CHAPTER 2

Honey Bee Viruses

Yan Ping Chen[1,*] and **Reinhold Siede**[†,‡]

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[1] To whom correspondence should be addressed. E-mail: judy.chen@ars.usda.gov

* USDA-ARS, Bee Research Laboratory, Beltsville, Maryland 20705
† Landesbetrieb Landwirtschaft Hessen, Bieneninstitut Kirchhain, 35274 Kirchhain, Germany
‡ Institute of Virology, Justus Liebig University, 35392 Giessen, Germany

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Abstract

Viruses are significant threats to the health and well-being of the honey bee, *Apis mellifera*. To alleviate the threats posed by these invasive organisms, a better understanding of bee viral infections will be of crucial importance in developing effective and environmentally benign disease control strategies. Although knowledge of honey bee viruses has been accumulated considerably in the past three decades, a comprehensive review to compile the various aspects of bee viruses at the molecular level has not been reported. This chapter summarizes recent progress in the understanding of the morphology, genome organization, transmission, epidemiology, and pathogenesis of honey bee viruses as well as their interactions with their honey bee hosts. The future prospects of research of honey bee viruses are also discussed in detail. The chapter has been designed to provide researchers in the field with updated information about honey bee viruses and to serve as a starting point for future research.

I. INTRODUCTION

The honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is found all over the world and plays an important role in the global economy by assisting in the pollination of a wide variety of food crops and by producing honey, beeswax, pollen, propolis, royal jelly, and other hive products. To ensure an adequate supply of bees for the pollination of agricultural crops and the production of hive products, a healthy and vigorous population of honey bees will be essential. However, like other animals, honey bees are inevitably subject to infection by a wide variety of pathogens that are responsible for significant colony losses. Among honey bee pathogens, viruses pose one of the major threats to the health and well-being of honey bees and have caused serious concerns for researchers and beekeepers.

Viruses were first identified as a new class of pathogens infecting honey bees when a US scientist, Dr. White, discovered that a filterable agent from diseased bee larvae could cause sacbrood disease in the honey bee (White, 1913). Since then, at least 18 viruses have been reported to infect honey bees worldwide (Allen and Ball, 1996; Ellis and Munn, 2005). Although knowledge of honey bee viruses is still limited compared to that of other well-studied insect viruses, such as baculoviruses, understanding

of virus infections in honey bees has grown considerably over the last three decades and a body of literature dealing with bee virus identification, physiochemical properties, natural history, transmission, incidence, and pathology has been accumulated. In this chapter, we describe recent progress in understanding morphology, genome organization, transmission, epidemiology, and pathogenesis of honey bee viruses as well as their interactions with their honey bee host. Infections of viruses in honey bees have been reviewed previously. The main goal of this chapter is to update previous findings with more recent work relating to the molecular biology of the honey bee viruses, however, some main features of earlier reviews: Bailey, 1976, 1981, 1982a; Bailey and Ball, 1991; Ball, 1996; Ball and Bailey, 1991, 1997.

II. COMMON HONEY BEE VIRUSES

Viruses could attack at different developing stages and castes of the honey bees, including eggs, larvae, pupae, adult worker bees, adult drones, and queen of the colonies. Although bee viruses usually persist as inapparent infections and cause no overt signs of disease, they can dramatically affect honey bee health and shorten the lives of infected bees under certain conditions (Ball and Allen, 1988; Martin, 2001). Of 18 viruses identified to attack honey bees, six viruses, namely, Deformed wing virus (DWV), *Black queen cell virus* (BQCV), *Sacbrood virus* (SBV), Kashmir bee virus (KBV), Acute bee paralysis virus (ABPV), and Chronic bee paralysis virus (CBPV) are the most common infections and have been objects of active research currently.

A. Deformed wing virus

DWV was first isolated from diseased adult bees in Japan (Bailey and Ball, 1991). The occurrence and distribution of DWV has since been worldwide. Except for Oceania, the infection of DWV so far has been reported in Europe, North America, South America, Africa, Asia, and the Middle East (Allen and Ball, 1996; Antúnez *et al*., 2006; Ellis and Munn, 2005). The infection of DWV has also been identified in *A. cerana* in China (Bailey and Ball, 1991).

DWV is one of a few bee viruses that cause well-defined disease symptoms in infected bees. Typical disease symptoms of DWV infection include shrunken, crumpled wings, decreased body size, and discoloration in adult bees. However, the mechanism by which the DWV causes the morphological deformities of the infected hosts is unclear. Aside from the adult stage, DWV infection is also detected in other stages of

bee development, including egg, larvae, and pupae. When pupae at the normally multiplies slowly and rarely kills the pupae, instead mostly causing deformity and early death in newly emerged adult bees. Adult honey bees infected with DWV usually appear normal but are believed to have a reduction in life span (Bailey and Ball, 1991; Ball and Bailey, 1997; Kovac and Crailsheim, 1988).

DWV appears to be the most prevalent infection in *A. mellifera* in recent years. Our 5-year field survey carried out in Beltsville, MD showed that DWV infection occurred in 100% of the apiaries investigated (Y. P. C., unpublished observation). Similar results were reported previously by Tentcheva *et al*. (2004b) who observed that DWV was detected in over 97% of French apiaries when the adult bee population was examined. A study on the prevalence and distribution pattern of viruses in Austria demonstrated that DWV was present in 91% of tested bee samples (Berényi *et al*., 2006). Although high prevalence of DWV is not geographically related, some seasonal variation in virus incidence was observed and the frequency of DWV infection in both adult bees and pupae increased considerably from summer to autumn during the year (Tentcheva *et al*., 2004a,b). The striking high incidence of DWV infection in honey bees obtained from these studies indicate that DWV is prevalent over a wide range of geographic locations and is likely to become an important cause of mortality in honey bee colonies whenever a viral disease outbreak occurs, and warrants further investigation in the epidemiology and pathogenesis of this pathogen.

Bee colonies infected with DWV are often found to be associated with the infestation of a parasitic mite, *Varroa destructor* (Anderson and Trueman, 2000). Both laboratory and field studies showed that the varroa mite is an effective vector of the DWV (Ball and Allen, 1988; Bowen-Walker *et al*., 1999; Martin *et al*., 1998; Nordström, 2003; Nordström *et al*., 1999; Shen *et al*., 2005b). Varroa mites acquire the virus from infected bees and transmit it to uninfected bees, which either develop morphological deformities or die after the mites feed on them for a period of time. Studies of virus status in varroa mites showed that DWV was present in 100% of varroa mites collected from Thailand (Chantawannakul *et al*., 2006) and that varroa mites appeared to be DWV positive in 100% of French apiaries (Tentcheva *et al*., 2004b). Evaluation of DWV infection in individual bees showed that DWV was detected in 69% of bees collected from mite-infested colonies in Poland (Topolska *et al*., 1995), and in over 90% of bees from mite-infested colonies in England (Ball, 2001). The high frequency of DWV in mites and mite-infested bee colonies suggests that the significant increase in prevalence of DWV infection in recent years is likely associated with the worldwide infestation of varroa mites in honey bees. It also suggests that the varroa mite may play a major role in colony collapse due to the outbreak of viral disease.

B. Sacbrood virus

SBV is the most widely distributed of all honey bee viruses. Since its first identification in the United States in 1913 (White, 1913), infection of SBV has been found on every continent where *A. mellifera* honey bees are present (Allen and Ball, 1996; Bradbear, 1988; Ellis and Munn, 2005).

SBV attacks both brood and adult stages of bees, but larvae about 2-day old are most susceptible to SBV infections (Ball and Bailey, 1997). SBV affects adult bees without causing obvious signs of disease, but the infected adult bees may have a decreased life span (Bailey, 1969; Bailey and Fernando, 1972). The initial spread of SBV within a colony occurs when nurse bees become infected while removing larvae killed by SBV. Virus particles accumulate in the hypopharyngeal glands of the nurse bees and infected nurse bees can then spread the virus throughout the colony by feeding larvae with their glandular secretion and exchanging food with other adult bees including foraging bees. Infected foraging bees spread the virus by passing it from their glandular secretions to the pollen loads as they collect pollen. Young larvae become infected with the virus by ingesting virus-contaminated food. The SBV starts to replicate in the larva, and the infected larva turns pale yellow after the brood cell is capped. As the disease progresses, the skin of the larva becomes leathery and the larva fails to pupate because it cannot digest the old cuticle. A large amount of fluid containing millions of SBV particles accumulates between the body of a diseased larva and its saclike skin. Affected larvae appear to be a water-filled sac when removed from the cell. Sacbrood derives its name from the saclike appearance of the diseased larvae.

Infection of SBV can be readily diagnosed in the field because of the characteristic symptoms produced in diseased brood. Typically, when bee colonies are heavily infected with SBV, there are a number of partially uncapped or completely uncapped brood cells scattered among capped brood that can be found on the brood frame. Dead larva becomes a dark, brittle scale can be easily removed from the brood cell, a characteristic that differs from a bacterium-caused brood disease, American foulbrood.

Prevalence of SBV in honey bees has been found to be prominently seasonal. Frequencies of SBV infection in spring and summer were significantly higher than in autumn (Anderson and Gibbs, 1988; Bailey *et al.*, 1981; Tentcheva *et al.*, 2004b). The incidence of SBV has been believed to be positively correlated with the number of susceptible brood and young workers in the colonies. During the seasons of spring and summer, the rich sources of pollen and nectar stimulate brood rearing and a great number of new workers hatch from the brood cells, providing opportunities for SBV to attack bees and multiply in the colonies. The seasonal variation in SBV indirectly reflects variable susceptibility of different bee developmental stages to the virus infection.

SBV infection has been associated with varroa mite infestation. SBV was detected in large amount of adult bees from varroa mite–infested colonies (Antúnez *et al*., 2006; Ball, 1989; Berényi *et al*., 2006). Detection of SBV in varroa mites (Chantawannakul *et al*., 2006; Shen *et al*., 2005a; Tentcheva *et al*., 2004b) indicates that varroa mites have the potential to transmit the virus in the bee colonies, although varroa mite as a vector in transmitting SBV has not yet been experimentally demonstrated.

A new strain of SBV has been identified in the eastern honey bee, *A. cerana*, from Thailand in 1982. Infection of Thai SBV (TSBV) was also detected in India. TSBV is serologically related to SBV but not physiochemically identical to SBV (Bailey, 1982b).

C. Black queen cell virus

BQCV was first isolated from dead queen larvae and prepupae sealed in their cells that had turned dark brown to black along with the walls of the cell (Bailey and Woods, 1977), hence the designation of the name. The infection of BQCV in bees has been reported in North America, Central America, Europe, Oceania, Asia, Africa, and the Middle East (Allen and Ball, 1996; Ellis and Munn, 2005).

BQCV mainly affects developing queen larvae and pupae in the capped-cell stage. High incidences of the virus infection are observed in queen-rearing colonies in spring and early summer (Laidlaw, 1979). Diseased larvae have a pale yellow appearance and a tough saclike skin, a disease symptom also seen in SBV-infected larvae. BQCV readily multiplies in the pupal stage of the honey bees. Infected pupae turn dark and die rapidly. The wall of the queen cell eventually becomes dark colored, a characteristic symptom of BQCV infection. Worker bees can also be infected by BQCV but normally do not exhibit outward disease symptoms. BQCV does not multiply in bees when the virus particles are ingested.

Our 5-year field survey in Beltsville, MD showed that BQCV was the second most common infection of honey bees in the field after DWV (Y. P. C., unpublished observation). In 1993, Anderson (1993) reported that BQCV was the most common cause of queen larvae mortality in Australia. A study conducted by Tentcheva *et al.* (2004b) indicated that BQCV infection was more prevalent in adult bees than in pupae and that the incidence of BQCV was higher in spring and summer than in autumn. This result was consistent with a previous finding by Laidlaw (1979) that BQCV was more prevalent in spring and summer during the year.

In the field, BQCV disease outbreak has been linked with infection of a protozoan, *Nosema apis*. When the incidence of *N. apis* infection was high during the spring and summer, the infection of BQCV was more prevalent in honey bees (Bailey, 1981). It has been observed that BQCV multiplied rapidly in adult bees infected with *N. apis* (Bailey, 1982a).

BQCV is believed to be transmitted to queen brood via glandular secretion of nurse bees during the feeding (Bailey, 1982a). *N. apis* infects midgut tissues of the adult bees, increasing the susceptibility of the alimentary tract to infection by BQCV. Bailey *et al.* (1981) reported that honey bees infected with BQCV were found to be infected with *N. apis* simultaneously from all parts of England and Wales during 1979. Field survey of Austrian apiaries showed that *N. apis* was found to be present in 78% of BQCV-positive bee samples and that 75% of *N. apis*–infected colonies were also infected with BQCV (Berényi *et al.*, 2006). Similar results were also obtained from a survey carried out in France (Tentcheva *et al.*, 2004b). Although positive association between the BQCV and *N. apis* infections has been documented in the field observations, definite experimental evidence for deciphering the mechanism of *N. apis* in activation and transmission of BQCV infection remains to be determined.

Varroa mites are thought to sometimes act as a vector for BQCV (Bailey, 1976). Detection of BQCV in varroa mites collected from a Thai honey bee apiary supports this assumption (Chantawannakul *et al.*, 2006). However, an investigation conducted by Tentcheva *et al.* (2004b) yielded a different result; BQCV was never detected in any of the varroa mites they examined. Further studies to confirm the role of varroa mites as a vector in BQCV transmission will be necessary.

D. Kashmir bee virus

The origin of KBV in the bee species is obscure. KBV was first isolated from adult western honey bees, *A. mellifera*, that were experimentally inoculated with an extract prepared from the diseased Asian honey bee (*A. cerana*) in Kashmir, northwestern region of India, hence the name (Bailey and Woods, 1977). Subsequently, KBV has been detected in *A. mellifera* collected from Australia (Bailey *et al.*, 1979). The detection of KBV in the natural population of *A. mellifera* in Australia was unexpected because *A. cerana*, which is assumed to be the original host of KBV, does not exist there. Later, strains of KBV have been found in *A. mellifera* from Canada and New Zealand (Allen and Ball, 1995; Anderson, 1985), Fiji (Anderson, 1990), Spain (Allen and Ball, 1995), and the United States (Bruce *et al.*, 1995; Hung *et al.*, 1995). The unexpected emergence of KBV in the countries such as Australia and New Zealand might be due to the importation of bees from North American or other countries where KBV is endemic. So far, infection of KBV in *A. mellifera* has also been documented in several countries in Europe and Oceania (Allen and Ball, 1996; Ellis and Munn, 2005; Siede *et al.*, 2005).

KBV attacks all stages of the bee life cycle (Hornitzky, 1981, 1982) and commonly persists within brood and adult bees as an inapparent infection (Anderson and Gibbs, 1988; Dall, 1985). The disease and mortality

caused by KBV infection occurs in different developing stages of bees without clearly defined disease symptoms. Among all of the viruses infecting honey bees, KBV is considered to be the most virulent under laboratory conditions. It multiplies quickly once a few viral particles are introduced into the bee hemolymph and can cause bee mortality within 3 days. However, KBV does not cause infection when adult bees are fed with food mixed with KBV particles. The virus probably invades the bees through the cuticle by direct contact between live bees (Bailey *et al.*, 1979).

KBV is genetically, serologically, and pathologically closely related to another bee virus ABPV. Infection of KBV in honey bees resembles infection caused by ABPV in several ways. For example, both viruses usually persist as inapparent infections in bees and replicate readily only when injected into the hemolymph of adult bees (Anderson, 1991). Immunodiffusion tests showed that strains of KBV from Canada and Spain were even more serologically closely related to ABPV than were other KBV strains (Allen and Ball, 1995). Molecular analysis revealed KBV and ABPV share about 70% sequence homology over the entire genome, although there are significant differences in several critical areas of the genomes between the two viruses (De Miranda *et al.*, 2004). Phylogenetic analyses suggest that KBV and ABPV are distinct viruses and can be inferred to be different species, even though there is no clear geographic and ecological separation between the two viruses (De Miranda *et al.*, 2004; Evans, 2001).

Incidence of KBV infection in honey bees is less prevalent, as compared with other highly prevalent bee viruses such as DWV, BQCV, and SBV. Field survey of honey bee viruses on a large geographic scale of France showed that KBV was found in 17% of the apiaries for adult population, and 5% of the apiaries for pupae versus 97% and 94% of the apiaries with DWV infection for adult and pupae, and 86% and 80% of apiaries with SBV infection for adult and pupae, 86% and 23% with SBV infection for adult and pupae, respectively (Tentcheva *et al.*, 2004b). Although KBV has been considered to be more widespread in the United States than in Europe (Allen and Ball, 1996), field survey from 2002 to 2006 in Maryland indicated that the incidence of KBV infection varied significantly from year to year with more than 50% of apiaries with KBV infection in 2002 and about 10–20% of the apiaries with KBV infection for the rest of the years (Y. P. C., unpublished observation).

Although KBV usually persists as an inapparent infection in honey bees, infection of KBV can be activated to a lethal level in the presence of varroa mites (Bailey *et al.*, 1979). A high mite-infestation level could result in high virulence in the bee colonies (Hung *et al.*, 1996b). It has been experimentally proven that varroa mites were effective vectors of KBV. They transmitted KBV in the same way as they transmitted DWV in bee colonies (Chen *et al.*, 2004b). Varroa mites acquired KBV from virus-infected bees and transferred the virus to virus-negative hosts during

feeding. Varroa mites also acquired virus from KBV-positive mites by cohabiting in the same cell with virus-positive mites via a bee host intermediary. A subsequent study conducted by Shen *et al*. (2005b) further supports the role of varroa mites as a vector in transmitting KBV in bees.

E. Acute bee paralysis virus

ABPV was first discovered during laboratory infectivity tests with CBPV (Bailey *et al*., 1963). When bees were experimentally inoculated with purified CBPV particles, the bees remained flightless and trembling for about 5–7 days before they died. In contrast, when healthy bees were injected with extract prepared from a group of apparently healthy bees and incubated for 5–6 days, most of the bees became flightless and died quickly. Virus particles were isolated from the extracts of those apparently healthy bees that caused bee acute paralysis, hence the designation of the name to distinguish it from CBPV (Bailey *et al*., 1963). Since its first identification, the presence of ABPV in honeybees of *A. mellifera* has been reported in North America, Central and South America, Europe, Oceania, Asia, Africa, and the Middle East (Allen and Ball, 1996; Ellis and Munn, 2005).

ABPV can be detected in both brood and adult stages of bee development. In the field, ABPV commonly occurred in apparently healthy adult bees, particularly during the summer, and infection of ABPV was rarely noticed to be associated with disease or mortality of bees (Bailey, 1965b; Bailey *et al*., 1981). Spread of ABPV in the colonies is probably via salivary gland secretion of infected adult bees when glandular secretions are fed to young larvae or mixed in the pollen. Infected larvae either die before they are sealed in brood cell if large amounts of virus particles were ingested, or survive to emerge as inapparently infected adult bees (Bailey and Ball, 1991).

ABPV is considered to be the second most-prevalent virus in Austria (Berényi *et al*., 2006), though it has been a sporadic infection in the United States only for the last 5 years based on our survey results (unpublished observation). ABPV has been identified as a major cause for the decline and collapse of bee colonies that were also infested with varroa mites in Europe and the United States (Antúnez *et al*., 2006; Bakonyi *et al*., 2002; Ball, 1989; Ball and Allen, 1988; Berényi *et al*., 2006; Faucon *et al*., 1992; Hung *et al*., 1996c; Kulincevic *et al*., 1990). The laboratory experiments by Ball (1989) demonstrated that varroa mites can act as a virus vector and transmit ABPV from severely infected bees to healthy adult bees and brood via feeding activities. Detection of ABPV in varroa mites further supports the possible role of varroa mites in the virus transmission (Allen *et al*., 1986; Bakonyi *et al*., 2002; Chantawannakul *et al*., 2006; Tentcheva *et al*., 2004b). In addition to acting as a vector of the virus, the varroa mite is also believed to serve as an activator of ABPV in infected bees.

Detection of large amounts of the virus in diseased or dead bees from colonies heavily infested with varroa mites suggests that infestation of varroa mites may stimulate the virus to replicate to the amounts sufficient to cause bee disease and mortality (Ball and Allen, 1988; Faucon *et al*., 1992; Hung *et al*., 1996c; Kulincevic *et al*., 1990). While varroa mites might activate ABPV replication, replication of the virus in bees can be also induced by some other factors. Previous studies showed that ABPV was present in bees from apiaries where no APBV-positive varroa mites were detected (Tentcheva *et al*., 2004b) and that replication of ABPV can be activated to detectable concentrations by injection of potassium phosphate buffer (Hung *et al*., 1996c), suggesting that the varroa mite is not the sole factor contributing to the disease outbreaks of ABPV infection.

F. Chronic bee paralysis virus

CBPV was identified as a cause of adult bee paralysis by Bailey *et al*. (1963) after long suspicion that the tracheal mite, *Acarapis woodi*, was the culprit of the paralysis. Later, CBPV was extracted from naturally paralyzed bees as one of the first viruses isolated from honey bees (Bailey *et al*., 1968). CBPV has since been detected in adult bees of *A. mellifera* from every continent except South America (Allen and Ball, 1996; Ellis and Munn, 2005).

CBPV mainly attacks adult bees and causes two forms of ''paralysis'' symptoms in bees (Bailey, 1975). The most common one is characterized by an abnormal trembling of the body and wings, crawling on the ground due to the flight inability, bloated abdomens, and dislocated wings. The other form is identified by the presence of hairless, shiny, and black-appearing bees that are attacked and rejected from returning to the colonies at the entrance of the hives by guard bees. Both forms of symptoms can be seen in bees from the same colony. The variation in the disease symptoms may reflect differences among individual bees in inherited susceptibility to the multiplication of the virus (Kulincevic and Rothenbuhler, 1975; Rinderer *et al*, 1975).

While CBPV causes the same symptoms of trembling and the inability to fly in infected bees that ABPV does, the two viruses are different in several ways: CBPV is the less virulent of the two viruses, as CBPV takes several days to kill the diseased bees while ABPV takes only 1 day; the shapes of the two viruses are different—CBPV particles are asymmetric and ABPV particles are isometric; there are many more virus particles of CBPV than of ABPV in naturally paralyzed bees (Bailey, 1965a).

Laboratory tests were carried out to investigate the infectivity of CBPV by injecting purified virus particles into the hemolymph of bees, spraying virus preparation on the surfaces of bees, or mixing virus particles with colony food (Bailey and Ball, 1991; Bailey *et al*., 1983). The results showed

that CBPV was readily transmitted to bees by topical application of virus particles after hairs on the surface of the body were denuded. The results also showed that CBPV is not readily replicated to the level sufficient to cause disease when the virus was introduced in bees via food. Accordingly, CBPV naturally spread best among bees when the colonies were the most crowded. The close contact of overcrowded bees breaks hairs from the cuticle, allowing CBPV to spread from diseased bees to healthy bees via their exposed epidermal cytoplasm. It is likely that any factors that result in decreased foraging activities and crowded conditions in the bee colonies may lead to disease outbreaks of CBPV.

It has been reported that CBPV is very widespread in Britain and infects most bees and causes mortality in bee colonies (Bailey *et al.*, 1981). The incidence of CBPV in Britain declined from 8% in 1947 to less than 2% by 1963 based on the samples submitted by beekeepers. The decrease in CBPV incidence coincided with the decline in the total number of bee colonies during that period of time (Bailey *et al.*, 1983). In Austria, CBPV was found to be present in different geographic regions and infection of CBPV was detected in 10% of bee colonies suffering from various diseases (Berényi *et al.*, 2006). A field survey in France showed that CBPV was the least prevalent of all examined viruses and that infection of CBPV was detected only in adult bees with the maximum frequency of 4% in the colonies. Infection of CBPV also did not appear to follow any seasonal pattern (Tentcheva *et al.*, 2004b). In the United States, incidence of CBPV has been very sporadic for the last 5 years and less than 1% of bees were identified with CBPV infection in the colonies. Field survey in France and Thailand showed that all examined varroa mites were negative for CBPV. This result suggests that the varroa mite is unlikely a vector of CBPV.

CBPV is often associated with the ''satellite'' virus, chronic paralysis virus associate (CPVA). CPVA is a single-stranded, isometric RNA satellite virus that is of unknown significance. It is serologically unrelated to CBPV but cannot multiply in the absence of CBPV (Ball *et al.*, 1985).

III. TAXONOMY

A. Virion properties

Aside from the filamentous virus and the *A. iridescent* virus, all honey bee viruses reported so far share a genome of positive-sense single-stranded RNA; icosahedral, pseudo $T = 3$ structure symmetry; and are free of a lipid-containing envelope although they differ somewhat in their biological properties. The outer shell of the capsid is composed of 60 repeated protomers, each consisting of a single molecule of three subunits VP1, VP2, and VP3. In addition to these three subunits, there is a smaller

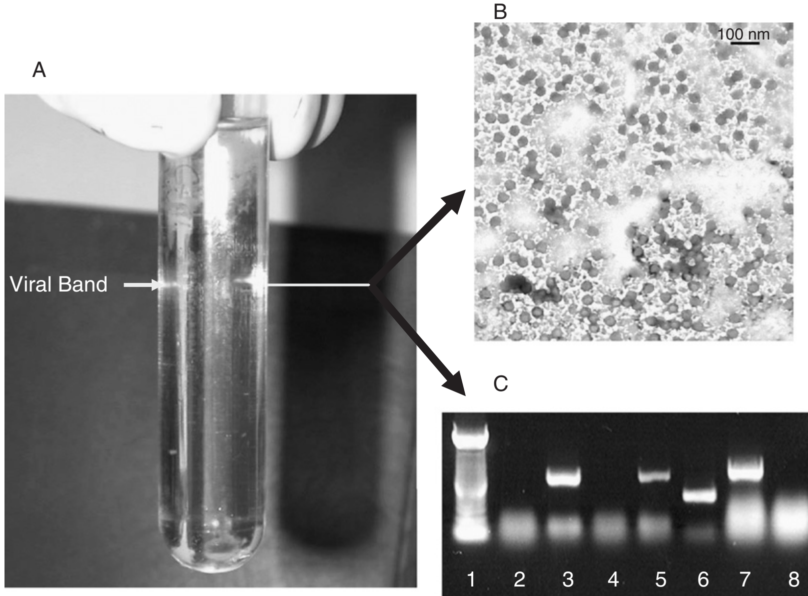

FIGURE 1 (A) Virus band after CsCl density gradient centrifugation. The virus-containing band was collected for subsequent electron micrograph and RT-PCR analyses. (B) Electron micrograph of honey bee virus particles. Bee viruses are spherical to slightly oval particles about 29 nm in diameter as determined from EM. Bar marker represents 0.1 μM. (C) The virus preparation used for this electron micrograph was also examined for the presence of six viruses: ABPV, BQCV, CBPV, DWV, KBV, and SBV by RT-PCR. The primers used in the study were the same as reported earlier (Chen *et al*., 2005). Four viruses, BQCV, DWV, KBV, and SBV, were detected in the virus preparation. Primer pair specific for BQCV, DWV, KBV, and SBV amplified a PCR fragment of 700, 702, 415, and 824 bp, respectively. Lane 1, 100-bp DNA ladder; Lane 2, ABPV; Lane 3, BQCV; Lane 4, CBPV; Lane 5, DWV; Lane 6, KBV; Lane 7, SBV; and Lane 8, Negative control (previously identified negative sample). As shown in electron micrograph, no significant difference in the virion size and morphology could be observed among the four different virus particles (modified from Chen *et al*., 2006a).

fourth protein VP4 that is present in the virions of some viruses such as BQCV and ABPV (Govan *et al*., 2000; Leat *et al*., 2000). VP4 is not exposed at the surface of the viral particle and is located on the internal surface of the fivefold axis below VP1. The capsid proteins play important roles in the protection of viral RNA from activities of RNases and irregular environments and in the determination of viral host specificity and tissue tropism.

Electron micrographs reveal that honey bee virions are spherical to slightly ovoid in shape, approximately 17–30 nm in diameter. The virions

possess a buoyant density in CsCl ranging from 1.33 to 1.42 g/ml, and a sedimentation coefficient between 100S and 190S (Bailey, 1976; Ball and Bailey, 1991). It is a common phenomenon that several viruses of similar size and shape coexist in natural populations of honey bees (Anderson and Gibbs, 1988; Chen *et al.*, 2004c). Purified virus preparations are therefore rarely free of contaminating viruses. As shown in Fig. 1, the virus preparation used for electron microscope analysis was determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) analysis to contain four different viruses BQCV, DWV, KBV, and SBV. No significant differences in virion size and morphology could be observed among the virus particles that comprised the four different viruses (Chen *et al.*, 2004c). This is in general agreement with previous EM studies of viruses isolated from bees (Bailey and Ball, 1991; Bailey and Woods, 1977) and bee mites (Kleespies *et al.*, 2000).

B. Genome organization and classification

The genomes of the positive-stranded RNA viruses are directly involved in several key viral processes including acting as mRNAs for translation of viral proteins, serving as templates for viral genome replication, and being assembled into progeny of viral particles along with structural proteins. Of course, genomes of honey bee viruses are involved in each of these processes. The replication of viruses occurs entirely in the cytoplasm of the host cell. The virus particle attaches to the surface of the host cell and interacts with a receptor on the host cell membrane and releases its RNA genome into the host cell. No viral enzymes/proteins enter the host cell along with the viral genome. Once inside the host cell, the RNA genome is translated into the protein precursors that undergo a cascade of cleavages to form structural and functional proteins for RNA replication. With the help of RNA-dependent RNA polymerase (RdRp), the positive-stranded RNA genome is copied to a negative-stranded intermediate, which serves as a template for replication of new genomic strands. When sufficient positive-stranded progeny RNAs and structure proteins are generated, they are packed into progeny viral particles. The progeny virions then travel to the cell surface where they are released.

Most honey bee viruses belong to the picorna-like virus superfamily and have the following characteristics in their genomic structure: (1) a single molecule of RNA genome coated with a capsid protein shell; (2) a small protein called VPg (viral *p*rotein *g*enome linked) covalently attached to the 5′ end of the viral RNA genome. VPg is responsible for stabilizing the 5′ end of the RNA genome and serves as a primer for replication and translation, contrary to cellular mRNAs where a methylated G cap is attached at the 5′end; (3) at the 5′ end, a long untranslated region (UTR) containing a ‘‘cloverleaf’’ secondary structure, presumably

involved in initiation of translation; (4) a string of adenylic acid residue linked to the 3′ end of the RNA genome and the length of the poly(A) tail is genetically determined and varies in different viruses; and (5) the 3′ terminal sequences of the genomic RNA that can be folded into a stem-loop structure presumably involved in RNA replication.

To date, the complete genome sequences of six honey bee viruses including ABPV (Govan *et al.*, 2000), BQCV (Leat *et al.*, 2000), DWV (Lanzi *et al.*, 2006), KBV (De Miranda *et al.*, 2004), Kakugo virus (KV) (Fujiyuki *et al.*, 2004), and SBV (Ghosh *et al.*, 1999), and partial genome sequences of CBPV (GenBank accession number: AF461061) have been reported. The genomic information of these viruses provides considerable insight into the basic gene structure and organization of honey bee viruses. The genome sizes of honey bee viruses range from 8550 to 10,140 bp, excluding the poly(A) tail. The genomes of bee viruses are enriched in AU (58.97–62.4%), compared to the content of GC (37.6–40.71%) (Table I). Genomes of SBV, DWV, and KV contain one large open reading frame (ORF), while genomes of ABPV, BQCV, and KBV contain two nonoverlapping ORFs. According to the gene order of the proteins, honey bee viruses are divided into two forms of genomic organization. The genomes of ABPV, BQCV, and KBV are monopartite bicistronic with the nonstructural proteins encoded in the 5′-proximal ORF and the structural proteins encoded in the 3′-proximal ORF. In contrast, the genomes of SBV, DWV, and KV are monopartite monocistronic with the structural proteins encoded in the 5′-proximal ORF and the nonstructural proteins encoded in the 3′-proximal ORF (Fig. 2). Based largely on their genomic organization, BQCV, KBV, and ABPV, formerly known as insect picorna-like viruses, are assigned to *Cripavirus*, a genus belonging to family Dicistroviridae. SBV and DWV are assigned to the genus *Iflavirus* which is a ''floating genus'' and not yet assigned to a family (Mayo, 2002).

Phylogenetic analysis using either amino acid sequence alignment of helicase or RdRp of viruses showed that KBV, APBV, and BQCV formed a common lineage with picorna-like viruses that infect plants, insects, and vertebrate. KBV is closely related to ABPV in the phylogenetic tree and BQCV tended to group together with KBV and ABPV but not closely related to them. DWV, KV, and SBV fell into a separate group, with DWV and KV more closely related to one another to SBV in the group (Fig. 3). KV is a novel picorna-like virus isolated from the brains of worker bees and has been associated with aggressive behaviors in worker bees (Fujiyuki *et al.*, 2004, 2005, 2006). Although there are significant differences in the L protein region of the RNA genomes (Lanzi *et al.*, 2006) and in the host pathology (Fujiyuki *et al.*, 2005, 2006; Rortais *et al.*, 2006) between KV and DWV, the species status of KV has not been defined so far because it shares the same host and high nucleotide sequence identity

TABLE I Genome of honey bee viruses

Viruses	Size (bp)	Base composition (%)				GenBank accession no.	References
		A	U	G	C		
ABPV	9470	30.3	30.4	20.5	18.8	AF150629	Govan *et al.*, 2000
BQCV	8550	29.2	30.6	21.6	18.5	AF183905	Leat *et al.*, 2000
DWV	10,140	29.5	32.3	22.4	15.8	NC004830	Lanzi *et al.*, 2006
SBV	8832	29.8	29.4	24.4	16.4	AF092924	Ghosh *et al.*, 1999
KBV	9524	33.8	28.6	20.2	17.4	NC004807	De Miranda *et al.*, 2004

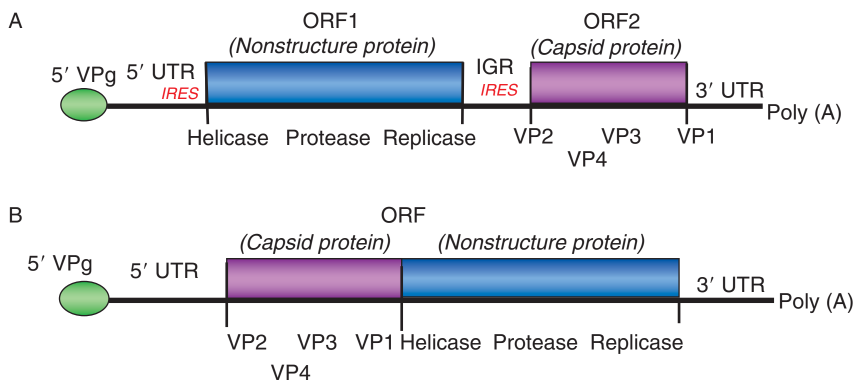

FIGURE 2 Schematic representation of genomes of honey bee viruses. The RNA genome is covalently attached by a genome-linked virion protein (VPg) at the 5′ and a poly(A) tail at 3′ ends. Genomes of honey bee viruses are organized in two different ways. (A) The genomes of ABPV, BQCV, and KBV are monopartite bicistronic with nonstructural genes at the 5′ end and structural genes at the 3′ end. The 5′ UTR and the untranslated intergentic region (IGR) between the two ORFs can initiate efficient translation as the internal ribosomal entry site (IRES). (B) The genome of SBV and DWV are monopartite monocistronic genomes with structural genes at the 5′end and nonstructural genes at the 3′ end (Chen *et al.*, 2006a).

(97%) with DWV. Further investigation of the virus biological properties such as antigenicity, natural cell, and tissue tropism will help to define whether KV is a species distinct from DWV or if KV and DWV are different variants of the same species.

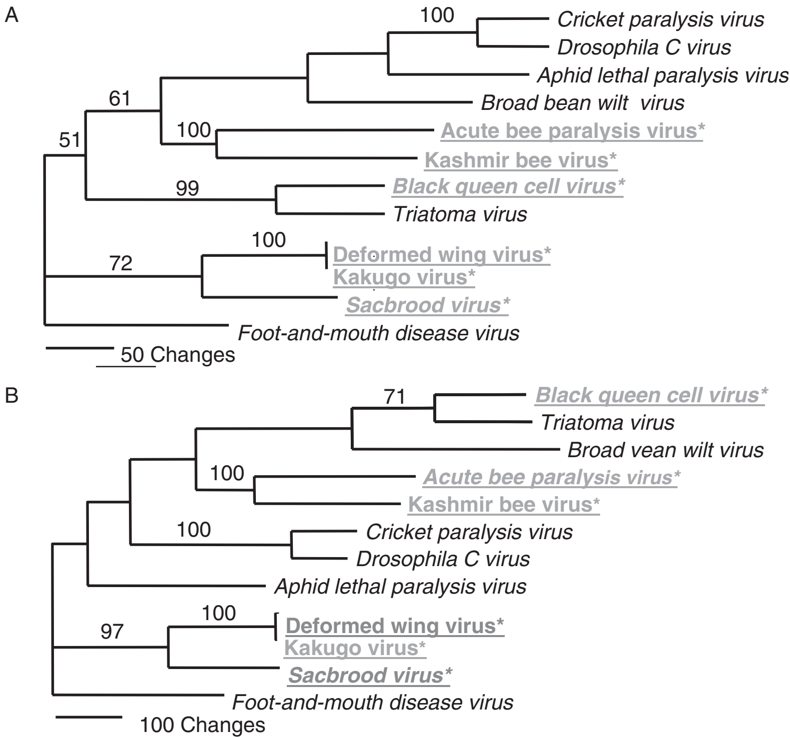

FIGURE 3 Phylogenetic trees derived from the putative helicase (A) and RdRp (B) amino acid sequences of the viruses. For both panels A and B, *Foot-and-mouth disease virus* was used as an out-group to root the trees. Bar lengths represent 50 inferred character state changes for the tree derived from the helicase domain and 100 inferred character state changes for the tree derived from the RdRp domain. Branch lengths are proportional to the number of inferred character state transformations. Numbers at each node represent bootstrap values as percentages of 100 and only bootstrap values greater than 50% are shown. Honey bee viruses are underlined and shown by asterisks. The names of viruses are abbreviated as follows: TV, *Triatoma virus*; ABPV, Acute bee paralysis virus; ALPV, *Aphid lethal paralysis virus*; BBWV, *Broad bean wilt virus*; BQCV, *Black queen cell virus*; CPV, *Cricket paralysis virus*; DCV, *Drosophila C virus*; DWV, Deformed wing virus; FMDV, *Foot-and-mouth disease virus*; KBV, Kashmir bee virus; KV, Kakugo virus; SBV, *Sacbrood virus* (Chen *et al*., 2004c).

The monopartite bicistronic genomes are also characterized by two ORFs that are separated by an untranslated intergenic region (IGR). Both 5′ UTR and the IGR contain highly structured RNA sequences that function as internal ribosomal entry sites (IRESs) for facilitating the cap-independent translation of the viral proteins, though no sequences and translation initiation mechanisms are the same for two IRES elements.

The 5′ UTR-IRES and IGR-IRES elements were first reported in picornaviruses by Jang *et al*. (1988) and Pelletier and Sonenberg (1988), respectively. Since then, IRES elements have been detected in genomes of several other positive-stranded RNA viruses (Hellen and Sarnow, 2001; Sasaki and Nakashima, 1999). Sequence alignments of the IGR of ABPV, BQCV, and KBV with other positive-stranded RNA viruses that were experimentally identified with IRESs (Sasaki and Nakashima, 1999) revealed a considerable level of sequence similarities and indicated the existence of IRES elements in the IGR of ABPV, BQCV, and KBV. Amino acid sequence analysis revealed that methionine is the initial amino acid in the translation of the capsid proteins of ABPV, BQCV, and KBV, in contrast to the non-AUG codons found in genes of capsid proteins of several other members of the Dicistroviridae (Domier *et al*., 2000; Sasaki and Nakashima, 2000; Wilson *et al*., 2000). There is no evidence that translation of proteins is mediated by IRES for the monopartite monocistronic genome.

IV. TRANSMISSION MODES

Viruses are obligate intracellular parasites that can only multiply inside living host cells utilizing the host cell's metabolic machinery. In order to survive, viruses must have ways to invade hosts and be transmitted from one host to another. Transmission processes determine the persistence and the spread of viruses in a population. In theory, transmission of a virus can occur horizontally or vertically, or both. In horizontal transmission, viruses are transmitted between different individuals of the same generation. In vertical transmission, viruses are passed vertically from mother to offspring via egg during its development through the follicle cells or after completion of egg development. Horizontal transmission of a virus can occur by the following means: foodborne transmission, fecal–oral transmission, venereal (sexual) transmission, airborne transmission, and/or vector-borne transmission. Vertical transmission can be further divided into transovum transmission in which viruses are transmitted on the surface of the egg and/or transovarian transmission in which viruses are transmitted within the egg.

Honey bees are eusocial insects and are characterized by the following traits: (1) they live in colonies consisting of overlapping generations: one mother queen and her successors, 20,000–60,000 workers and several hundred drones; (2) there is a reproductive division of labor, that is, sterile workers contribute their entire lives to support reproduction of a single egg-laying queen in the colony; and (3) each member of the bee colony works together in a highly structured social order and engages in extensive coordinating activities, including rearing brood, defending

against invaders, foraging for food, and constructing the combs. The densely crowded populations and high contact rate between colony members in honey bee colonies provide an ideal environment for transmission of pathogens. Because of the importance of the transmission processes in the dynamics of virus infections, elucidation of virus transmission in honey bees represents one of the rapidly developing research fields. Our understanding of bee virus transmission has markedly advanced, and intricate routes of transmission have been identified and documented in honey bees during the last 5 years.

A. Horizontal transmission

1. Foodborne transmission

Foodborne transmission is a means of spreading infection that occurs after eating virus-contaminated food and is the most common route of virus transmission. Natural food in honey bee colony consists of honey, pollen, and royal jelly. The foraging worker bees collect the nectar from flowers and store it in their stomach ''honey sacs.'' After returning to the colonies, foraging bees regurgitate the nectar and pass it on to nurse bees that add an enzyme to convert the nectar into honey used as an energy component of the bee diet. The worker bees also visit flowers to collect pollen that is brought back to the hive as a load on the hind leg and used as a protein source for bee brood to grow. Both honey and pollen are also stored in the combs of the hive for the winter months when nectar and pollen sources are scarce. Royal jelly is a secretion of the hypopharyngeal and mandibular glands of nurse bees. It is used by the nurse bees to feed the queen bee and young larvae. Although trophallactic chain is an important cohesive force in honey bee colonies, trophallactic activities of honey bees, including processing nectar, packing pollen, feeding the brood, and attending the queen, offer the potential for foodborne transmission of pathogens. It is very likely that contamination of food by viruses can occur during foraging or processing by virus-infected workers and that foodborne infection can take place by eating virus-contaminated food. Under conditions of high population density, high contact rate, and high trophallactic rate, direct foodborne transmission may be a significant route for spreading viruses in bee colonies. Evidence of the foodborne transmission pathway in bees has been provided by detection of viruses in food resources. Early studies demonstrated virus transmission to larvae via brood feeding by the detection of viruses in the thoracic gland and hypopharyngeal gland of honey bees (Bailey, 1969; Bailey and Ball, 1991). A study conducted by Shen *et al*. (2005a) showed that two viruses, KBV and SBV, were detected in colony food including honey, pollen, and royal jelly as well as in all developmental stages of bees, suggesting the involvement of colony food in the spread of virus infections. Similar findings

were reported by Chen *et al*. (2006a) who found that two viruses, BQCV and DWV, were detected in honey and six viruses, including ABPV, BQCV, CBPV, DWV, KBV, and SBV, were detected in pollen samples. The two viruses BQCV and DWV found in the honey were also present in over 80% of the examined brood and adult workers in the bee colonies where the colony food was collected. Although ABPV, CBPV, KBV, and SBV were detected in pollen samples, the same viruses were not detected in the bees and their glandular secretion, royal jelly (Chen *et al*., 2006a). These results suggest that bees ingesting virus-contaminated food such as pollen might not always be necessarily infected. The successful infection of a virus may depend on the amount of the virus introduced into the bees and the pathogenic nature of the virus. When a virus is activated to replicate to the amount sufficient to cross the epithelial barrier of the digestive tract and invade different parts of bee body, infection of the virus will likely be detected in different parts of bee and bee products such as royal jelly.

2. Fecal–oral transmission

Fecal–oral transmission spreads pathogens by transferring feces of diseased hosts to uninfected hosts via ingestion and is strongly suspected in environments with overcrowded conditions. Honey bee colonies with densely crowded populations should be a favorable condition for this transmission route. Evidence of a fecal-borne transmission route of viruses in honey bees has been provided by the detection of viruses in feces and digestive tracts of bees. Chen *et al*. (2006b) demonstrated the presence of two viruses BQCV and DWV in the feces freshly defecated by individual queens. Among samples examined for viruses, 100% of feces samples tested positive for the presence of BQCV, and 90% of feces samples tested positive for the presence of DWV. Findings by Chen *et al*. (2006b) were consistent with previous reports that viruses were found in the feces of worker bees (Bailey and Gibbs, 1964; Hung, 2000). Detection of viruses in feces of bees suggests the possibility of the existence of foodborne transmission in honey bees, where infected bees eliminate viruses in their feces and uninfected bees can be infected by feeding on feces-contaminated food or by cleaning the infected bees' feces accumulated in the hive. Oral infection of viruses by contaminated food can be further traced by examination of the digestive gut for virus infections. The studies showed that the same viruses found in feces were also detected in the digestive tract of the bees, providing further evidence of the ingestion of virus-contaminated food and the existence of foodborne or fecal–oral transmission routes in honey bees (Chen *et al*., 2006b). In addition, quantification of virus load in different bee tissues indicated that virus titer was significantly higher in the digestive tract than other tissues tested (Chen *et al*., 2006b), indicating that the digestive tract was the primary site

of virus accumulation and the epithelial cell lining of the digestive tract may constitute the major portal for the spread of virus infection in bees.

3. Venereal transmission

Venereal transmission is a type of infection in which pathogens are transmitted between two sexes during mating. In honey bees, each virgin queen mates with 10 or more drones and semen acquired from multiple drones is stored in the spermatheca, a special pouch in each queen's body. After mating, queens return to the colonies and release a small amount of sperm at a time to fertilize their eggs. After vitellogenesis and egg maturation are completed, the queens start to lay eggs. If drones in honey bee colonies are infected with viruses, the mating can pose an opportunity for horizontal transmission of viruses from infected drones to queens via semen, which in turn further contributes to the transovarial transmission of viruses from queens to their eggs. The detection of viruses in adult drones (Chen *et al.*, 2004a), semen (Chen *et al.*, 2006a; Yue *et al.*, 2006), and in the spermatheca of queens (Chen *et al.*, 2006b) implies the existence of venereal transmission in honey bees. However, it is unclear at this point whether virus infection in queens is a result of foodborne transmission or venereal transmission or both. Further studies will be required to define the role of drones in the spread of virus infections to queens.

4. Airborne transmission

Airborne transmission is a method of spreading infection through aerosol-containing infectious agents that can remain suspended in the air for long periods. Pathogens carried in aerosol are disseminated by air currents and inhaled by susceptible hosts in a localized area. In a honey bee colony, worker bees function as a single unit to maintain a steady temperature within 0.5 °C of 35 °C (Simpson, 1961). During the winter seasons when the ambient temperature is below the temperature range, bees cluster together and raise their metabolic rate to conserve and generate heat. During the summer season when the ambient temperature is above the temperature range, worker bees collect water as well as nectar, evaporate it, and establish air currents through the colony to reduce the internal colony temperature and to prevent the brood nest from overheating. The special thermoregulation mechanism of honey bees creates an active circulating environment within the bee colonies, which might provide opportunities for transmission of viruses via the airborne route. A study carried out by Lighthart *et al.* (2005) reported that honey bees not only absorb airborne bacterial spores but also viruses and showed that honey bees induced to fly in a miniature wind tunnel absorbed aerosol that carried a virus, bacteriophage MS2. Although there is no epidemiological or laboratory data on airborne transmission of honey bee viruses, the results demonstrated in studies of Lighthart *et al.* imply the possibility

that honey bee viruses can be carried by aerosol and spread in the bee colonies through the infected bees to susceptible bees in the colonies. To prove this hypothesis, further studies will be needed.

5. Vector-borne transmission

Vector-borne transmission is an indirect route of horizontal transmission and involves an intermediate biological host, a vector, which acquires and transmits viruses from one host to another. The varroa mite is an obligate parasite of the honey bee attacking different developmental stages and castes of bees and is considered to be the most important pest of honey bees around the world. The entire life cycle of the varroa mite is spent with their honey bee hosts. Female mites feed on the bee larvae and lay eggs of both sexes in the brood cells. Developing mites feed on immature bees. After the mites mature, male and female mites mate inside of the capped brood cell. The male dies after copulation and females emerge from the brood cell along with their bee host and seek another host to repeat the life cycle. The feeding of varroa mites can result in a decline in host vigor, immunity, weight, shorter bee life span, and the eventual destruction of the colonies within a few years (De Jong *et al*., 1982; Korpela *et al*., 1992; Kovac and Crailsheim, 1988; Weinberg and Madel, 1985; Yang and Cox-Foster, 2005). In addition to its direct impact on host health, the feeding of mites on bees provides entry for diseases; both nymph and adult mites feed on bees using their piercing mouthparts to penetrate the body walls of bees to suck the hemolymph. The mites can therefore act as vectors for pathogens during the feeding. The detection of several bee viruses in varroa mites indicates the possible role of varroa mites as vectors in the transmission of viruses among honey bees (Chantawannakul *et al*., 2006; Fujiyuki *et al*., 2006; Hung and Shimanuki, 1999; Ongus *et al*., 2004; Shen *et al*., 2005b; Tentcheva *et al*., 2004a,b; Yue and Genersch, 2005). Previous field investigations reported that viral infections in honey bees have been involved in the collapse of bee colonies also infested with varroa mites (Allen and Ball, 1996; Ball and Allen, 1988; Kulincevic *et al*., 1990). Several viral disease outbreaks including ABPV, CBPV, slow paralysis virus (SPV), BQCV, KBV, Cloudy wing virus (CWV), SBV, and DWV have been documented to be associated with the infestation of varroa mites (Allen and Ball, 1996; Allen *et al*., 1986; Ball and Allen, 1988; Martin, 2001; Martin *et al*., 1998, Tentcheva *et al*., 2004b). The term ''bee parasitic mite syndrome'' has been used to describe a disease complex in which colonies are simultaneously infected with viruses and infested with varroa mites (Shimanuki *et al*., 1994). The observation of positive correlation between the levels of varroa mite infestation and the levels of virus concentration in infected bees suggests that vector-borne transmission exists in honey bees and that the varroa mite is not only a vector but also an activator of bee viruses (Ball and Allen, 1988).

The frequent observations of the association of varroa mite infestation with virus infections in honey bees led to laboratory experiments to further define the role of varroa mites in vectoring virus infections. The fact that varroa mites act as vectors in acquiring and transmitting viruses from severely infected individuals to healthy bees in bee colonies has been experimentally demonstrated in several studies. Bowen-Walker *et al*. (1999) provided the first circumstantial evidence that the varroa mite was an effective vector of DWV in bee colonies. Using serological methods, they demonstrated that varroa mites obtained DWV from infected bees and acted as vectors to transmit the virus to uninfected bees, which consequently developed morphological deformities or died after the mites fed on them for certain periods of time. Subsequent studies conducted by Chen *et al*. (2004b) provided strong evidence that the varroa mite is a vector in transmitting KBV to bees. By collecting mites from the KBV-infected colonies and experimentally introducing variable numbers of mites into the individually sealed brood cells of the KBV-negative colonies, a significant positive relationship between the percentage of pupae becoming virus positive and the number of mites introduced per brood cell were found. The more donor mites that were introduced, the greater the incidence of virus was detected in the recipient brood. Representative results obtained from one transmission experiment showed the following results: in the group with no mite introduction, all brood were virus negative; in the group with one, two, three, and four mites introduced per cell, 20%, 40%, 60%, and 100% brood were KBV positive, respectively. This study definitely showed that varroa mites are capable of transmitting KBV to bee brood. Additional observations were made in the same study. Evaluation of the transmission efficiency of the virus revealed that virus frequency in the mites was directly correlated with the number of mites per cell. The more mites introduced into each brood cell, the higher the chance of all mites becoming KBV positive, as long as at least one mite had KBV. While 37% of mites involved in the single mite introductions were determined to be KBV positive 5 days after their introduction into the cells, this percentage rose to 60% in two-mite introductions, 72% in three-mite introductions, and 94% in four-mite introductions. This result suggests that not only do mites transmit viruses to their bee hosts, but noninfected mites can also acquire viruses by cohabiting in a cell with virus-positive mites, presumably via a honey bee intermediary. Therefore, mites emerging from multiple-infested cells can play a disproportionate role in the spread of viruses within the colony. Shen *et al*. (2005b) provided further evidence for the role of varroa mites in transmitting KBV and DWV in honey bee colonies. In their studies, titers of DWV and KBV were found to be significantly higher in mite-infested bee samples, and the elevated virus titers in mite-infested bees were suggested to be a result of virus replication in infected bees due to the suppression of host

immunity by varroa mite infestation. The laboratory experiments, coupled with the field observations, provide unequivocal evidence of the existence of a vector-borne transmission pathway in honey bees and prove that the varroa mite is an effective vector and activator of honey bee viruses.

Although both field and laboratory studies have confirmed that the varroa mite is an effective vector in transmitting and activating bee virus infections, the mechanism of mite-mediated transmission of bee viruses is uncertain. In general, vector-borne transmission of a pathogen can occur in two ways. Mechanical vector-borne transmission occurs when the vector transmits the pathogen from one host to another but does not support the replication of the pathogen. The pathogen is short-lived in a mechanical vector which is only a carrier of the pathogen and not essential in the life cycle of the pathogen. Biological vector-borne transmission, on the other hand, occurs when a vector is persistently infected with the pathogen and the pathogen multiplies in the body of the vector before it is passed to another host. A biological vector may even be an essential part of the pathogen's life cycle. Ongus *et al.* (2004) reported the discovery of a new virus from varroa mites, namely, *Varroa destructor-1* (VDV-1), and demonstrated that VDV-1 replicates in varroa mites as shown by RT-PCR amplification of the negative strand of VDV-1-specific PCR fragment and by scattered occurrence of paracrystalline structures of viral particles in the cytoplasm of varroa mites in histological sections. Their studies also showed that DWV sharing 83–84% nucleotide sequence identity with VDV-1 and that DWV was found to be replicated in varroa mites. Findings that viruses replicate in the varroa mite and that viruses are present in mite saliva suggest that the varroa mite is likely a biological vector of bee viruses (Ongus *et al*., 2004; Shen *et al*., 2005b). Further studies of the pathogenecity of VDV-1 in honey bees would shed more light on the mechanism regulating virus–vector–host interactions and transmission processes of the virus.

B. Vertical transmission

Vertical transmission in which viruses are passed vertically from mother to offspring has long been known to occur in mammals, vertebrates, arthropods, and plants (reviewed in Mims, 1981). Vertical transmission routes of viruses in honey bees were proposed by Fries and Camazine (2001) based on a honey bee disease model. However, it is difficult to demonstrate vertical transmission experimentally by inoculating virus-negative queens with purified viruses and then estimating the filial infection rates or recovering the viruses from the queens' progeny due to the following reasons: (1) most honey bee queens are virus carriers and it is difficult to obtain virus-negative queens for virus inoculation; and

(2) honey bees are often attacked by multiple viral infections, therefore, it is difficult to purify virus particles that contain only a single virus.

Despite limitations, evidence of a vertical transmission pathway has been documented in several reported studies (Chen *et al.*, 2005, 2006b; Shen *et al.*, 2005a). The detection of multiple viruses in queens suggests that a vertical transmission pathway exists within the bee colony and that eggs have the opportunity to obtain viruses from an infected queen (Chen *et al.*, 2005; Shen *et al.*, 2005a). Quantification of virus titer in the ovaries of queens showed that virus concentration in ovaries was relatively low when compared to other examined tissues. The weak virus signals detected in ovaries suggests that virus infections in ovaries were retained in a nonreplicate or latent stage so that viruses would not be propagated to the level that would have a deleterious effect on the embryos (Chen *et al.*, 2006b).

The detection of virus in eggs, the developmental stage not normally associated with any direct and indirect horizontal transmission routes, provides evidence of vertical transmission in honey bees (Chen *et al.*, 2004a; Shen *et al.*, 2005a). Further, the detection of viruses in surface-sterilized eggs excludes the possibility of transovum transmission and suggests the existence of a transovarial transmission pathway in which viruses infect ovarian tissues of the queen and disseminate in developing eggs before oviposition. In addition, the detection of a virus-positive signal in larvae and a virus-negative signal in the royal jelly of the same bee colonies excluded the possibility of foodborne transmission contributing to virus infections in the larval stages of bees and suggest possible vertical transmission.

Field surveys of virus status of both mother queens and their offspring showed more evidence of vertical transmission in honey bees. When queens were found to be positive for certain viruses in bee colonies, the same viruses were detected in their eggs, larvae, and adult worker bees, though neither queens nor their offspring exhibited any overt symptoms of disease. Meanwhile, when queens were negative for certain viruses, these viruses could not be detected in their offspring. These data provide an additional line of evidence that vertical transmission of viruses from queens to their progeny is highly likely in honey bees (Chen *et al*, 2006b).

C. Discussion

The mode of transmission is a major determinant of a virus' virulence. Evolution of virulence is governed by competition between two transmission pathways (Ewald, 1983, 1987, 1994; Lipsitch *et al.*, 1996). With horizontal transmission, virulence will increase through production of high numbers of pathogens. The greater the number of pathogens produced, the higher the opportunities for host exploitation and thereby the higher

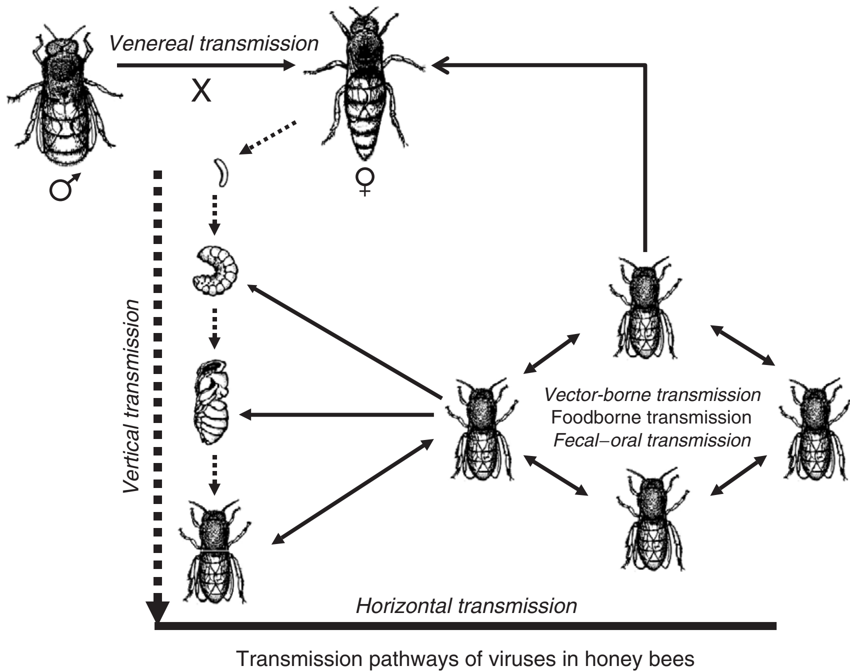

FIGURE 4 Schematic representation of virus transmission routes in honey bees. Virus transmission in honey bees appears to involve both horizontal and vertical transmission pathways. Viruses infect different bee hosts of the same generation by horizontal transmission via the following means: foodborne transmission, fecal–oral transmission, venereal (sexual) transmission, and/or vector-borne transmission. Viruses are also vertically transmitted from infected queen to their offspring. Both transmission pathways are believed to be the important survival strategies for persistence and establishment of viruses in bee population. Solid lines represent horizontal transmission and dotted lines represent vertical transmission (modified from Chen *et al*., 2006a).

rate of transmission. Hence, selection favors high virulence of pathogens. In contrast, virulence of a pathogen decreases under vertical transmission because the fitness of the pathogen is directly dependent on the survival and reproduction of its hosts and any reduction in host reproductive potential will cause a reduction in the reproduction of pathogen. Hence, vertical transmission is associated with low virulence and latent infection. However, if the replication rates of viruses are too high, high virulence will result in high pathogen-induced host mortality, and hosts will lose fitness before producing enough progeny to infect more hosts. On the other hand, if the replication rate is too low, the pathogen will lose opportunities to infect new hosts and thus will lose fitness. Therefore,

a pathogen's fitness is the result of pathogen–host interactions and trade-offs between horizontal and vertical transmission.

Both horizontal and vertical transmission pathways have been proved to be involved in virus transmission in honey bees. Viruses infect different bee hosts of the same generation via foodborne transmission, fecal–oral transmission, venereal transmission, and vector-borne transmission. Viruses can also infect offspring of the current host via vertical transmission (Fig. 4). Both transmission pathways are important survival strategies for viruses not only for their long-term persistence in bee population but also for their establishment in nature. Viruses choose the appropriate transmission pathway based on the developmental, physiological, ecological, and epidemiological conditions. When colonies are under noncompetitive and healthy conditions, viruses remain in bee colonies via vertical transmission and exist in a persistent or latent state without causing honey bees to show any overt signs of infections. Alternatively, when honey bees live under stressful conditions such as infestation of varroa mites, coinfection of other pathogens such as *N. apis*, or decline in food supply, viruses switch to horizontal transmission and start to replicate. High numbers of produced virions then become much more infectious, leading to the death of hosts and possible collapse of the whole bee colony.

V. PATHOGENESIS

While transmission concerns the spread of viruses in a population, pathogenesis deals with the processes by which viruses infect and cause disease in their target hosts. A virus infection depends on a number of pathogen and host factors as well as environmental factors that affect pathogenesis. The outcomes of the virus infection exhibited in the hosts vary, ranging from inapparent infections to severe infections or acutely lethal diseases. Among the wide spectrum of consequences of the virus infections, latent or persistent infections are the most common and are considered to be a state of balanced pathogenicity where multiplication of viruses is arrested by the host's defense mechanism but the viruses themselves remain in the host for long periods of time without producing a manifesting infection. Viruses in the latent state can be replicated if hosts are put under irregular conditions or other environmental stresses, leading to the outbreak of overt diseases. The asymptomatic virus carriers constitute major sources for the transmission of viruses in a population and have great epidemiological importance.

Elucidation of virus pathogenesis requires investigation of many biological features of the viruses and their respective hosts. While transmission pathways of honey bee viruses have been well studied, not much is known about the pathogenesis of viruses in honey bees. In this section, we focus on the current available information involving pathogenic processes of virus infections in honey bees.

A. Causal relationship between a virus and a disease

One of the biggest challenges of studying virus pathogenesis in honey bees is linking the virus infection with a particular disease and therefore evaluating the economic impact of the virus infection. In the field, honey bees are often infected by multiple viruses simultaneously, most of these viruses usually persisting as latent infections in the bee hosts. In addition, virus infections in honey bees are often associated with the infection of other pathogens and infestation of parasites. Therefore, it is difficult to prove that one disease is indeed caused by a particular virus and not the result of mixed virus infections when hosts harbor multiple viruses. However, studies with DWV have revealed that quantification of virus loads using sequence-based methods provides a new way for proving disease causation in infected bees (Chen, 2005; Chen *et al*., 2004a). Detection of the virus by RT-PCR assay showed that DWV was present not only in 100% of the adult bees with symptoms of wing deformity and reduced body size, but also in 70% of the apparently healthy adult bees. This result fulfills one of Koch's postulates, a scientific standard for causal evidence created by Dr. Robert Koch (1884), that the pathogen is present in every case of the diseased individual. The quantification of virus titers by TaqMan real-time quantitative RT-PCR showed that the DWV concentration in bees with the disease symptoms was 4.4-fold higher than in apparently healthy adult bees and that there was no direct correlation between doses of coexisting viruses other than DWV and the appearance of disease symptoms. These results indicate that DWV titers in infected adult bees are the determinants for the appearance of the disease. This result satisfies the molecular revision of Koch's postulates by Fredericks and Relman (1996) that if sequence detection predicts disease and copy numbers of the pathogen correlates with disease severity, then the relationship between a pathogen and a disease is more likely to be causal. The study with DWV clearly demonstrates that the determination of viral load can link the causal association between a virus and a disease when multiple viruses coexist in the same host. Future efforts to determine the critical threshold of the virus concentration required to induce the disease will help to define viral dose requirements for host pathological responses in order to monitor disease development in honey bee colonies.

B. Tissue tropism

The ability of a virus to invade the tissues of a host is a fundamental requirement for a successful infection. The term ''tissue tropism'' is referred to as the specificity of a virus to infect and replicate in particular cells or tissues. Tissue tropism is determined mostly by (1) the chemical affinity of the virus attachment protein with virus-specific receptors on

the surface of a host cell; (2) the suitability of viral entry sites to support virus replication; and (3) the ability of the virus to escape from the host's immediate immunity and thereby to establish long-term chronic or latent infections. The first step of virus infection is the interaction between the viral capsid protein and the specific receptor on the surface of the susceptible host cell to allow the viral RNA to enter the cell cytoplasm. Despite the fact that virus entry processes have been well established for several family members of the Picornaviridae, such as *Poliovirus* (Basavappa *et al*., 1998; Mendelsohn *et al*., 1989), molecular mechanisms of receptor recognition that determine the tissue tropism of honey bee viruses are currently unknown. Research on studying cellular aspects of the pathogenesis of honey bee viruses is largely impeded by the lack of certified virus-free bees and an *in vitro* cultivation system. While a long-term cultivation of honey bee cells has been reported (Bergem *et al*., 2006), a permanent cell line derived from honey bees is not yet available for bee virus propagation. Studies of the mechanisms of tissue tropism that underlie virus binding and spreading to different host cells require a full understanding of the structural features of a virus particle. The atomic structure of a virus particle by X-ray diffraction offers an opportunity to elucidate the molecular determinants of the virus that are necessary for the recognition of receptors and the specificity of tissue tropism. One essential requirement for the crystallization and determination of a virus atomic structure is that viruses need to be propagated in a cell culture and purified to a very high degree. At present it is very difficult to obtain bee viruses in high purity because bee viruses are usually grown *in vivo* and there is always the chance that preparation of any particular bee virus may be contaminated with one or more additional viruses. In addition, determination of the presence or absence of virus-specific receptor molecules on the surface of host cells and characterization of the interactions between receptors and a particular virus is not even possible without an *in vitro* system. Due to these difficulties, our knowledge of tissue tropism of honey bee viruses is mostly limited to ultrastructural studies of virus cytopathology. There have been attempts to culture embryonic bee cells in a highly nutritive medium (Mitsuhashi, 2001, 2002) and in a classical medium (Bergem *et al*., 2006). Cell migration from the explants was observed. The cells could be maintained for a period of several weeks but passaging of the bee cells failed. Although to date there are no continuous cell lines nor heterogenous cell lines derived from honey bees for the proliferation of bee viruses, the protocols for bee tissue cultures and setup of primary cultures have been developed (Kaatz *et al*., 1985; Kreissl and Bicker 1992; Malun *et al*., 2003; Rachinsky and Hartfelder, 1998).

Bee viruses exhibit some differences in their tissue tropism in their bee hosts. Although bee viruses multiply abundantly and fatally when injected into bee hemolymph, the initial infection site of most honey

bee viruses usually occurs through the cuticle by direct contact between healthy and infected bees or in the alimentary tract when bees ingest virus-contaminated food. For example, KBV, CBPV, and ABPV are most likely transmitted contagiously between crowded live bees via the cytoplasm of broken cuticular hairs, while SBV causes infection in bees when both young adult bees and larvae ingest the virus particles mixed in with their food (Bailey and Ball, 1991; Bailey *et al.*, 1979, 1983; Ball and Bailey, 1991). These viruses are then further transmitted to brood via the glandular secretions of infected worker bees during feeding. Although BQCV does not multiply readily when ingested by both worker bees and larvae, it replicates abundantly in adult bees when they are also infected with *N. apis* (Bailey, 1982a). Since *N. apis* often causes gastrointestinal infections in bees, it is believed that infection of *N. apis* increases the susceptibility of the alimentary tract to infection by BQCV, indicative of the alimentary tract as an initial infection site for BQCV.

Honey bee viruses are able to spread their infections systemically from initial sites to secondary target tissues of the host via the blood circulation or nervous systems. KBV infects and replicates in most tissues of an infected bee, including the fore- and hindgut epithelial tissue, alimentary canal musculature, epidermis, tracheal epithelium, hemocytes, oenocytes, and treacheal end cells. However, no evidence of KBV multiplication has been found in the nerve tissues (Dall, 1987). SBV most commonly accumulates in the hypopharyngeal glands of worker bees, but virus particles have also been found in the cytoplasm of fat, muscle, and tracheal-end cells of larvae (Lee and Furgala, 1967). CBPV has a particular tropism for nervous tissues. This is probably why infection of CBPV is often associated with paralysis behavior in infected bees. The CBPV particles can also be found in the alimentary tract, mandibular, and hypopharyngeal glands. However, CBPV does not appear in the cytoplasm of fat or muscle tissues (Giauffret *et al.*, 1966, 1970; Lee and Furgala, 1965). ABPV particles have been seen in the cytoplasm of fat body cells, the brain, and hypopharyngeal glands of acutely paralyzed bees (Bailey and Milne, 1969; Furgala and Lee, 1966). Localization of DWV infection in queens and drones by *in situ* hybridization and RT-PCR methods showed that DWV infection is spread throughout the whole body, including the queen ovaries, queen fat body, spermatheca, and drone seminal vesicles (Chen *et al.*, 2006b; Fievet *et al.*, 2006). Nothing is known about the cytopathological effects of BQCV in honey bees.

C. Host range

A virus' host range is generally referred to as the range of host species that a virus is capable of infecting, although host cell types that a virus infects can also be considered to be a host range in a broad sense. The successful

infection and replication of a virus in a host is a complex process involving the interaction and coevolution of virus and host (Moya *et al.*, 2000). Host species specificity is a genetic property of a virus and any changes in host specificity can occur through virus mutation. RNA viruses show the highest mutation rates among all pathogens, roughly one nucleotide per genome is incorrectly reproduced in each replication (Bonhoeffer and Sniegowski, 2002). The high mutation rates of RNA viruses are due to error-prone replication, since there is no proofreading mechanism for RdRps. Error-prone replication along with a short replication time and large population size leads to high levels of genetic diversity of RNA viruses and the formation of viral quasispecies. The viral quasispecies is a population of genetic variants of virus organized in a way that a central master sequence, the most frequent and fittest genotype, is surrounded by a cloud of mutant sequences. Such an organization provides an evolutionary advantage to RNA viruses and allows the viruses to evolve and adapt to new environments and challenges during infection and sometimes to cross species barriers to new hosts. Host expansion is an important evolutionary force for a virus population and allows viruses to expand their ecological niche to a great diversity of resources and to reduce competition among competitors.

Honey bee viruses display a host range that is not restricted to their original *A. mellifera* host. Apart from the European honey bee, *A. mellifera*, infections of SBV, KBV, and DWV have been reported in the eastern honey bee *A. cerana*. Except for CBPV, the other five common bee viruses, DWV, SBV, BQCV, KBV, and ABPV, are found to be harbored by the varroa mite. The host range of ABPV was shown to extend to at least three bumble bee species (Bailey and Gibbs, 1964). KBV also has alternate hosts in nature and infection of KBV has been detected in bumble bees (*Bombus* spp.) from New Zealand and German wasps (*Vespula germanica*) from Australia (Anderson, 1991). Current detection of DWV, in bumble bees, *B. terrestris* and *B. pascuorum*, demonstrated the ability of DWV to expand its host range (Genersch *et al.*, 2006). Evidence that honey bee viruses exploit multiple host species in their habitat reflects the genetic variability and quasispecies nature of bee viruses. When a virus is expanded to a different host or ecological niche, a new variant to adapt to changes of the new environment may already be formed in a viral population. Further, the widespread nature of a mixed virus infection in honey bees implies that viruses sharing the same physiological niches have the potential to undergo genetic changes by recombination or reassortment, leading to the formation of genetic variants or emergence of new viral species. Further studies on genetic variability of honey bee viruses would shed more light on the pathogenesis of bee virus infections.

VI. HOST DEFENSE MECHANISMS

A virus causes infection by invading host cells, multiplying new virions, and exiting the host cell to attack others. As part of their survival strategies, hosts have evolved effective mechanisms to defend against viral invaders by employing multifaceted immune responses. Virulence and pathogenesis are the consequences of the complex interactions between the infecting virus and host immunity. Vertebrates deal with viral infections by two types of immune responses, innate/nonspecific and adaptive/specific responses. The innate immune response is a rapid response to prevent the spread of viruses during the early phase of the invasion. The innate immune response includes synthesis of interferons to inhibit virus replication and the induction of natural killer (NK) cells to lyse virus infected cells. The adaptive immune response has two components, the humoral and cell-mediated responses. The humoral response attacks viruses when they are present in the host's circulation by B-lymphocyte-produced antibodies (immunoglobulins). The cell-mediated response destroys virus-infected cells by T-lymphocyte-produced cytokines once viruses have resided inside of the host cells. The adaptive immune response can also result in the production of ''memory cells'' which endow the immune system with the ability to respond much more rapidly and effectively to a subsequent infection of the same virus, which provides long-term protection against a given virus. In insects, NK cells, antibodies, cytotoxic T cells, and memory cells are all lacking and the entire immune system is innate. In general, insects utilize three lines of defense to combat infections: physical and chemical barriers, humoral immune responses, and cellular immune responses. In insect cellular immune responses, hemocytes confer cellular immunity to insects and hemocytic response is mediated by phygocytosis, nodule formation, and encapsulation of microbes. The insect humoral response is characterized by the activation of the phenoloxidase cascade and biosynthesis of antimicrobial peptides. The hemocytic and phenoloxidase responses are rapid and present the first line of defense behind the physical and chemical barriers, while the synthesis of antimicrobial peptides is much slower and begins to appear some hours after the actual infection has been recognized. Together, these responses constitute an effective defense system to protect insects from challenges by numerous invaders (Schmid-Hempel, 2005).

While the humoral and cellular immune responses to bacterial and fungal infections have been characterized and documented in honey bees, relatively little is known concerning how honey bees recognize and fight viral infections. However, we believe that honey bees do possess effective defense mechanisms that protect them from virus infections.

The commonly observed phenomenon that viruses persist in apparently healthy colonies as latent infections is a good indication that honey bees have the innate ability to resist the multiplication of virus infections.

Recent work has indicated that RNA interference (RNAi) is a natural, conserved mechanism of antiviral immunity in plants, vertebrates, and insects (Keene *et al*., 2004; Li *et al*., 2002; Voinnet, 2001). RNAi is an RNA-dependent gene silencing process triggered by a long double-stranded RNA (dsRNA). When dsRNA is introduced into a cell, a specific RNaseIII endonuclease, Dicer, binds and cleaves dsRNA to produce double-stranded fragments of 20–25 base pairs with 2-nt 3′ overhangs, called small interfering RNAs (siRNAs). The siRNAs are integrated into the RNA-induced silencing complex (RISC) to activate the RISC. Activated RISC bind to homologous mRNA and cause sequence-specific degradation of the target mRNA. Positive-stranded RNA viruses appear to be potentially vulnerable to RNAi because the viruses replicate their genomes through complementary strands resulting in dsRNA replication intermediates that are attractive targets for siRNAs. Since the genomes of most honey bee viruses are positive-stranded RNA molecules, we would expect RNAi to also be an important defense mechanism against viruses in honey bees.

A. Colony-level defense

The honey bee colony is considered to be a superorganism since a bee colony often acts as a single unit to share labor, specialize in tasks, and coordinate efforts. The homogeneous genetic structure, close physical contact, and extensive social interactions among individuals make bee colonies especially vulnerable to the infection and transmission of pathogens. On the other hand, the highly elaborate social organization of bee colonies poses a special advantage for bee immunity to defend against the infection of pathogens and to improve the survival of the colonies (Evans and Pettis, 2005; Fries and Camazine, 2001; Naug and Camazine, 2002). Therefore, it is sometimes necessary to look at the host defense mechanisms at the colony level. Completion of genome sequences of the honey bee shows that *A. mellifera*, compared to *Anopheles* and *Drosophila*, has fewer paralogs for genes related to innate immunity, with about one-third of the total number of genes found in *Anopheles* and *Drosophila* for 17 immune-related gene families (Evans *et al*., 2006; Honey Bee Genome Sequencing Consortium, 2006). Honey bee immunity against intruders is constituted not only by individual-level defense regulated by immune-related genes, but also by the colony-level defense mechanism. Compared to other nonsocial insects, the reduction of immune-related genes in honey bees may be a result of strengthened colony-level defense.

Hygienic behavior is characterized by the rapid detection of diseased and dead brood, uncapping of the brood cell, and removal of the affected brood by worker bees. The hygienic behavior of worker bees is an important aspect of the honey bee's immunity and has been shown to be effective against American foulbrood, chalkbrood, nosema, and varroa mites in colonies (Gilliam *et al*., 1983; Park *et al*., 1937; Peng *et al*., 1987; Rothenbuhler, 1964; Spivak and Reuter, 2001; Woodrow and Holst, 1942). In addition, hygienic activity has been shown to be an effective defensive strategy against virus infections in honey bees. For example, adult worker bees could quickly detect larvae with SBV infection and remove them from the colony to prevent further spread of the infection (Bailey *et al*., 1964). The cleaning or mutual grooming behaviors displayed by worker bees are believed to be responsible for the spontaneous disappearance of SBV infection in the field during the summer when bee colonies are large and foraging activity is high and the ratio of larvae to adult bees is diminishing (Bailey *et al*., 1964). The worker bees in the colonies have also been observed to display aggressive behaviors toward bees affected with CBPV (Drum and Rothenbuhler, 1983). The spontaneous disappearance of CBPV infection in bee colonies has also been associated with bee hygienic behavior provoked by the virus infection (Bailey, 1967).

Honey bees have been observed to generate a brood comb fever in response to invasion by the heat-sensitive pathogen *Ascosphaera apis* before larvae are killed (Starks *et al*., 2000). This fever-producing behavior is a special social defense strategy displayed in honey bees. Brood comb fever can elevate the colony environmental temperature to a level that is above the optimum growing temperature for a microorganism so that the growth and replication of the microorganism are arrested. The higher temperature can also result in the increase of bee metabolism, thereby speeding up the immune activities of individual bees against the microbial infections.

Additionally, honey bees improve their resistance to disease infections by producing antimicrobial substances in their hive products. Propolis is a resinous substance collected from tree sap or other plant sources and then mixed with wax by honey bees. Propolis has been identified to be rich in a group of biologically active antioxidants called flavonoids, which promote natural immunity and cell regeneration (Greeneway *et al*., 1990). It has been shown that propolis not only functions as a cement to seal nest cracks or cavities but also has antimicrobial properties that help the hive block out viruses, bacteria, and other microorganisms (Kujumgiev *et al*., 1999; Miorin *et al*., 2003). Another important feature of honey bees' natural defense is the antimicrobial activity of colony food, including honey, pollen, and royal jelly. The antibiotic agents (also called ''inhibin'') inhibit the development of bacteria and fungi in stored food (Burgett, 1997).

Glucose oxidase is an enzyme known to possess antimicrobial activity against insect pathogens. Glucose oxidase is expressed specifically in the hypopharyngeal gland of honey bees and secreted into the royal jelly, providing protection to bee brood from infection of microorganisms (Ohashi *et al.*, 1999; Santos *et al.*, 2005). Although the antimicrobial properties of colony food to bacterial and fungal infections have been documented, there have been no reports regarding antiviral activities of the colony food in honey bees. Identification of neopterin which displayed some antiviral properties against Coxsackie B virus, a member of the Picornavirus, in royal jelly implies that colony food may have antiviral effects against viruses (Bratslavska *et al.*, 2007; Hamerlinck, 1999). The future identification and characterization of antiviral agents from bees and colony food will be a significant contribution to the management of virus diseases in honey bees.

B. Individual-level defense

1. Physical and chemical barriers

Honey bee viruses usually enter the host through the alimentary tract during feeding or trauma on the body surface, though they can also directly enter the blood circulation via bites by varroa mites or other insects. Like other insects, honey bees can utilize both physical and chemical barriers as a primary line of passive defense to avoid infection. Both physical and chemical barriers confer nonspecific immunity to honey bees. The physical barrier includes the outer cuticle exoskeleton, the chitinous linings of the trachea, the cuticle lining of the foregut and hindgut, and the peritrophic membrane of the midgut. The rigid epidermal cuticle physically separates internal tissues from the external environment and thereby provides protection against microbial invasion. The peritrophic membrane, a chitinous matrix lining of the midgut, constitutes a second interface protecting internal tissues from external environment and also functions as a permeability barrier to keep pathogens that enter the alimentary canal with food from entering the hemocoel through the gut wall. Additionally, the biochemical environment of the gut can form a chemical barrier to inhibit the multiplication and spread of pathogens to other body tissues.

2. Cellular immune response

Although the physical and chemical barriers usually keep pathogens from entering the body, pathogens occasionally break through these defenses and begin to multiply. Whenever physical and chemical barriers are breached, honey bees can actively protect themselves from infection by employing an innate immunity response which represents a second line of defense and occurs immediately on infection. The primary goal of

the immune system is the recognition of pathogens and differentiation of nonself from self molecules. Once a microorganism is recognized as foreign, the immune system is activated to mount a defensive response to kill or eliminate the intruder. Insects lack immunoglobulin-based immune responses. The recognition of nonself is achieved by pattern recognition receptors (PRRs) that are germline-encoded immune proteins that recognize the pathogen-associated molecular patterns (PAMPs) presented on the surface of microorganisms. There are two families of PRRs: the peptidoglycan recognition proteins (PGRPs) and the Gram-negative binding proteins (GNBPs). The binding of PAMPs to PGRPs and GNBPs activates the proteolytic cascades involving serine protease and serpins. These cascades trigger an intracellular humoral pathway that controls antimicrobial peptide expression and a variety of unspecific cell defense reactions including phagocytosis, nodule formation, encapsulation and melanization, which entails immediate action against foreign intruders.

Phagocytosis is the primary response of hemocytes to small microorganisms such as bacteria. It involves the binding of hemocyte proteins to bacterial or fungal polysaccharides, changes in hemocyte number and morphology, and intracellular killing of pathogens. Nodule formation is a multi-hemocyte-cooperated cellular immune response. Hemocytes may entrap a large number of bacteria in hemocyte aggregates called nodules. Nodule formation is an important mechanism for cleaning large doses of microorganism in the hemolymph. When a foreign invader is too large to be phagocytosed or to be formed into a nodule, it becomes encapsulated by a capsule-like envelope that is made of multiple layers of hemocytes or a melanin coat or both. Encapsulation is the most effective cell-mediated immune mechanism in defending against large intruders such as parasitoid. Hemocyte-mediated killing mechanism is often accomplished by phenoloxidase activity and melanization. Melanization is triggered by the activation of a phenoloxidase cascade. A key enzyme, phenoloxidase, converts phenols into quinines, which subsequently polymerize to melanin. Melanin is deposited around a foreign invader before more hemocytes are recruited, leading to the eventual formation of a melanized cell capsule accompanied by elevated levels of nitric oxide, superoxide anion, and hydrogen peroxide in the host. However, there is another sort of encapsulation, cellular encapsulation, that does not depend on oxygen and can occur without any sign of melanization. Killing by cellular encapsulation probably depends on the lysozyme hydrolytic mechanisms (Carton and Nappi, 2001; Dimopoulos, 2003; Dunn, 1986; Lavine and Strand, 2002).

The cell-mediated immune response to fungus infections has been characterized in honey bees (Glinski and Buczek, 2003). Two critical enzymes, phenol oxidase and glucose dehydrogenase that play an important role in melanin synthesis and are necessary for defense against

intruding microorganisms and parasites, are present in the hemolymph of the honey bees (Yang and Cox-Foster, 2005; Zufelato *et al.*, 2004). The genes that encode serine protease and serpins, which involve in the binding of PAMPs to PGRPs and GNBPs, have been identified in the genome of the honey bee, suggesting that honey bees have an innate immune system that enable them to defend against various microorganisms and parasites (Honey Bee Genome Sequencing Consortium, 2006; Zou *et al.*, 2006). However, how honey bees combat viral infections via cell-mediated defense reaction remains undefined.

3. Humoral immune response

Insect humoral immune responses involve secretion of antimicrobial peptides by fat bodies that is functionally equivalent to the mammalian liver, into the hemolymph in response to challenges to the immune system. Most of our knowledge of the insect humoral immune response is derived from studies of *Drosophila*. To date, seven classes of antimicrobial peptides, including attacin, cecropin, defensin, diptericin, drosocin, drosomycin, and metchnikowin, have been identified in *Drosophila*, and their expression has been found to be regulated by two NF-κB signaling pathways, Toll pathway and immune deficiency (Imd) pathway (reviewed by Bulet *et al.*, 2004; Leclerc and Reichhart, 2004). The humoral signaling pathway is also triggered by the binding of PAMPs to PGRPs and GNBPs which is involved in the upstream infection recognition. The Toll pathway has long been recognized to be a critical signaling pathway during Gram-positive bacterial and fungal infections. The Toll transduction cascade is activated when the ligand, Spätzle, binds to the transmembrane Toll receptor and induces the recruitment of a protein complex consisting of MyD88, Tube, and Pelle. The recruitment of the protein complex leads to the proteasome-dependent degradation of cactus. The degradation of cactus allows translocation of two NF-κB transcription factors, Dif and Dorsal, to the nucleus, causing rapid expression of gene-encoding antimicrobial peptides such as defensin, drosomycin, and metchnikowin. Imd signaling pathway, in contrast, is specific for Gram-negative bacteria although it is activated in a similar fashion to the Toll pathway. The Imd pathway activates a transcription factor, Relish, and the processed Relish then enters the nucleus where it regulates the expression of the gene-encoding antibacterial peptides such as attacin, cecropin, diptericin and drosocin. A study by Zambon *et al.* (2005) reported that both the Toll and Imd pathways were activated in *Drosophila* by an infection of *Drosophila X virus* (DXV), a dsRNA virus. Their studies showed that Toll pathway was required for the inhibition of DXV replication and that the inactivation of the Toll pathway could result in increases in virus titer and death in infected flies. This study clearly indicates that the Toll pathway was an essential part of the antiviral response in *Drosophila*.

Another study conducted by Dostert *et al*. (2005) showed that infection of *Drosophila C virus* (DCV), a member of the genus *Cripavirus* and the family Dicistroviridae, that several honey bee viruses also belong to, induced a set of genes distinct from those regulated by the Toll and Imd pathways and triggered a Janus Kinase-signal transducer and activator of transcription (Jak-STAT) DNA-binding activity. Therefore, they suggested that a Jak-STAT signaling pathway is required for an antiviral response in *Drosophila* (Dostert *et al*., 2005). The Jak-STAT pathway is ubiquitous amongst vertebrates. The signaling pathway takes part in the regulation of cellular responses to a variety of cytokines and growth factors to alter gene expression. The binding of a cytokine or growth factor to its receptor activates Jak, a cytoplasmic tyrosine kinase, and triggers it to phosphorylate and stimulate STAT, a gene regulatory protein, to detach from the receptor and translocate to the nucleus. Different STATs accumlated in the nucleus form hetero- and homodimers that induce expression of their target genes. Studies by Dostert *et al.* clearly indicated that in addition to Toll and Imd pathways for defense against bacterial and fungal infections, another evolutionarily conserved innate immune pathway, Jak-STAT pathway, exists in *Drosophila* and participates in the function of antiviral infections.

Several antimicrobial peptides including abaecin, apidaecin, hymenoptaecin, and defensin have been identified in the hemolymph of honey bees on induction of bacterial infections (Casteels *et al*., 1989, 1990; Casteels-Josson *et al*., 1994). These peptides do appear to be involved in the bee immune response to pathogen infections. A recent genome-wide analysis of honey bee immunity indicates that honey bees possess orthologues for the core members involved in different recognition and signaling pathways including Toll, Imd, Jak-STAT, as well as JNK, which is also a pivotal actor in the *Drosophila* immune response and involves the activation of transcriptional factor, Basket, though the functions of most honey bee components in these pathways remain to be validated (Evans *et al*., 2006). The data generated from *Drosophila* studies indicate that insects have an effective innate immune system that is able to respond not only to bacterial and fungal infections but also to viral infections. Knowledge of the antiviral immunity demonstrated in *Drosophila* should provide us with important insight into the relationship between virus infections and host immune responses in honey bees.

VII. MANAGEMENT OF VIRUS INFECTIONS

Viral disease outbreaks as well as inapparent viral infections can seriously affect the profitability of the beekeeping industry. Beekeepers are advised to take measures to limit viral infections, although as with any other

animal and plant viruses, chemotherapies for killing bee viruses are currently not possible. An integrated pest management program for bee diseases caused by viruses should include at least the following three components: (1) accurate diagnosis of diseases that allows rapid development and implementation of control strategies, (2) good beekeeping management practice that enhances honey bees' natural immunity to virus infections, and (3) selecting and breeding of disease-resistant strains of honey bees.

A rapid and accurate diagnosis of virus infections is a critical component of the virus surveillance and control program. It will help to determine the epidemiology of bee viral infections and to monitor honey bee colonies for viruses to prevent the spread of diseases. For many years, the detection and identification of viral infection in honey bees were based largely on serological methods like Ouchterlony gel diffusion, indirect fluorescent antibody (IFA), and enzyme-linked immunosorbent assay (ELISA) tests (Allen and Ball, 1995; Allen *et al.*, 1986; Anderson, 1984). The development of molecular methods has revolutionized the diagnosis of viral diseases and provided powerful tools for specific, sensitive, and rapid identification of viruses. The RT-PCR method has become a standard method for detection, quantification, and phylogenetic analysis of honey bee viruses (Bakonyi *et al.*, 2002; Benjeddou *et al.*, 2001; Evans, 2001; Grabensteiner *et al.*, 2001; Hung *et al.*, 1996a; Ribiere *et al.*, 2002; Stoltz *et al.*, 1995; Tentcheva *et al.*, 2004a). With increasing genomic information of bee viruses, we would expect that nucleic acid–based methods such as Northern blotting, real-time RT-PCR, microarray analysis, and other emerging methods will continue to serve as predominant tools for the diagnosis of viral diseases in honey bees.

Good bee management practice is fundamental for enhancing honey bees' natural immunity, which is the most useful tool in combating viral diseases. Stressful circumstances can favor outbreaks of viral diseases, thus any efforts that strengthen the colony health are expected to reduce the risk of virus infections. Since the varroa mite has been proven to be an effective vector in transmitting and activating viruses, timely and efficient control of the varroa mite population will reduce the incidence of viral diseases. A mathematical model proposed by Sumpter and Martin (2004) predicts that virus-associated winter collapses can be avoided if bee colonies are treated with varroacides in the summer to decrease the ABPV and DWV loads below a critical level. In addition to controlling the vector population, effective management of bee viral diseases can be achieved by maintaining good sanitation practices, feeding bees with the proper quantity and quality of food, and replacing combs and queens when the problem is serious.

Selection and breeding of disease resistant bee strains are an effective way to defend against viral attacks in honey bees. Several traits of honey bees, such as hygienic behavior and suppressed mite reproduction (SMR), are important behavioral mechanisms of disease resistance (Harbo and Harris, 2005; Lapidge *et al*., 2002). The highly hygienic bees can efficiently suppress the virus infection and *V. destructor* infestation by quickly recognizing and removing the diseased brood and varroa mites from combs. Nonhygienic bee lines show a slower removal response to diseased bee brood than bee stocks selected for hygienic traits (Spivak and Gilliam, 1998). Such hygienic behavior strongly depends on gene effects and has been the basis for breeding programs. The development of an integrated program to select bee populations with desirable traits, to preserve honey bee germplasm, and to arrange the mating of queens and drones will provide an important tool to breed for disease-resistant genotypes and hold great promise for colony-level disease resistance. In addition, with the completion of the honey bee genomic sequence, it becomes possible to conduct gene-based selection for genotypes with defensive and hygienic behaviors and to characterize the genes that confer disease resistance and to genetically manipulate the genes to enhance the disease resistance in honey bees.

VIII. FUTURE DIRECTIONS

In recent years, progress in honey bee virus research has been impressive. However, infections of viruses in honey bees have not been fully characterized at the molecular level and there are many gaps in our knowledge of the key processes underlying the dynamics of virus transmission, epidemiology, pathogenesis, and host immunity to virus infections. For example, what mechanisms regulate the virus transmission process, how viral gene expression contributes to disease pathogenesis, and how host immune responses regulate virus survival and replication? In addition, recent progress in the understanding of bee virus infections is limited to the aforementioned six honey bee viruses; the other previously identified honey bee viruses such as Filamentous virus, *A. iridescent* virus, Cloudy wing virus, Bee virus X, Bee virus Y, Arkansas bee virus, Egypt bee virus, slow paralysis virus, Thai Sacbrood, and Berkeley bee picornavirus remain poorly characterized. Moreover, identified viruses can act in new and unexpected ways and new viruses keep emerging, forming additional challenges in the elucidation of viral infections. The availability of the bee genome sequence in conjunction with new technologies opens exciting possibilities for exploring new aspects of virus life in the host and foretells future advances in bee virus research.

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REFERENCES

Allen, M., and Ball, B. (1995). Characterisation and serological relationships of strains of Kashmir bee virus. *Ann. Appl. Biol.* **126:**471–484.

Allen, M., and Ball, B. (1996). The incidence and world distribution of honey bee viruses. *Bee World* **77:**141–162.

Allen, M., Ball, B., White, R. F., and Antoniw, J. F. (1986). The detection of acute paralysis virus in *Varroa jacobsoni* by the use of a simple indirect ELISA. *J. Apic. Res.* **25:**100–105.

Anderson, D. L. (1984). A comparison of serological techniques for detecting and identifying honeybee viruses. *J. Invertebr. Pathol.* **44:**233–243.

Anderson, D. L. (1985). Viruses of New Zealand honey bees. *New Zealand Beekeeper* **188:**8–10.

Anderson, D. L. (1990). Pests and pathogens of the honeybee (*Apis mellifera* L.) in Fiji. *J. Apic. Res.* **29:**53–59.

Anderson, D. L. (1991). Kashmir bee virus – a relatively harmless virus of honey bee colonies. *Am. Bee J.* **131:**767–770.

Anderson, D. L. (1993). Pathogen and queen bees. *Australasian Beekeeper* **94:**292–296.

Anderson, D. L., and Gibbs, A. J. (1988). Inapparent virus infections and their interactions in pupae of the honey bee (*Apis mellifera* Linnaeus) in Australia. *J. Gen. Virol.* **69:**1617–1625.

Anderson, D. L., and Trueman, J. W. H. (2000). *Varroa jacobsoni* (Acari:Varroidae) is more than one species. *Exp. Appl. Acarol.* **24:**165–189.

Antúnez, K., D'Alessandro, B., Corbella, E., Ramalllo, G., and Zunino, P. (2006). Honeybee viruses in Uruguay. *J. Invertebr. Pathol.* **93:**67–70.

Bailey, L. (1965a). Paralysis of the honey bee Apis mellifera Linnaeus. *J. Invertebr. Pathol.* **7:**132–140.

Bailey, L. (1965b). The occurrence of chronic and acute bee paralysis viruses in bees outside Britain. *J. Invertebr. Pathol.* **7:**167–169.

Bailey, L. (1967). The incidence of virus diseases in the honey bee. *Ann. Appl. Biol.* **60:**43–48.

Bailey, L. (1969). The multiplication and spread of sacbrood virus of bees. *Ann. Appl. Biol.* **63:**483–491.

Bailey, L. (1975). Recent research on honey bee viruses. *Bee World* **56:**55–64.

Bailey, L. (1976). Viruses attacking the honey bee. *Adv. Virus Res.* **20:**271–304.

Bailey, L. (1981). ''Honey Bee Pathology.'' Academic Press, London.

Bailey, L. (1982a). Viruses of honeybees. *Bee World* **63:**165–173.

Bailey, L. (1982b). A strain of sacbrood virus from *Apis cerana*. *J. Invertebr. Pathol.* **39:**264–265.

Bailey, L., and Ball, B. V. (1991). ''Honey Bee Pathology.'' Academic Press, San Diego, CA.

Bailey, L., and Fernando, E. F. W. (1972). Effects of sacbrood virus on adult honey bees. *Ann. Appl. Biol.* **72:**27–35.

Bailey, L., and Gibbs, A. J. (1964). Acute infection of bees with paralysis virus. *J. Insect. Pathol.* **6:**395–407.

Bailey, L., and Milne, R. G. (1969). The multiplication regions and interaction of acute and chronic bee-paralysis viruses in adult honey bees. *J. Gen. Virol.* **4:**9–14.

Bailey, L., and Woods, R. D. (1977). Two more small RNA viruses from honey bees and further observations on sacbrood and acute bee-paralysis viruses. *J. Gen. Virol.* **37:**175–182.

Bailey, L., Gibbs, A. J., and Woods, R. D. (1963). Two viruses from adult honey bees (*Apis mellifera* Linnaeus). *Virology* **21:**390–395.

Bailey, L., Gibbs, A. J., and Woods, R. D. (1964). Sacbrood virus of the larval honey bee (*Apis mellifera* Linnaeus). *Virology* **23:**425–429.

Bailey, L., Gibbs, A. J., and Woods, R. D. (1968). The purification and properties of chronic bee-paralysis virus. *J. Gen. Virol.* **2:**251–260.

Bailey, L., Carpenter, J. M., and Woods, R. D. (1979). Egypt bee virus and Australian isolates of Kashmir bee virus. *J. Gen. Virol.* **43:**641–647.

Bailey, L., Ball, B. V., and Perry, J. N. (1981). The prevalence of viruses of honey bees in Britain. *Ann. Appl. Biol.* **97:**109–118.

Bailey, L., Ball, B. V., and Perry, J. N. (1983). Honeybee paralysis: Its natural spread and its diminished incidence in England and Wales. *J. Apic. Res.* **22:**191–195.

Bakonyi, T., Farkas, R., Szendröi, A., Dobos-Kovács, M., and Rusvai, M. (2002). Detection of acute bee paralysis virus by RT-PCR in honey bee and *Varroa destructor* field samples: Rapid screening of representative Hungarian apiaries. *Apidologie* **33:**63–74.

Ball, B. (1996). Honeybee viruses: A cause for concern? *Bee World* **77:**117–119.

Ball, B. V. (1989). *Varroa jacobsoni* as a virus vector. *In* ''Present Status of Varroatosis in Europe and Progress in the Varroa Mite Control'' (R. Cavalloro, ed.), pp. 241–244. ECSC-EEC-EAEC, Luxembourg.

Ball, B. V. (2001). Viruses and varroa. *Bee Craft* **83:**20–23.

Ball, B. V., and Allen, M. F. (1988). The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni*. *Ann. Appl. Biol.* **113:**237–244.

Ball, B. V., and Bailey, L. (1991). Viruses of honey bees. *In* ''Atlas of Invertebrate Viruses'' (J. R. Adams and J. R. Bonami, eds.), pp. 525–551. CRC press, Boca Raton, FL.

Ball, B. V., and Bailey, L. (1997). Viruses. *In* ''Honey Bee Pest, Predators, & Diseases'' (R. A. Morse and K. Flottum, eds.), pp. 11–31. The A. I. Root Co., Medina, OH.

Ball, B. V., Overton, H. A., Buck, K. W., Bailey, L., and Perry, J. N. (1985). Relationships between the multiplication of chronic bee-paralysis virus and its associate particle. *J. Gen. Virol.* **66:**1423–1429.

Basavappa, R., Gómez-Yafal, A., and Hogle, J. M. (1998). The poliovirus empty capsid specifically recognizes the poliovirus receptor and undergoes some, but not all, of transitions associated with cell entry. *J. Virol.* **72:**7551–7556.

Benjeddou, M., Leat, N., Allsopp, M., and Davison, S. (2001). Detection of acute bee paralysis virus and black queen cell virus from honeybees by reverse transcriptase PCR. *Appl. Environ. Microbiol.* **67:**2384–2387.

Berényi, O., Bakonyi, T., Derakhshifar, I., Köglberger, H., and Nowotny, N. (2006). Occurrence of six honey bee viruses in diseased Austrian Apiaries. *Appl. Environ. Microbiol.* **72:**2414–2420.

Bergem, M., Norberg, K., and Aamodt, R. M. (2006). Long-term maintenance of *in vitro* cultured honeybee (*Apis mellifera*) embryonic cells. *BMC Dev. Biol.* **6:**17.

Bonhoeffer, S., and Sniegowski, P. (2002). Virus evolution: The importance of being erroneous. *Nature* **420:**367–369.

Bowen-Walker, P. L., Martin, S. J., and Gunn, A. (1999). The transmission of deformed wing virus between honey bees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni* Oud. *J. Invertebr. Pathol.* **73:**101–106.

Bradbear, N. (1988). World distribution of major honeybee diseases and pests. *Bee World* **69:**15–39.

Bratslavska, O., Platace, D., Miklaševičs, E., Fuchs, D., and Martinsons, A. (2007). Influence of neopterin and 7,8-dihydroneopterin on the replication of Coxsackie type B5 and influenza A viruses. *Med. Microbiol. Immunol.* **196**(1)**:**23–29.

Bruce, W. A., Anderson, D. L., Calderone, N. W., and Shimanuki, H. (1995). A survey for Kashmir bee virus in honey bee colonies in the United States. *Am. Bee. J.* **135:**352–354.

Bulet, P., Stocklin, R., and Menin, L. (2004). Anti-microbial peptides: From invertebrates to vertebrates. *Immunol. Rev.* **198:**169–184.

Burgett, D. M. (1997). Antibiotic systems in nectar, honey, and pollen. *In* ''Honey Bee Pests, Predators, and Diseases'' (R. A. Morse and K. Flottum, eds.), pp. 455–468. The A. I. Root Co., Medina, OH.

Casteels, P., Ampe, C., Jacobs, F., Vaeck, M., and Tempst, P. (1989). Apidaecins: Antibacterial peptides from honeybees. *EMBO J.* **8:**2387–2391.

Casteels, P., Ampe, C., Riviere, L., Van Damme, J., Ellicone, C., Fleming, M., Jacobs, F., and Tempst, P. (1990). Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (*Apis mellifera*). *Eur. J. Biochem.* **187:**381–386.

Casteels-Josson, K., Zhang, W., Capaci, T., Casteels, P., and Tempst, P. (1994). Acute transcriptional response of the honeybee peptide-antibiotics gene repertoire and required post-transcriptional conversion of the precursor structures. *J. Biol. Chem.* **269:**28569–28575.

Carton, Y., and Nappi, A. J. (2001). Immunogenetic aspects of the cellular immune response of *Drosophila* against parasitoids. *Immunogenetics* **52:**157–164.

Chantawannakul, P., Ward, L., Boonham, N., and Brown, M. (2006). A scientific note on the detection of honeybee viruses using real-time PCR (TaqMan) in Varroa mites collected from a Thai honeybee (*Apis mellifera*) apiary. *J. Invertebr. Pathol.* **91:**69–73.

Chen, Y. P. (2005). Koch's postulates and quantitative RT-PCR as a new approach for proving viral disease causation in the honeybee, *Apis mellifera* L. *Proceedings of the Bee Research and Virus in Europe (BRAVE) Meeting*, pp. 161–173.

Chen, Y. P., Higgins, J. A., and Feldlaufer, M. F. (2004a). Quantitative analysis of deformed wing virus infection in the honey bee, *Apis mellifera* L. by real-time RT-PCR. *Appl. Environ. Microbiol.* **71:**436–441.

Chen, Y. P., Pettis, J. S., Evans, J. D., Kramer, M., and Feldlaufer, M. F. (2004b). Transmission of Kashmir bee virus by the ectoparasitic mite, *Varroa destructor*. *Apidologie* **35:**441–448.

Chen, Y. P., Zhao, Y., Hammond, J., Hsu, H. T., Evans, J. D., and Feldlaufer, M. F. (2004c). Multiple virus infections in the honey bee and genome divergence of honey bee viruses. *J. Invertebr. Pathol.* **87:**84–93.

Chen, Y. P., Pettis, J. S., and Feldlaufer, M. F. (2005). Detection of multiple viruses in queens of the honey bee, *Apis mellifera* L. *J. Invertebr. Pathol.* **90:**118–121.

Chen, Y. P., Evans, J. D., and Feldlaufer, M. F. (2006a). Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera*. *J. Invertebr. Pathol.* **92:**152–159.

Chen, Y. P., Pettis, J. S., Collins, A., and Feldlaufer, M. F. (2006b). Prevalence and transmission of honey bee viruses. *Appl. Environ. Microbiol.* **72:**606–611.

Dall, D. J. (1985). Inapparent infection of honeybee pupae by Kashmir and sacbrood bee viruses in Australia. *Ann. Appl. Biol.* **106:**461–468.

Dall, D. J. (1987). Multiplication of Kashmir bee virus in pupae of the honeybee, *Apis mellifera*. *J. Invertebr. Pathol.* **49:**279–290.

De Jong, D., De Jong, P. H., and Goncalves, L. S. (1982). Weight loss and other damage to developing worker honey bees from infestation with *Varroa jacobsoni*. *J. Apic. Res.* **21:**165–167.

De Miranda, J. R., Drebot, M., Tylor, S., Shen, M., Cameron, C. E., Stolz, D. B., and Camazine, S. M. (2004). Complete nucleotide sequence of Kashmir bee virus and comparison with acute bee paralysis virus. *J. Gen. Virol.* **85:**2263–2270.

Dimopoulos, G. (2003). Insect immunity and its implication in mosquito-malaria interactions. *Cell. Microbiol.* **5:**3–14.

Domier, L. L., McCoppin, N. K., and D'Arcy, C. J. (2000). Sequence requirements for translation initiation of *Rhopalosiphum padi* virus ORF2. *Virology* **268:**264–271.

Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., Hoffmann, J. A., and Imler, J.-L. (2005). The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. *Nat. Immunol.* **6:**946–953.

Drum, N. H., and Rothenbuhler, W. C. (1983). Non stinging aggressive responses of worker honey-bees to hivemates, intruder bees and bees affected with chronic bee paralysis. *J. Apic. Res.* **22:**256–260.

Dunn, P. E. (1986). Biochemical aspects of insect immunity. *Annu. Rev. Entomol.* **31:**321–339.

Ellis, J. D., and Munn, P. A. (2005). The worldwide health status of honey bees. *Bee World* **86:**88–101.

Evans, J. D. (2001). Genetic evidence for coinfection of honey bees by acute bee paralysis and kashmir bee viruses. *J. Invertebr. Pathol.* **78:**189–193.

Evans, J. D., and Pettis, J. S. (2005). Colony-level effects of immune responsiveness in honey bees, *Apis mellifera*. *Evolution* **59:**2270–2274.

Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J.-L., Jiang, H., Kanost, M., Thompson, G. J., Zou, Z., and Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect. Mol. Biol.* **15:**645–656.

Ewald, P. W. (1983). Host-parasite relations, vectors and the evolution of disease severity. *Annu. Rev. Ecol. Syst.* **14:**465–485.

Ewald, P. W. (1987). Transmission modes and evolution of the parasitism-mutualism continuum. *Ann. JVT Acad. Sci.* **503:**295–306.

Ewald, P. W. (1994). ''Evolution of Infectious Diseases.'' Oxford University Press, Oxford, UK.

Faucon, J. P., Vitu, C., Russo, P., and Vignoni, M. (1992). Diagnosis of acute paralysis: Application to epidemic honeybee diseases in France in 1990. *Apidologie* **23:**139–146.

Fievet, J., Tentcheva, D., Gauthier, L., de Miranda, J., Cousserans, F., Colin, M. E., and Bergoin, M. (2006). Localization of deformed wing virus infection in queen and drone *Apis mellifera* L. *Virol. J.* **3:**16.

Fredericks, D. N., and Relman, D. A. (1996). Sequence-based identification of microbial pathogens: A reconsideration of Koch's postulates. *Clin. Microbiol. Rev.* **9:**18–33.

Fries, I., and Camazine, S. (2001). Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. *Apidologie* **32:**199–214.

Fujiyuki, T., Takeuchi, H., Ono, M., Ohka, S., Sasaki, T., Nomoto, A., and Kubo, T. (2004). Novel insect picorna-like virus identified in the brains of aggressive worker honeybees. *J. Virol.* **78:**1093–1100.

Fujiyuki, T., Takeuchi, H., Ono, M., Ohka, S., Sasaki, T., Nomoto, A., and Kubo, T. (2005). Kakugo virus from brains of aggressive worker honeybees. *Adv. Virus Res.* **65:**1–27.

Fujiyuki, T., Ohka, S., Takeuchi, H., Ono, M., Nomoto, A., and Kubo, T. (2006). Prevalence and phylogeny of Kakugo virus, a novel insect picorna-like virus that infects the honeybee (*Apis mellifera* L.), under various colony conditions. *J. Virol.* **80:**11528–11538.

Furgala, B., and Lee, P. E. (1966). Acute bee paralysis virus, a cytoplasmic insect virus. *Virology* **29:**346–348.

Genersch, E., Yue, C., Fries, I., and de Miranda, J. R. (2006). Detection of deformed wing virus, a honey bee viral pathogen, in bumble bee (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. *J. Invertebr. Pathol.* **91:**61–63.

Ghosh, R. C., Ball, B. V., Willcocks, M. M., and Carter, M. J. (1999). The nucleotide sequence of sacbrood virus of the honey bee: An insect picorna-like virus. *J. Gen. Virol.* **80:** 1541–1549.

Giauffret, A., Duthoit, J. L., and Caucat, M. J. (1966). Histological study of nervous tissue from honey bees infected with ''black disease.'' *Bull. Apic. Doc. Sci. Tech. Inf.* **9:**221–228.

Giauffret, A., Duthoit, J. L., and Tostain-Caucat, M. J. (1970). Ultrastructure of cells of bees infected with the virus of black disease paralysis. Study of the cellular inclusions. *Bull. Apic. Doc. Sci. Tech. Inf.* **13:**115–126.

Gilliam, M., Taber, S., III, and Richardson, G. V. (1983). Hygienic behavior of honey bees in relation to chalkbrood disease. *Apidologie* **14:**29–39.

Glinski, Z., and Buczek, K. (2003). Response of the Apoidea to fungal infections. *Apiacta.* **38:**183–189.

Govan, V. A., Leat, N., Allsopp, M., and Davison, S. (2000). Analysis of the complete genome sequence of acute bee paralysis virus shows that it belongs to the novel group of insect-infecting RNA viruses. *Virology* **277:**457–463.

Grabensteiner, E., Ritter, W., Carter, M. J., Davison, S., Pechhacker, H., Kolodziejek, J., Boecking, O., Derakshhifar, I., Moosbeckhofer, R., Licek, E., and Nowotny, N. (2001). Sacbrood virus of the honeybee (*Apis mellifera*): Rapid identification and phylogenetic analysis using reverse transcription-PCR. *Clin. Diagn. Lab. Immunol.* **8:**93–104.

Greeneway, W., Scaysbroock, T., and Whatley, F. R. (1990). The composition and plant origins of propolis: A report work at Oxford. *Bee World* **71:**107–118.

Hamerlinck, F. F. (1999). Neopterin: A review. *Exp. Dermatol.* **8:**167–176.

Harbo, J. R., and Harris, J. W. (2005). Suppressed mite reproduction explained by the behavior of adult bees. *J. Apic. Res.* **44:**21–23.

Hellen, C. U., and Sarnow, P. (2001). Internal ribosome entry sites in eukaryotic mRNA molecules. *Genes Dev.* **15:**1593–1612.

Honey Bee Genome Sequencing Consortium (2006). Insights into social insects from the genome of the honey bee *Apis mellifera*. *Nature* **443:**931–949.

Hornitzky, M. (1981). The examination of honey bee virus in New South Wale. *Australasian Beekeeper* **82:**261–262.

Hornitzky, M. (1982). Bee diseases research. *Australasian Beekeeper* **84:**7–10.

Hung, A. C. F. (2000). PCR detection of Kashmir bee virus in honey bee excreta. *J. Apic. Res.* **39:**103–106.

Hung, A. C. F., and Shimanuki, H. (1999). A scientific note on the detection of Kashmir bee virus in individual honeybees and *Varroa jacobsoni* mites. *Apidologie* **30:**353–354.

Hung, A. C. F., Adams, J. R., and Shimanuki, H. (1995). Bee parasitic mite syndrome (II): The role of Varroa mite and viruses. *Am. Bee. J.* **135:**702–704.

Hung, A. C. F., Ball, B. V., Adams, J. R., Shimanuki, H., and Knox, D. A. (1996a). A scientific note on the detection of American strain of acute paralysis virus and Kashmir bee virus in dead bees in one US honey bee (*Apis mellifera* L.) colony. *Apidologie* **27:**55–56.

Hung, A. C. F., Shimanuki, H., and Knox, D. A. (1996b). The role of viruses in bee parasitic mite syndrome. *Am. Bee J.* **136:**731–732.

Hung, A. C. F., Shimanuki, H., and Knox, D. A. (1996c). Inapparent infection of acute paralysis virus and Kashmir bee virus in the U.S. honey bees. *Am. Bee J.* **136:**874–876.

Jang, S. K., Kräusslich, H. G., Nicklin, M. J. H., Duke, G. M., Palmenberg, A. C., and Wimmer, E. (1988). A segment of the 5′ nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during *in vitro* translation. *J. Virol.* **62:**2636–2643.

Kaatz, H. H., Hagedorn, H. H., and Engels, W. (1985). Culture of honey bee organs: Development of a new medium and the importance of tracheation. *In vitro Cell. Dev. Biol.* **21:** 347–352.

Keene, K. M., Foy, B. D., Sanchez-Vargas, I., Beaty, B. J., Blair, C. D., and Olson, K. E. (2004). RNA interference acts as a natural antiviral response to O'nyong-nyong virus (Alphavirus; Togaviridae) infection of *Anopheles gambiae*. *Proc. Natl. Acad. Sci. USA* **107:** 17240–17245.

Kleespies, R. G., Radtke, J., and Bienefeld, K. (2000). Virus-like particles found in the ectoparasitic bee mite *Varoa jacobsoni* Oudemans. *J. Invertebr. Pathol.* **75:**87–90.

Koch, R. (1884). Die Aetiologie der Tuberkulose. *Mitt. Kais Gesundheitsamt.* **2:**1–88.

Korpela, S., Aarhus, A., Fries, I., and Hansen, H. (1992). *Varroa jacobsoni* Oud. in cold climates: Population growth, winter mortality and influence on the survival of honey bee colonies. *J. Apic. Res.* **31:**157–164.

Kovac, H., and Crailsheim, K. (1988). Life span of *Apis mellifera Carnica* Pollm. Infested by *Varroa jacobsoni* in relation to season and extent of infestation. *J. Apic. Res.* **27:**230–238.

Kreissl, S., and Bicker, G. (1992). Dissociated neurons of the pupal honeybee brain in cell culture. *J. Neurocytol.* **21:**545–556.

Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., and Popov, S. (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *J. Ethnopharmacol.* **64:**235–240.

Kulincevic, J. M., and Rothenbuhler, W. C. (1975). Selection for resistance and susceptibility to hairless-black syndrome in the honeybee. *J. Invertebr. Pathol.* **25:**289–295.

Kulincevic, J., Ball, B., and Mladjan, V. (1990). Viruses in honey bee colonies infested with *Varroa jacobsoni*: First findings in Yugoslavia. *Acta Vet.* **40:**37–42.

Laidlaw, H. H. (1979). Contemporary queen rearing. Dadant and Sons, Hamilton, IL.

Lanzi, G., de Miranda, J. R., Boniotti, M. B., Cameron, C. E., Lavazza, A., Capucci, L., Camazine, S. M., and Rossi, C. (2006). Molecular and biological characterization of deformed wing virus of honey bees (*Apis mellifera* L.). *J. Virol.* **80:**4998–5009.

Lapidge, K. L., Oldroyd, B. P., and Spivak, M. (2002). Seven suggestive quantitative trait loci influence hygienic behaviour of honey bees. *Naturwissenschaften* **89:**565–568.

Lavine, M. D., and Strand, M. R. (2002). Insect hemocytes and their role in immunity. *Insect. Biochem. Mol. Biol.* **32:**1295–1309.

Leat, N., Ball, B., Govan, V., and Davison, S. (2000). Analysis of the complete genome sequence of black queen-cell virus, a picorna-like virus of honey bees. *J. Gen. Virol.* **81:**2111–2119.

Leclerc, V., and Reichhart, J. M. (2004). The immune response of *Drosophila melanogaster*. *Immunol. Rev.* **198:**59–71.

Lee, P. E., and Furgala, B. (1965). Chronic bee paralysis virus in the nerve ganglia of the adult honey bee. *J. Invertebr. Pathol.* **7:**170–174.

Lee, P. E., and Furgala, B. (1967). Electron microscopic observations on the localization and development of sacbrood virus. *J. Invertebr. Pathol.* **9:**178–187.

Li, H., Li, W. X., and Ding, S. W. (2002). Induction and suppression of RNA silencing by an animal virus. *Science* **296:**1319–1321.

Lighthart, B., Prier, K. R. S., and Bromenshenk, J. J. (2005). Flying honey bees absorb airborne viruses. *Aerobiologia* **21:**147–149.

Lipsitch, M., Siller, S., and Nowak, M. A. (1996). The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution* **50:**1729–1741.

Malun, D., Moseleit, A. D., and Grünewald, B. (2003). 20-Hydroxyecdysone inhibits the mitotic activity of neuronal precursors in the developing mushroom bodies of the honeybee, *Apis mellifera*. *J. Neurobiol.* **57:**1–14.

Martin, S., Hogarth, A., van Breda, J., and Perrett, J. (1998). A scientific note on *Varroa jacobsoni* Oudemans and the collapse of *Apis mellifera* L. colonies in the United Kingdom. *Apidologie* **29:**369–370.

Martin, S. J. (2001). The role of Varroa and viral pathogens in the collapse of honey bee colonies: A modelling approach. *J. Appl. Ecol.* **38:**1082–1093.

Mayo, M. A. (2002). Virus Taxonomy—Houston 2002. *Arch. Virol.* **147:**1071–1076.

Mendelsohn, C. L., Wimmer, E., and Racaniello, V. R. (1989). Cellular receptor for poliovirus: Molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell* **56:**855–865.

Mims, C. A. (1981). Vertical transmission of viruses. *Microbiol. Rev.* **45:**267–286.

Miorin, P. L., Levy Junior, N. C., Custodio, A. R., Bretz, W. A., and Marcucci, M. C. (2003). Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca angustula* against *Staphylococcus aureus*. *J. Appl. Microbiol.* **95:**913–920.

Mitsuhashi, J. (2001). Development of highly nutritive culture media. *In Vitro Cell. Dev. Biol.* **37:**330–337.

Mitsuhashi, J. (2002). ''Invertebrate Tissue Culture Methods.'' Springer Lab Manual, Spring-Verlag, Tokyo.

Moya, A., Elena, S. F., Bracho, A., Miralles, R., and Barrio, E. (2000). The evolution of RNA viruses: A population genetic view. *Proc. Natl. Acad. Sci. USA* **97:**6967–6973.

Naug, D., and Camazine, S. (2002). The role of colony organization on pathogen transmission in social insects. *J. Theor. Biol.* **215:**427–439.

Nordström, S. (2003). Distribution of deformed wing virus within honey (Apis mellifera) brood cells infected with the ectoparasitic mite *Varroa destructor*. *Exp. Appl. Acarol.* **29:**293–302.

Nordström, S., Fries, I., Aarhus, A., Hansen, H., and Korpela, S. (1999). Virus infections in Nordic honey bee colonies with no, low or severe *Varroa jacobsoni* infections. *Apidologie* **30:**475–484.

Ohashi, K., Natori, S., and Kubo, T. (1999). Expression of amylase and glucose oxidase in the hypopharyngeal gland with an age-dependent role change of the worker honeybee (*Apis mellifera* L.). *Eur. J. Biochem.* **265:**127–133.

Ongus, J. R., Peters, D., Bonmatin, J.-M., Bengsch, E., Vlak, J. M., and Van Oers, M. M. (2004). Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite *Varroa destructor*. *J. Gen. Virol.* **85:**3747–3755.

Park, O. W., Pellett, F. C., and Paddock, F. B. (1937). Disease resistance and American foulbrood. *Am. Bee J.* **77:**20–25.

Pelletier, J., and Sonenberg, N. (1988). Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* **334:**320–325.

Peng, Y. S., Fang, Y., Xu, S., Ge, L., and Nasr, M. E. (1987). Response of foster Asian Honey bee (*Apis cerana* Fabr.) colonies to the brood of European honey bee (*Apis mellifera* L.) infested with parasitic mite *Varroa jacobsoni* Oudemanns. *J. Invertebr. Pathol.* **49:**259–264.

Rachinsky, A., and Hartfelder, K. (1998). *In vitro* biosynthesis of juvenile hormone in larval honey bees: Comparison of six media. *In Vitro Cell. Dev. Biol. Anim.* **34:**646–648.

Ribiere, M., Triboulot, C., Mathieu, L., Aurieres, C., Faucon, J.-P., and Pepin, M. (2002). Molecular diagnosis of chronic bee paralysis virus infection. *Apidologie* **33:**339–351.

Rinderer, T. E., Rothenbuhler, W. C., and Kulincevic, J. M. (1975). Responses of three genetically different stocks of the honey bee to a virus from bees with hairless-black syndrome. *J. Inverteb. Pathol.* **25:**297–300.

Rothenbuhler, W. C. (1964). Behaviour genetics of nest cleaning in honey bees. IV. Responses of F1 and backcross generations to disease-killed brood. *Am. Zool.* **4:**111–123.

Rortais, A., Tentcheva, D., Papachristoforou, A., Gauthier, L., Arnold, G., Colin, M. E., and Bergoin, M. (2006). Deformed wing virus is not related to honey bees' aggressiveness. *Virol. J.* **3:**61.

Santos, K. S., dos Santos, L. D., Mendes, M. A., de Souza, B. M., Malaspina, O., and Palma, M. S. (2005). Profiling the proteome complement of the secretion form hypopharyngeal gland of Africanized nurse-honeybees (*Apis mellifera* L.). *Insect. Biochem. Mol. Biol.* **35:**85–91.

Sasaki, J., and Nakashima, N. (1999). Translation initiation at the CUU codon is mediated by the internal ribosome entry site of an insect picorna-like virus *in intro*. *J. Virol.* **73:**1219–1226.

Sasaki, J., and Nakashima, N. (2000). Methionine-independent initiation of translation in the capsid protein of an insect RNA virus. *Proc. Natl. Acad. Sci. USA* **97:**1512–1515.

Schmid-Hempel, P. (2005). Evolutionary ecology of insect immune defense. *Annu. Rev. Entomol.* **50:**529–551.

Shen, M. Q., Cui, L. W., Ostiguy, N., and Cox-Foster, D. (2005a). Intricate transmission routes and interactions between picorna-like viruses (Kashmir bee virus and sacbrood virus) with the honeybee host and the parasitic varroa mite. *J. Gen. Virol.* **86:**2281–2289.

Shen, M. Q., Yang, X. L., Cox-Foster, D., and Cui, L. W. (2005b). The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. *Virology* **342:**141–149.

Shimanuki, H., Calderone, N. W., and Knox, D. A. (1994). Parasitic mite syndrome: The symptoms. *Am. Bee J.* **134:**827–828.

Siede, R., Derakhshifar, I., Otten, C., Berényi, O., Bakonyi, T., Köglberger, H., and Büchler, R. (2005). Prevalence of Kashmir bee virus in Europe. *J. Apic. Res.* **44:**131–132.

Simpson, J. (1961). Nest climate regulation in honeybee colonies. *Science* **133:**1327–1333.

Spivak, M., and Gilliam, M. (1998). Hygienic behaviour of honey bees and its application for control of brood diseases and varroa—Part II. Studies on hygienic behaviour since the Rothenbuhler era. *Bee World* **79:**169–186.

Spivak, M., and Reuter, G. S. (2001). Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior. *Apidologie* **32:**555–565.

Starks, P. T., Blackie, C. A., and Seeley, T. D. (2000). Fever in honeybee colonies. *Naturwissenschaften* **87:**229–231.

Stoltz, D., Shen, X.-R., Boggis, C., and Sisson, G. (1995). Molecular diagnosis of Kashmir bee virus infection. *J. Apic. Res.* **34:**153–160.

Sumpter, D., and Martin, S. (2004). The dynamics of virus epidemics in Varroa-infested honey bee colonies. *J. Anim. Ecol.* **73:**51–63.

Tentcheva, D., Gauthier, L., Jouve, S., Canabady-Rochelle, L., Dainat, B., Cousserants, F., Colin, M. E., Ball, B. V., and Bergoin, M. (2004a). Polymerase chain reaction detection of deformed wing virus (DWV) in *Apis mellifera* and *Varroa destructor*. *Apidologie* **35:**431–439.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M. E., and Bergoin, M. (2004b). Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. *Appl. Environ. Microbiol.* **70:**7185–7191.

Topolska, G., Ball, B. V., and Allen, M. (1995). Identification of viruses in bees from two Warsaw apiaries. *Medycyna Weterynaryina* **51:**145–147.

Voinnet, O. (2001). RNA silencing as a plant immune system against viruses. *Trends Genet.* **17:**449–459.

Weinberg, K. P., and Madel, G. (1985). The influence of the mite *Varroa jacobsoni* Oud. on the protein concentration and haemolymph volume of the brood of the worker bees and drones of the honey bee, *Apis mellifera*. *Apidologie* **16:**421–436.

White, G. F. (1913). Sacbrood, a Disease of Bees. *US Department of Agriculture, Bureau of Entomology*. Circular No. 169.

Wilson, J. E., Powell, M. J., Hoover, S. E., and Sarnow, P. (2000). Naturally occurring dicistronic cricket paralysis virus RNA is regulated by two internal ribosome entry sites. *Mol. Cell. Biol.* **20:**4990–4999.

Woodrow, A. W., and Holst, E. C. (1942). The mechanism of colony resistance to American foulbrood. *J. Econ. Entomol.* **35:**327–330.

Yang, X., and Cox-Foster, D. L. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: Evidence for host immunosuppression and viral amplification. *Proc. Nat. Acad. Sci. USA* **102:**7470–7475.

Yue, C., and Genersch, E. (2005). RT-PCR analysis of deformed wing virus in honeybees (*Apis mellifera*) and mites (*Varroa desctructor*). *J. Gen. Virol.* **86:**3419–3424.

Yue, C., Schröder, M., Bienefeld, K., and Genersch, E. (2006). Detection of viral sequences in semen of honeybees (*Apis mellifera*): Evidence for vertical transmission of viruses through drones. *J. Invertebr. Pathol.* **92:**105–108.

Zambon, R. A., Nandakumar, M., Vakharia, V. N., and Wu, L. P. (2005). The Toll pathway is important for an antiviral response in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **102:**7257–7262.

Zou, Z., Lopez, D. L., Kanost, M. R., Evans, J. D., and Jiang, H. (2006). Comparative analysis of serine protease-related genes in the honey bee genome: Possible involvement in embryonic development and innate immunity. *Insect. Mol. Biol.* **15:**603–614.

Zufelato, M. S., Lourenco, A. P., Simões, Z. L. P., Jorge, J. A., and Bitondi, M. M. (2004). Phenoloxidase activity in *Apis mellifera* honey bee pupae, and ecdysteroid-dependent expression of the prophenoloxidase mRNA. *Insect. Biochem. Mol. Biol.* **34:**1257–1268.