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Horizontal and vertical transmission of viruses in the honey bee, *Apis mellifera* ☆

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Abstract

The most crucial stage in the dynamics of virus infections is the mode of virus transmission. In general, transmission of viruses can occur through two pathways: horizontal and vertical transmission. In horizontal transmission, viruses are transmitted among individuals of the same generation, while vertical transmission occurs from mothers to their offspring. Because of its highly organized social structure and crowded population density, the honey bee colony represents a risky environment for the spread of disease infection. Like other plant and animal viruses, bee viruses use different survival strategies, including utilization of both horizontal and vertical routes, to transmit and maintain levels in a host population. In this review, we explore the current knowledge about the honey bee viruses and transmission routes of bee viruses. In addition, different transmission strategies on the persistence and dynamics of host–pathogen interactions are also discussed.
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Keywords: *Apis mellifera*; Viruses; Varroa mite; Horizontal transmission; Vertical transmission

1. Introduction

Viruses are a group of obligate, intracellular parasites that are found in virtually all life forms. Viruses lack a system for their own metabolism and must live and develop inside living host cells. Within host cells, viruses take over the metabolism of the host and utilize the host cell's machinery and components to make whatever is needed to produce their own progenies, virions. This process harms the host, resulting in the disease infection or even death of the host. Because of their profound impact on host health, viruses represent a major challenge to public health and agriculture societies.

A very crucial aspect of the dynamics of virus infections and evolution of host–pathogen interactions is the mode of transmission. Transmission processes determine the spread and the persistence of pathogens in a population. Knowledge of how the virus infection spreads is fundamental to design an appropriate disease control program. In general, transmission of a virus can occur horizontally or vertically, or both. In horizontal transmission, viruses are transmitted among individuals of the same generation. Horizontal transmission can be further classified as direct or indirect. Horizontal transmission by a direct route includes airborne infection, food-borne infection, and venereal (sexual) infection, whereas transmission by an indirect route involves an intermediate biological host, like a mosquito vector, which acquires and transmits virus from one host to another. In vertical transmission, viruses are passed vertically from mother to offspring via egg, either on the surface of the egg (transovum transmission) or within the egg (transovarian transmission). It has been suggested that these different transmission modes play a crucial role in determining the virulence of a pathogen (Clayton and Tompkins, 1994; Ewald, 1994). Typically, horizontal transmission favors overt expression of the disease and increases infection prevalence under certain conditions, such as high

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host population density and high pathogen replication rate. In contrast, vertical transmission is a mechanism for long-term virus persistence and favors evolution of benign infection. The outcome of any virus infection can reflect the balance between the two transmission processes.

The honey bee (*Apis mellifera* L.) is the most important pollinating insect species and plays a vital role in US agriculture by assisting in the pollination of a wide variety of crops and by producing honey and other hive products, with an annual market value exceeding 15 billion dollars (Morse and Calderone, 2000). However, like all living organisms, honey bees are exposed to a diverse array of pathogens including viruses, which are significant threats to their health and well-being. So far, at least 18 viruses have been reported to attack honey bees worldwide and dramatically affect honey bee health under certain conditions (Ball and Allen, 1988; Martin, 2001). Honey bees are social insects and live in colonies consisting of two generations: one mother queen and her successors, 20,000–60,000 workers and several hundreds of drones. Individual bees in the colony work together in a highly structured social order and engage in numerous coordinating activities including defending invaders, building combs, foraging for food, clearing brood cells, rearing offspring, and attending the queen. Because of densely crowded populations and a high contact rate between colony members related to feeding and chemical communication, honey bee colonies provide great opportunities for disease transmission.

Although there are many gaps in the knowledge of the key processes underlying virus transmission dynamics, elucidation of bee virus transmission modes represents a rapidly developing research area, and our understanding of virus transmission and epidemiology in honey bees has grown considerably over the last decade. In this review, we provide a brief overview of the current knowledge regarding honey bee viruses and transmission routes of honey bee viruses, and discuss the opportunities that lie ahead in the study of bee virus transmission and epidemiology.

2. Honey bee viruses

2.1. Morphology, genome structures, and classification of bee viruses

Except for filamentous bee virus, all honey bee viruses reported so far are spherical to oval shaped 20–30 nm in diameter, isometrically symetrical, non-occluded, and possess a buoyant density in CsCl ranging from 1.33 to 1.42 g/ml, and a 100–190S sedimentation coefficient (Bailey, 1976). Because of their similar characteristics, honey bee viruses are difficult to distinguish morphologically under the electron microscope (Fig. 1).

Honey bee viruses are positive-sense single-stranded RNA viruses belonging to the picorna-like virus superfamily and have the following common features. The viral genome is composed of a single-stranded RNA molecule coated with capsid proteins. The RNA genome is covalently attached by a genome-linked virion protein (VPg) at the 5′ and a polyA tail at 3′ ends. At the 5′ end, there is a

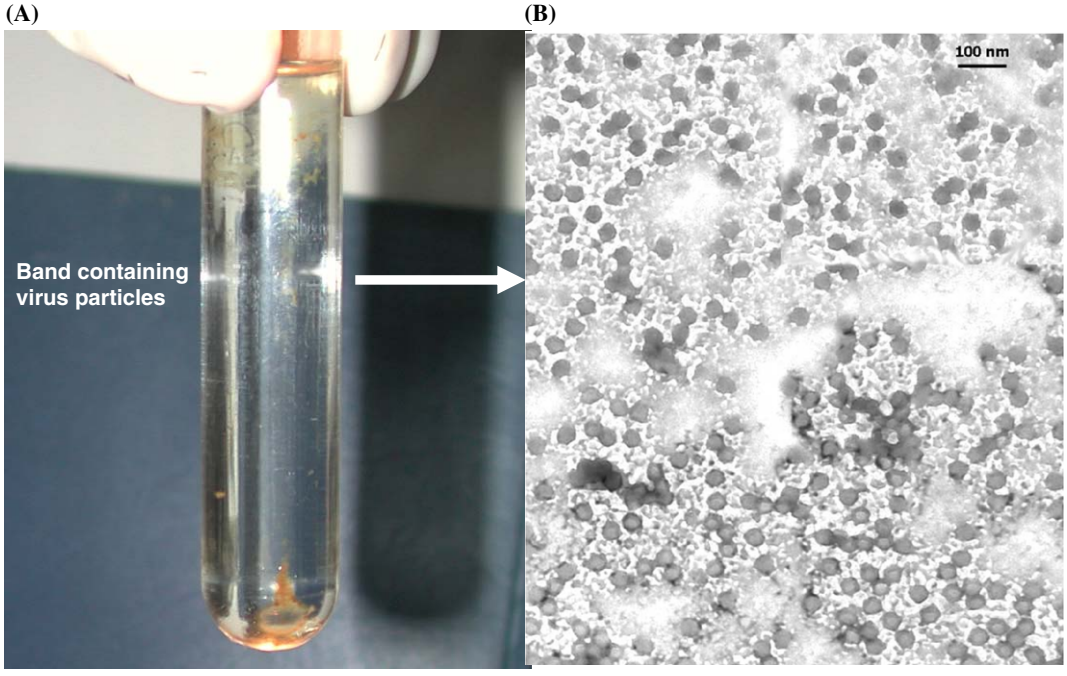

Fig. 1. (A) Virus band after CsCl density gradient centrifugation. Supernatant containing viruses mixed with CsCl solution to an initial density of 1.37 g/ml and centrifuged at 40,000 rpm for 18 h at 10 °C. The virus-containing band was collected for subsequent electron micrograph analysis. (B) Electron micrograph of honey bee virus particles. Bee viruses are spherical to slightly oval particles about 29 nm in diameter as determined from EM. The virus preparation used for this electron micrograph was determined by RT-PCR to contain four different viruses, BQCV, DWV, KBV, and SBV. No significant difference in the virion size and morphology could be observed among the four different virus particles. Bar marker represents 0.1 μM.

long untranslated region (UTR) containing a 'cloveleaf' secondary structure. The replication of viruses occurs in the cytoplasm of the host cell. The virus particle attaches to surface of the host cell and interacts with a receptor on the host cell membrane and injects its RNA genome into the host cell. Once inside the host cell, the RNA genome is translated into a single polyprotein that is subsequently cleaved into structural proteins and functional proteins for RNA replication. With the help of RNA-dependent RNA polymerase, the positive-stranded RNA genome is copied to a negative-stranded intermediate, which serves as a template for replication of new genomic strands that are assembled into progeny viral particles.

To date, the complete genome sequences of five honey bee viruses, acute bee paralysis virus (ABPV) (Govan et al., 2000, GenBank Accession No. AF150629), black queen cell virus (BQCV) (Leat et al., 2000, GenBank Accession No. AF183905), deformed wing virus (DWV) (GenBank Accession No. NC-004830), Kashmir bee virus (KBV) (de Miranda et al., 2004, GenBank Accession No. NC-004807), and sacbrood virus (SBV) (Ghosh et al., 1999, GenBank Accession No. AF092924) have been reported as well as partial genome sequences of chronic bee paralysis virus (CBPV). According to the gene order of the proteins, honey bee viruses are divided into two groups. The genomes of ABPV, BQCV, and KBV are monopartite bicistronic with non-structural genes at the 5′ end and structural genes at the 3′ end, while the genome of SBV and DWV are monopartite monocistronic genomes with structural genes at the 5′ end and non-structural genes at the 3′ end (Fig. 2). For monopartite bicistronic genome, the 5′ UTR and the untranslated intergentic region (IGR) between the two ORFs can initiate efficient translation as the internal ribosomal entry site (IRES). However, there is no evidence of that translation of protein is mediated by IRES for monopartite monocistronic genome. Based on their genomic organization, BQCV, KBV, and ABPV are assigned to a new virus family, *Dicistroviridae*, whereas SBV and DWV are assigned to the genus Iflavirus, which is a "floating genus" that has not yet been assigned to a family (Mayo, 2002).

2.2. Honey bee virus infections

Viruses infect all developmental stages of the bee including eggs, brood, and adults. Under field conditions, most bee viruses usually persist as latent infections and cause no overt signs of disease. Moreover, bee colonies can be attacked by more than one virus simultaneously and multiple viral infections have been reported in living bees (Anderson, 1990; Benjeddou et al., 2001; Chen et al., 2004c; Evans, 2001; Hung et al., 1996). Therefore, it is very difficult to identify bee virus infections and differentiate mixed virus infections based only on field observation. For many years, detection and identification of viral infection in honey bee colonies were based largely on serological methods like Ouchterlony gel diffusion, indirect fluorescent antibody (IFA) and enzyme-linked immunosorbent assay (ELISA) tests (Allen et al., 1986; Allen and Ball, 1995; Anderson, 1984). While these efforts resulted in an extensive database of known viruses, the use of serological methods in virus research is problematic. First, their low specificity can misclassify related viruses (Mansy et al., 1999; Rinderer and Green, 1976). For example, both ABPV and strains of KBV are serologically related, so antiserum produced from

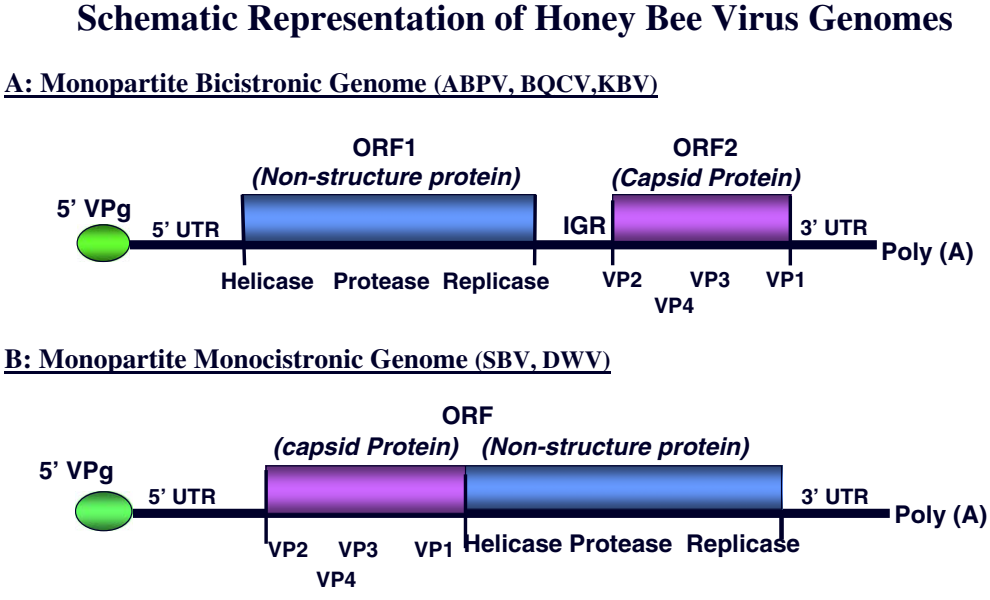

Fig. 2. Genomes of honey bee viruses. The RNA genome is covalently attached by a genome-linked virion protein (VPg) at the 5′ and a polyA tail at 3′ ends. Genomes of honey bee viruses are organized in two different ways. (A) The genomes of ABPV, BQCV, and KBV are monopartite bicistronic with non-structural 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 non-structural genes at the 3′ end.

ABPV interacted not only with the ABPV but also with Canadian and Spanish strains of KBV. The resemblance of both viruses in the serological reaction could cause confusion in the diagnosis of inapparent viral infection (Allen and Ball, 1995). Second, serological assays are limited by low sensitivity. While capable of detecting viruses in diseased bees, they are inadequate for detecting latent infections. Finally, serological techniques require the production and distribution of antibodies, making repeatability across different research groups difficult. The development of molecular technologies has revolutionized the diagnosis of honey bee virus infections. Stoltz et al. (1995) developed specific primer pairs for KBV and used reverse transcription polymerase chain reaction (RT-PCR) methodology to detect KBV infection in honey bee samples. The high sensitivity and specificity of RT-PCR offered a means to circumvent the problems associated with the use of serological tests. The availability of genome sequences of six honey bee viruses makes the molecular detection and characterization of these viruses possible. Currently, molecular methods have been used frequently for diagnosis of honey bee virus infections and these molecular techniques have become the mainstay of current research (Bakonyi et al., 2002a,b; Benjeddou et al., 2001; Chen et al., 2004a,c; Evans, 2001; Genersch, 2005; Grabensteiner et al., 2001; Hung et al., 1996, 2000; Ribiere et al., 2002; Tentcheva et al., 2004a).

3. Horizontal transmission of honey bee viruses

3.1. Direct food-borne transmission

Horizontal transmission of viruses via food-borne infection can take place by eating pathogen-contaminated food and passing out viruses from the gut with feces. Under conditions of high population density, high physical contact rates, and high trophallaxis rates, direct food-borne transmission may be a significant route for spreading diseases. Several behaviors of honey bees such as feeding brood, attending the queen, packing pollen, and processing nectar favor the probability of food-borne transmission.

Evidence of food-borne transmission route of viruses in honey bees has been provided by the detection of viruses in food resources, gut, and feces. Our recent study of colony food resources as possible resources in the transmission of bee viruses showed that six viruses including ABPV, BQCV, CBPV, DWV, KBV, and SBV were found in pollen samples and two viruses including BQCV and DWV were detected in the honey. However, we were unable to detect viruses in colonies' foods, royal jelly (Fig. 3). The results of our studies share some similarities with the findings of Shen et al. (2005a), who detected KBV and SBV in all developmental stages of bees and food resource including brood food, honey, pollen, and royal jelly. The variable presence of viruses in brood food, royal jelly could be due to differences in the virus infection status of bee colonies. The infections of KBV and SBV were not prevalent in the bees when

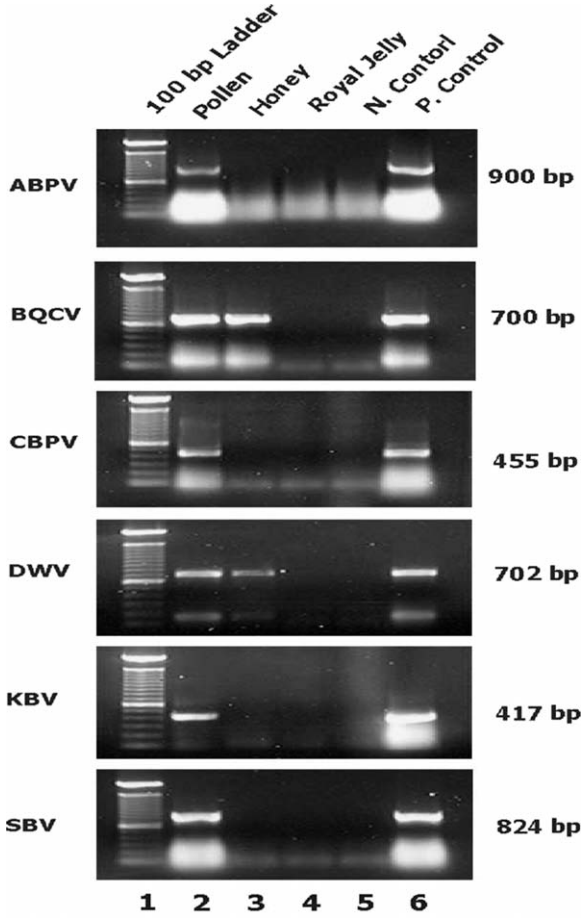

Fig. 3. Detection of viruses in colony foods. Colony foods including pollen, honey, and royal jelly were examined for presence of six viruses. Primer pairs specific for ABPV, BQCV, CBPV, DWV, KBV, and SBV were used separately to amplify RT-PCR products of 900, 700, 455, 702, 417, and 824 bp, respectively. Negative (H_2O) and positive controls (previously identified positive sample) were included in each run of the RT-PCR.

we examined the virus status of colony foods. If worker bees are not infected with one virus, it is unlikely the same virus will be detected in the royal jelly, a grandular secretion of worker bees.

Oral infection of viruses by contaminated foods can be traced by examination of the virus status of the digestive tract, the gut. We conducted a study to investigate the presence of viruses in the gut of queens and proved the presence of bee viruses, DWV and BQCV, in the gut tissue. Quantification of virus load in different tissues indicated that virus titers detected in gut were significantly higher than other examined tissues including hemolymph, ovaries, head, spermatheca, and eviscerated body (Chen et al., 2006). These results support the evidence of ingestion of virus contaminated-food and role of feeding in food-borne virus transmission.

An additional line of evidence for food-borne transmission is the detection of bee viruses in the feces of queen bees (Chen et al., 2006). Individual queens were isolated in a Petridish to allow them to defecate and then the clear fecal

materials were collected and RT-PCR was performed to test for the presence of bee viruses in the feces. The results indicated that same viruses detected in the gut were also detected in feces. Among samples examined, 100% of feces samples tested positive for the presence of BQCV, and 90% of feces samples tested positive for presence of DWV. Evidence of detection of viruses in feces of worker bees has been reported previously (Bailey and Gibbs, 1964; Hung, 2000). The results obtained from our study were consistent with previous findings and showed that viruses could be found in the feces of bees, suggesting food-borne transmission. However, whether feces serves as a source of contamination that results in a fecal-oral transmission route in the bee colonies is not certain and further study is needed.

3.2. Venereal transmission

Venereal transmission is a type of horizontal transmission in which viruses are transmitted between two sexes during mating. However, virus transmission via this route has not been studied in detail and there are no published studies demonstrating venereal transmission. Nevertheless, detection of viruses in adult drones (Chen et al., 2004a), semen (unpublished result), and in the spermatheca of queens (Chen et al., 2006), implies that venereal transmission may play an important role. However, additional experiments will clearly be required to decipher the role of the drone in virus transmission.

3.3. Indirect transmission of viruses by the parasitic mite, *Varroa destructor*

The *Varroa* mite, *Varroa destructor* (Anderson and Trueman, 2000) is an obligate parasite of the honey bee and has been catastrophic for the US beekeeping industry since its first detection in North America in 1987. *Varroa* mites attack adults (workers, drones, and queens) and brood, of which the drone brood is distinctly preferred (Bailey and Ball, 1991). Both adult mites and nymphs use their piercing mouth-parts to penetrate the body wall of developing bees. The repeated feeding on bee hemolymph shorten bee life span and can result in a decline in host immunity, colony vigor, and the eventual death of the colonies within a few years (DeJong et al., 1982; Korpela et al., 1992; Kovac and Crailsheim, 1988; Weinberg and Madel, 1985; Yang and Cox-Foster, 2005). So far, *Varroa* mites have killed at least 1/3 of the managed honey bee colonies and almost all of the feral honey bee colonies since becoming established in the US. Because mites feed and move regularly between brood and adult bees, they have the potential to act as vectors for bee viruses. Viral infections in honey bee colonies have often been reported to be involved in the collapse of bee colonies infested with *V. destructor* (Allen and Ball, 1996; Ball and Allen, 1988; Kulincevic et al., 1990). In the field, several viral disease outbreaks have been documented to be associated with the infestation of *V. destructor* including ABPV, CBPV, slow paralysis virus (SPV), BQCV, KBV, cloudy Wing Virus (CWV), SBV, DWV (Ball and Allen, 1988; Allen and Ball, 1996; Martin, 2001; Martin et al., 1998; Tectcheva et al., 2004b). The association of virus infection with *V. destructor* infestation in honey bee colonies presents the most intricate aspect in its biology and causes great concern for researchers and beekeepers.

The role of *Varroa* mites as a vector in acquiring and transmitting viruses from severely infected individuals to healthy bees in bee colonies has been experimentally demonstrated in several studies. The experiments investigating *Varroa* mite as a vector in horizontal transmission of bee viruses starts with the work of Bowen-Walker et al. (1999). Using serological methods, they demonstrated that *Varroa* mites obtained DWV from infected bees and acted as a vector to transmit the virus to uninfected bees, which developed morphological deformities or died after mites fed on them for certain periods of time. This study provided strong circumstantial evidence that *Varroa* mite is an effective vector of DWV in bee colonies. Unequivocal evidence of *Varroa* mite as a vector of bee viruses was shown with KBV (Chen et al., 2004b). Using KBV specific primers and RT-PCR, we identified honey bee colonies that were infected with KBV and infested with *Varroa* mites, and colonies free of virus and mites. To investigate the vectoring capability of *Varroa*, we collected live mites from the virus-infected colonies and experimentally introduced one, two, three or four of these mites into the sealed cells of individual bee brood from the virus-free colonies. We found a direct relationship between virus frequency and the number of mites to which recipient bees were exposed. The more donor mites that were introduced per cell, the greater the incidence of virus that was detected in the recipient bee brood. This was most evident in bees exposed to four mites, which resulted in 100% infection (Fig. 4). Recipient brood that were not exposed to mites showed no signs of infection. Our experimental design involving the introduction of varying numbers of mites allowed us to make a second novel discovery. Following the experiment, it was apparent that virus frequency in mites was directly correlated with the number of mites per cell. Thus, not only did mites transmit viruses to their bee hosts, we have evidence of horizontal mite-to-mite transmission of viruses, presumably via a honey bee intermediary. Mites emerging from multiple-infested cells could therefore play a disproportionate role in the spread of viruses within the colony. The studies document evidence that *Varroa* mite is an effective vector in transmitting KBV. Studies carried out subsequently by Shen et al. (2005b) confirmed previous findings about the role of *Varroa* mite in transmitting KBV and DWV in honey bee colonies.

Although *Varroa* mites have been confirmed as vectors in transmitting and activating bee virus infections, the mechanism of mite-mediated transmission is uncertain. Observations of the replication of viruses in *Varroa* mite and presence of viruses in mite saliva suggest that *Varroa* mite is likely a biological vector for bee viruses (Ongus et al., 2004; Shen et al., 2005b). However, the molecular mechanism regulating virus-vector interactions and

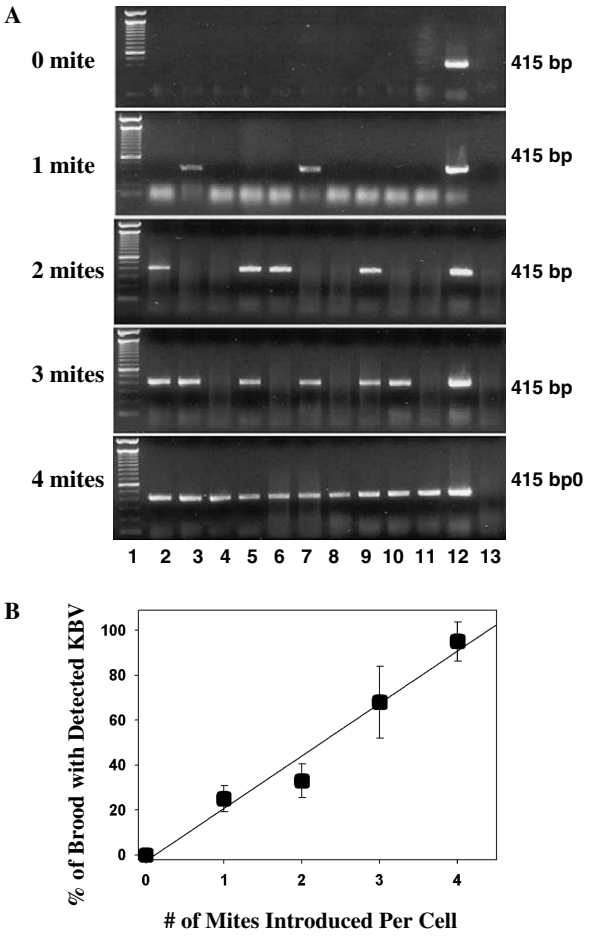

Fig. 4. Transmission of KBV by *Varroa* mites. (A) One representative transmission experiment. One, two, three, and four mites collected from mite donor colonies (high virus) were introduced into developing brood in recipient colony (no virus). Five days after exposure to mites, RNA was extracted from brood and subjected to RT-PCR. Band at 415 bp indicates presence of Kashmir bee virus (KBV). Lane 1 represents 100 bp DNA ladder. Lanes 2–11: RNA extracted from bee pupae exposed to mites. Lane 12, positive control. Lane 13, negative control. (B) Correlation between virus frequency and the number of mites introduced to each brood cell.

transmission processes is not known in any detail. To clarify this, further study will be needed.

4. Vertical transmission of honey bee viruses

Vertical transmission, another mechanism of virus transmission in nature, has been shown to occur in honey bees. Our previous study with DWV revealed that this virus could be detected in all developmental stages of honey bees, including adults, pupae, larvae, and eggs (Chen et al., 2005). Detection of DWV in eggs and in larval stages that are not normally associated with *Varroa* mite infestation led us to postulate that queens in bee colonies might be infected with viruses and that the viruses might be transmitted vertically from queens to eggs. In addition, the detection of negative signals for viruses in royal jelly (Fig. 3), which is used for feeding newly hatched larvae, excluded the possibility of food contributing to virus infections in larval stages of bees. Subsequent studies of virus infection in the queen of colonies confirmed our assumption and indicated that queens can be infected with viruses and that multiple viruses can be simultaneously detected in a single queen (Chen et al., 2005). The detection of viruses in queens suggests that a vertical transmission pathway exists within the bee colony and that eggs have the opportunity to obtain viruses from infected queen.

An additional indication of a vertical transmission of viruses was obtained through the detection of viruses in the ovaries of the queens and surface-sterilized eggs (Chen et al., 2006). 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. Quantification of virus titer in ovaries showed that virus concentration in ovaries was relatively low when compared to gut tissue (Chen et al., 2006). The weak virus signals detected in ovaries suggests that virus infections in ovaries were retained in a non-replicate or latent stage so that viruses would not be propagated to the level that would have a deleterious effect on embryos. In the same study, we examined the virus status of both mother queens and their offspring, including eggs, larvae, and adults simultaneously to further prove the existence of vertical transmission pathway. When queens were found to be positive for certain viruses, 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 specific viruses, these viruses could not be detected in their offspring. These data suggest that transmission of viruses from queens to their progeny is highly likely.

5. Conclusion

Mode of transmission is a crucial factor in the evolution of virulence and the dynamics of host–pathogen interactions. It is hypothesized that there is a conflict in the selective pressures between horizontal and vertical modes of transmission (Ewald, 1983, 1987, 1994; Lipsitch et al., 1996). Horizontal transmission is strongly dependent on the production of high numbers of pathogens. The greater the number of pathogens produced, the higher the opportunities for host exploitation and thereby a higher rate of transmission. Hence, selection favors high virulence of pathogens. In contrast, vertically transmitted pathogens are directly dependent upon the survival and reproduction of their hosts. Any reduction in host survival and reproduction will cause the reduction in the pathogens. Hence vertical transmission is associated with low virulence and latent infection. However, if the replication rate of pathogens is too high, the high virulence will result in high pathogen-induced host mortality, and hosts will lose fitness before production of enough pathogens to infect more hosts. On the other hand, if pathogen replication is too low, the pathogen will lose opportunities to infect new hosts. Therefore,

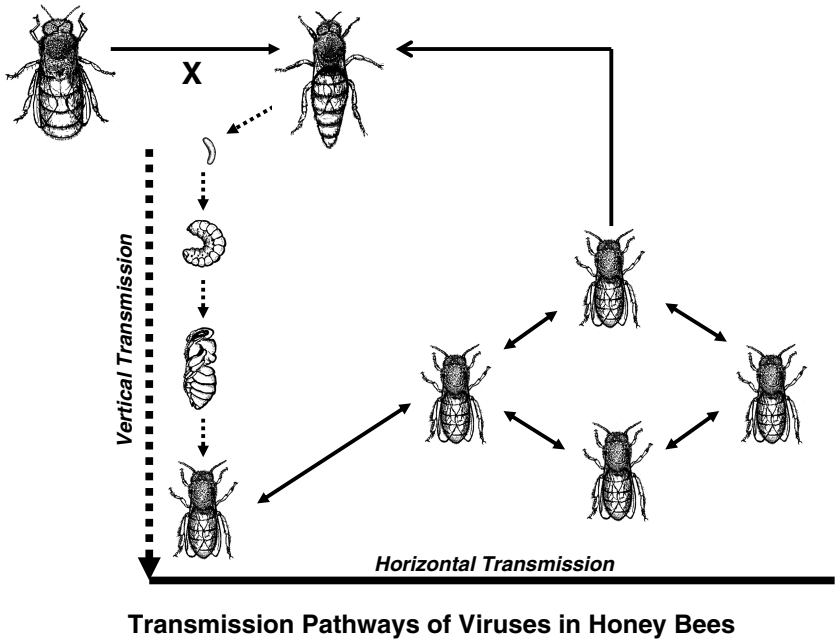

Fig. 5. Transmission of viruses in honey bees. Solid lines represent horizontal transmission and dotted lines represent vertical transmission. Honey bee viruses use different survival strategies, including utilization of both horizontal route including vector-borne infection, food-borne infection, and venereal transmission and vertical routes to transmit and maintain themselves in the host population.

the virulence of a pathogen is the result of pathogen–host interactions and evolutionary trade-offs between horizontal and vertical transmission.

Virus transmission in honey bees appears to involve food-borne transmission, venereal transmission, vector-borne transmission, and mother-to-offspring transmission (Fig. 5). Both vertical and horizontal transmission pathways are believed to be important survival strategies for honey viruses not only for their long-term persistence in bee population but also for their establishment in nature, leading to the following model for viral epidemiology. When colonies are under non-competitive and healthy conditions, viruses maintain in bee colonies via vertical mechanism of transmission and exist in 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 infestations of *Varroa* mite, co-infection of other pathogens, and a decline in food supply which can result in reduction of host growth rate, viruses appear to leave their latent state. High numbers of produced virions then become much more infectious via horizontal transmission mechanism, leading to the death of hosts and possible collapse of the whole bee colony.

Although we have evidence that bee viruses use both horizontal and vertical mechanisms of transmission in their life history, there are many gaps in our knowledge of the key processes underlying the dynamics of virus transmission and host–pathogen interactions. For examples, it is not known how a honey bee host's immune responses regulate virus survival, transmission, and replication. What are the mechanisms that regulate latent virus infections versus acute virus infections? What are the effects of vertically transmitted sublethal infections on host bees? Do vertically transmitted low-level virus infections exert any effects on host development, reproduction, and longevity? How does viral gene expression contribute to host pathogenesis, and which gene products from honey bee host are necessary for aiding viral replication and movement? Newly developed tools and genetic resources for bee viruses and bee will allow researchers to begin answering these more difficult questions.

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