to ensure optimal pathogen survival in the individual host and the host population. Too low a level of pathogen reproduction within the host (i.e. low-level virulence of the pathogen) may have little impact on host fitness but impairs transmission. In contrast, a high level of pathogen reproduction (high-level virulence) yields high transmission but host longevity may be too short to accomplish efficient transmission. Hence, virulence influences transmission success but at the same time transmission also influences virulence.

Previously we introduced the two main categories of transmission: horizontal transmission and vertical transmission. The trade-off model outlined above shows that less virulent pathogens, that have little impact on host fitness or longevity, will be better able to establish vertical transmission routes which rely on host survival and reproduction. By contrast, highly virulent pathogens that kill their host rather quickly will favour horizontal transmission routes, providing immediate pathogen transmission. Analysed from a reverse perspective, horizontal transmission allows for, but does not necessarily select for, the development of more virulent forms of the pathogen with a high negative impact on host fitness whereas vertical transmission requires less virulent forms that allow the next host generation to be produced (Day, 2003; Ebert and Bull, 2003; Fries and Camazine, 2001; Lipsitch and Moxon, 1997; Lipsitch and Nowak, 1996; Lipsitch et al., 1995).

Given that virulence evolution is oriented along a cost-benefit axis and that, therefore, a transmission-virulence trade-off exists, it should be possible to infer the degree of virulence of DWV from its transmission routes (Table 2). DWV is vertically transmitted at the individual-insect level through drones and queens as well as at the colony level by reproductive swarming of infected colonies

(Chen et al., 2006b; de Miranda and Fries, 2008; Yue et al., 2006, 2007). These vertical transmission routes do not result in any clinical symptoms in individuals or in any detectable negative impact on host fitness at the individual and colony levels, confirming the assumption that vertically transmitted pathogens are rather less virulent. In addition, direct horizontal transmission routes do exist. DWV could be detected in larval food suggesting horizontal transmission through feeding and trophallaxis (Yue and Genersch, 2005). Since DWV-infected workers can develop from DWV-negative eggs in the absence of *V. destructor*, but in the presence of contaminated food (Nordström, 2003; Yue et al., 2007) horizontal transmission through larval food might be effective although still resulting in a covert infection (Yue et al., 2007). DWV could be detected by *in situ*-hybridization in the midgut epithelium of adult bees and the midgut content was shown to be full of mature virus particles (Fievet et al., 2006). These results were consistent with the RT-PCR detection of DWV in faeces (Chen et al., 2006b) indicating that DWV replicates in the midgut and is shed into the midgut lumen. This process apparently takes place without causing massive destruction of midgut cells or noticeably affecting digestion, because no diarrhea or congestion or wasting (Dionne et al., 2006) has been reported in association with DWV infection, which raises questions about the manner of DWV release from infected cells. However, given that no detectable DWV infection could be initiated by feeding DWV-contaminated sucrose (Iqbal and Mueller, 2007) the efficiency and relevance of a faecal–oral transmission route to the overall epidemiology of DWV can be questioned.

None of these transmission routes result in any detectable or measurable negative impact on the fitness of bees and colonies. The picture is different only in relation to the indirect horizontal transmission of DWV with *V. destructor* as an arthropod vector. While transmission of DWV in the absence of *V. destructor* causes asymptomatic, covert infections, horizontal vectorial transmission of DWV by *V. destructor* through injection of the virus into bee hemolymph leads to overt acute and lethal infections. In addition, there is evidence that vectorial transmission of DWV to adult bees also affects host fitness through an overt chronic infection, although the associated neurological symptoms (learning deficits) constitute a less obvious, sub-lethal pathology (Iqbal and Mueller, 2007).

In summary, DWV is normally, i.e. in the absence of parasitic mites, a virus of minor virulence, allowing infected brood to develop through the pupal stage to adulthood (Bailey and Ball, 1991; Ball and Bailey, 1997). It is this low virulence that accounts for DWV being the main virus associated with varroa infestations, because more virulent viruses such as CBPV, ABPV, KBV, BQCV, SBV, and slow paralysis virus kill the brood too quickly for varroa to complete its development on the bee pupae and transmit the virus to new hosts (Martin, 2001; Sumpter and Martin, 2004). The vertical transmission of DWV correlates with its low intrinsic virulence as predicted by the above discussed trade-off model for the evolution of pathogen virulence. Additional mite-independent, direct horizontal routes through feeding and trophallaxis, although potentially allowing for the development of more virulent forms, did not select for increased virulence in the case of DWV. However, horizontal indirect transmission through mites as virus vectors fits the predictions from the trade-off model that horizontal transmission routes allow for more virulent forms of a pathogen to evolve.

Conflicts of interest

There are no conflicts of interest to be declared.

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