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Short Communication

New evidence that deformed wing virus and black queen cell virus are multi-host pathogens

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ABSTRACT

The host-range breadth of pathogens can have important consequences for pathogens' long term evolution and virulence, and play critical roles in the emergence and spread of the new diseases. *Black queen cell virus* (BQCV) and *Deformed wing virus* (DWV) are the two most common and prevalent viruses in European honey bees, *Apis mellifera*. Here we provide the evidence that BQCV and DWV infect wild species of honey bees, *Apis florea* and *Apis dorsata*. Phylogenetic analyses suggest that these viruses might have moved from *A. mellifera* to wild bee species and that genetic relatedness as well as the geographical proximity of host species likely play an important role in host range of the viruses. The information obtained from this present study can have important implication for understanding the population structure of bee virus as well as host-virus interactions.

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1. Introduction

It has been known for some time that more than 60% of pathogens are capable of infecting multiple host species and that multi-host pathogens appear to play a key role in causing emerging diseases in human, domestic animals, wildlife, and agricultural crops (Daszak et al., 2000; Pedersen et al. 2005; Woolhouse et al., 2001). The specificity of pathogen-host interactions defines the host range of multi-host pathogens and is a key element of disease epidemiology. Therefore, studying the potential host range of pathogens is highly relevant for understanding pathogen population dynamics and designing effective disease management practices, and is of fundamental interest from health, agricultural and biodiversity perspectives.

In recent years, there has been increased concern about virus infections in populations of honey bees, largely due to the recent observation of a correlation between an emerging bee-infecting virus and some honey bees suffering honey bee Colony Collapse Disorder (CCD; Cox-Foster et al. 2007). The issue of host specificity of honey bee viruses has been raised, since the host range of a virus can have significant effects on the evolution of its fitness and virulence. Previous studies have shown that *Deformed wing virus* (DWV) and *Black queen cell virus* (BQCV), two viruses originally identified in European honey bees, *Apis mellifera* (reviewed in Chen and Reinhold, 2007), can cause infection in several species of bumble bees, including *Bombus terrestris*, *Bombus pascuorum*, and *Bombus huntii* (Genersch et al., 2006; Li et al., 2011; Peng et al., 2011). Other viruses commonly found in *A. mellifera* including *Acute bee paralysis virus* (ABPV) and *Kashmir bee virus* (KBV) were also found to infect different species of bumble bees (Bailey and Gibbs, 1964; Anderson, 1991). A recent study regarding inter-taxa virus transmission in the pollinator community reported the detection of DWV, BQCV, *Israeli acute paralysis virus* (IAPV), *Kashmir bee virus* (KBV), and *Sacbrood virus* (SBV) in multiple non-apis hymenopteran species and in pollen pellets from forager bees (Singh et al., 2010). The ability of honey bee viruses to infect multiple host species indicates the complex aspect of host-virus dynamics and evolution in natural populations.

The dwarf honeybee *Apis florea* and the giant honeybee *Apis dorsata* are valuable natural pollinators found in tropical forests of southeastern Asia. Like their cavity-nesting and multiple-comb-building sister species, *A. mellifera* and *Apis cerana*, wild populations of *A. florea* and *A. dorsata* are threatened by the adverse effects of parasites and pathogens in conjunction with environmental degradation (Oldroyd and Nanork, 2009). Parasitic mites in the genus *Varroa* attack multiple honey bee species and these mites are known to be a significant vector of viruses in the European honey bee, *A. mellifera*, leading to colony decline and collapse. Another parasitic mite of Asian origin, *Tropilaelaps clareae*, was originally described from *A. dorsata* and was linked to the infestation of DWV in European honey bee host (Forsgren et al., 2009). In order to gain deep insight into the evolution and transmission of honey bee

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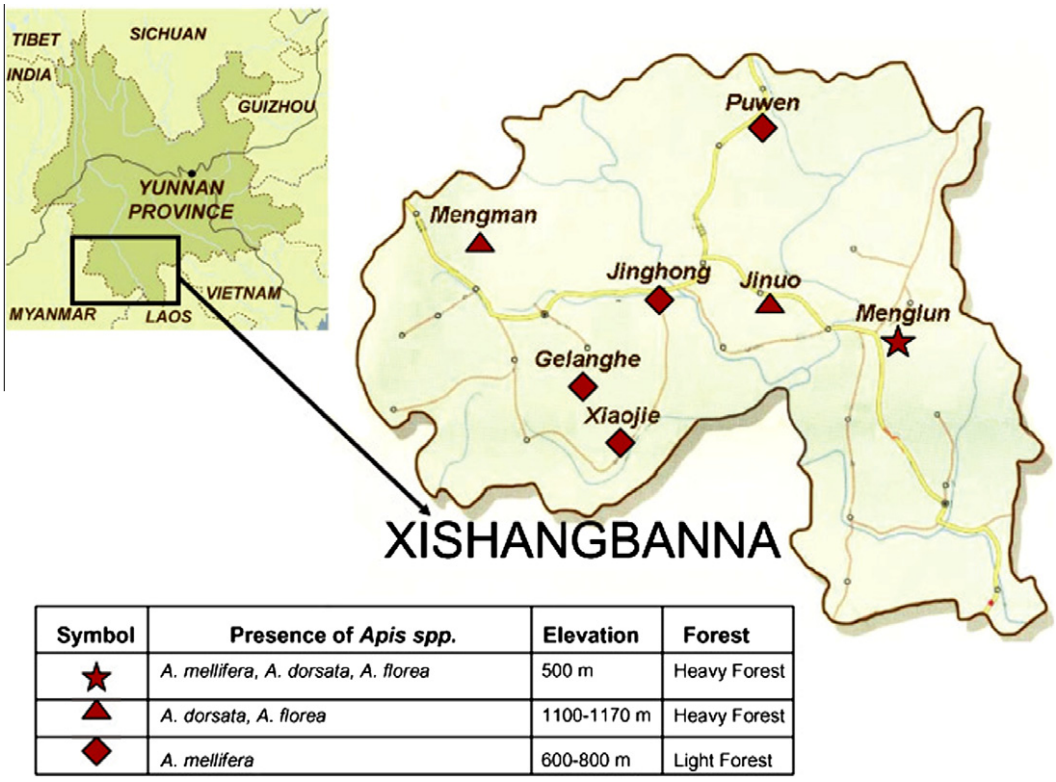

Symbol	Presence of *Apis spp.*	Elevation	Forest
★	*A. mellifera, A. dorsata, A. florea*	500 m	Heavy Forest
▲	*A. dorsata, A. florea*	1100-1170 m	Heavy Forest
◆	*A. mellifera*	600-800 m	Light Forest

Fig. 1. Distribution of *Apis florea* and *A. dorsata* in Xishuangbanna, Yunnan province, China. *Apis florea* and *A. dorsata* are distributed in areas labeled with stars and triangles.

viruses, virus infections in *A. florea* and *A. dorsata* were investigated in the present study.

Honey bee species of *A. florea* and *A. dorsata* were collected individually in seven different geographic regions of Xishuangbanna county, Yunnan province, China (Fig. 1). In the field, live bees were individually collected and stored in plastic tubes and then placed in a tank of liquid nitrogen before being transported back to the laboratory. All samples were stored at −80 °C until molecular analysis was conducted. Total RNA was isolated from individual bees using Trizol® Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The concentration and purity of extracted RNAs were measured by NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Total RNA samples extracted from individual bees were examined for the presence of seven viruses including *Acute bee paralysis virus* (ABPV), BQCV, *Chronic bee paralysis virus* (CBPV), DWV, IAPV, KBV and SBV. Promega's Access RT-PCR system (Madison, WI) was used for specific amplification of individual viruses following the protocol provided with the system. Most of the oligonucleotide primers were used as previously described (Chen et al., 2005). The primers used to amplify RNA-Dependent RNA Polymerase (RdRp) region of DWV (6526–6772) were: sense: 5′-GAGATTGAAGCGCATGAACA-3′; and antisense: 5′-GAAAGCCGAGTTGAAGATGA-3′. This pair of primers resulted in fragments 302 nt long were designed in this study. The specificities of RT-PCR were confirmed by performing sequencing analysis of amplified PCR products. The nucleotide sequences of BQCV and DWV from *A. florea* and *A. dorsata* determined in this study have been deposited in the NCBI GenBank and assigned the accession numbers shown in Fig. 2A and B. The viral sequences in this study, together with virus sequences from *A. mellifera* hosts that were retrieved from GenBank database, were then used to infer phylogenetic relationships. Phylogenetic trees were constructed using the neighbor-joining algorithm within the MEGA4 program (Tamura et al., 2007). The statistical significance of each obtained tree topology was evaluated by bootstrap re-sampling analysis for 500 replicates.

Bee sample collection showed that the distribution of *A. florea* and *A. dorsata* in Xishuangbanna overlap. Xishuangbanna has been well known to be a biodiversity hotspot in China and is assigned as an international biosphere protection area. There are diverse varieties of flowering plants and flowing trees in this area. Honey bees help to maintain the natural diversity of flora as well as crop production via pollinating activities. In our study, both *A. florea* and *A. dorsata* were predominantly found in or near areas of high elevation (1100–1170 m) with heavy forest coverage. In addition, *A. florea* and *A. dorsata* were also found in low-elevation areas (~500 m) with a heavy forest cover where they share the open air and nesting habits with *A. mellifera*. However, both species could not be found at the mid-elevation areas (600–800 m) with farming practices and urbanization (Fig. 1). Of seven viruses examined, two viruses, BQCV and DWV, were detected in both *A. florea* and *A. dorsata*. Among 190 *A. dorsata* adults sampled and examined, 21.6% of samples ($N = 41$) were positive for BQCV and 11.6% of samples were DWV positive. Among 134 *A. florea* adults sampled and examined, 52% ($N = 70$) were detected to be positive for BQCV and 15.6% of samples ($N = 21$) were DWV positive. Co-infections of BQCV and DWV were identified in 6% of *A. florea* and 5.3% of *A. dorsata*. The comparison of DWV showed that the nucleotide sequence difference between *A. florea* isolates and *A. dorsata* isolates was less than 1% and that the sequence difference between *A. florea* isolates and *A. mellifera* isolates or between *A. dorsata* isolates and *A. mellifera* isolates was in 2–4% range. The comparison of BQCV nucleotide sequences at the 3′ UTR (7897–8537) showed that the difference between *A. florea* isolates and *A. dorsata* isolates was also less than 1% but that the sequence difference between *A. florea* isolates and *A. mellifera* isolates or between *A. dorsata* isolates and *A. mellifera* isolates was in 11–15% range. The phylogenetic analysis showed that, for both BQCV and DWV, viruses amplified

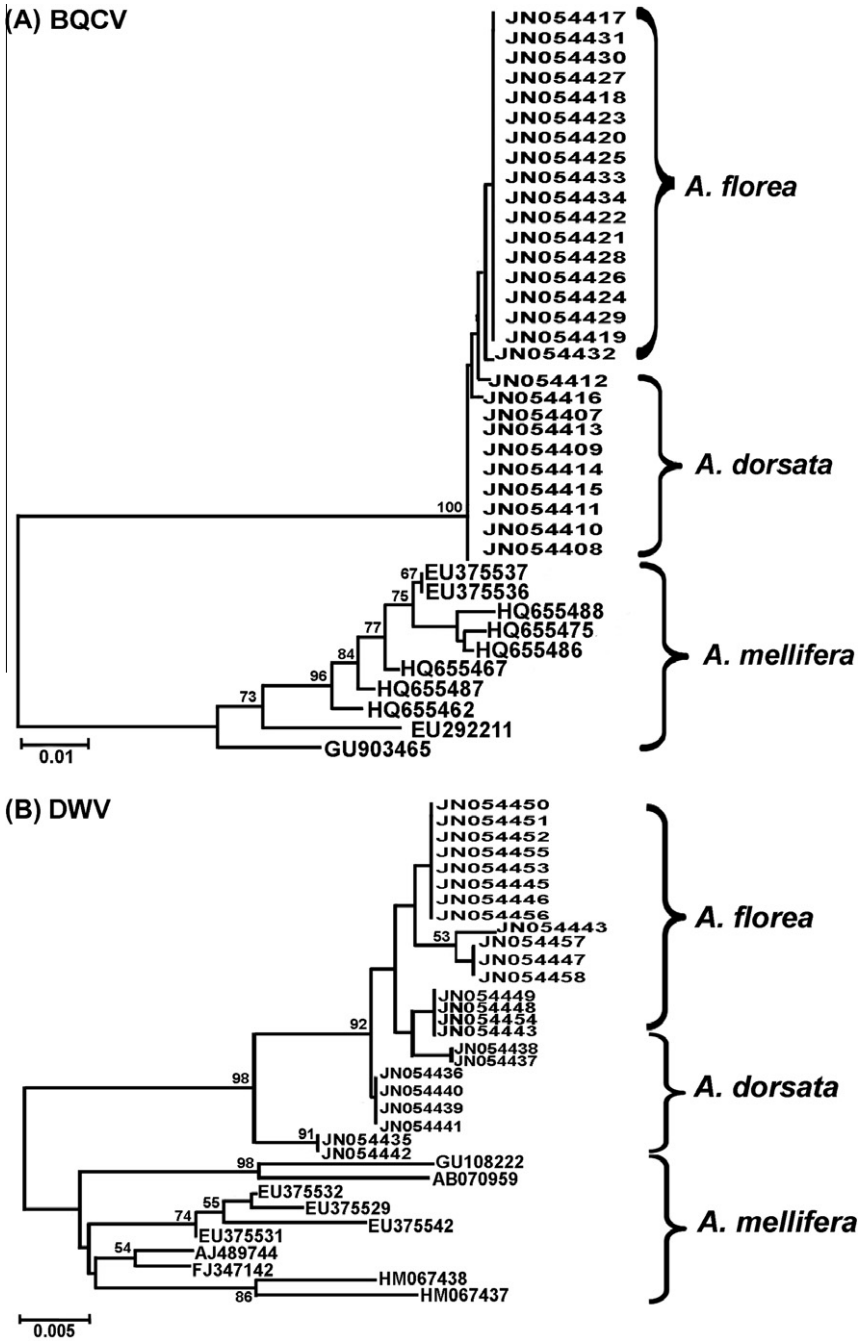

Fig. 2. Evolutionary relationships of BQCV taxa (A) and DWV taxa (B). For both BQCV and DWV, the evolutionary history was inferred using the Neighbor-Joining method. The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic trees. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Numbers in the nodes correspond to bootstrap values. Phylogenetic analyses were conducted in MEGA4. The optimal tree of BQCV with the sum of branch length = 0.16393472 is shown in (A) and the optimal tree of DWV with the sum of branch length = 0.19757939 is shown in (B).

from *A. florea* and *A. dorsata* clustered together with sequences from *A. dorsata* branching out first. The sequences of BQCV or DWV from *A. mellifera* grouped together and formed a distinct clade in the phylogenetic trees (Fig. 2A and B).

BQCV and DWV are the two most common and prevalent virus infections in European honey bees, *A. mellifera,* worldwide and DWV has been associated with honey bee colony declines (Genersch et al., 2010; vanEngelsdorp et al., 2010). Both BQCV and DWV are positive-stranded RNA viruses and belong to the virus family *Dicistroviridae* and *Iflaviridae*, respectively. RNA viruses are characterized by their high mutation rates due to error-prone RNA-dependent RNA polymerase that lacks proofreading activity. High mutations provide RNA viruses with enormous capacity to adapt to complex environments and facilitate their successful invasion of new host populations. As a result, RNA viruses are the most likely resources of genetic diversity in nature, and comprise 37% of all emerging and reemerging pathogens (Woolhouse and Gowtage-Sequeria, 2005). Our present study showed that BQCV and DWV can cause infection in wild species of honey bees, *A. florea* and *A. dorsata,* which migrate routinely between high- and low-elevation areas where there are heavy forest covers. The high degree of genetic homology of the viruses between *A. florea* and *A. dorsata*

reflects the similarly ecological environment shared by these two host species. These results provides further evidence that BQCV and DWV are multi-host pathogens and have variable host ranges, reflecting the highly adaptive nature of RNA viruses. Compared to BQCV and DWV isolated from hosts of *A. mellifera*, viruses from *A. florea* and *A. dorsata* are more evolutionarily derived in the phylogenetic tree, suggesting that the viruses might have moved from *A. mellifera* to *A. florea* and *A. dorsata*. This result is in line with previous reports (Li et al., 2011; Peng et al., 2011; Singh et al., 2010) that the geographical proximity or overlap of the host species and genetic relatedness of the hosts likely played a role in host range expansion of these viruses and the viruses could be transmitted through pollen between different host species. The successful establishment of viruses in different host species is likely to involve non-synonymous nucleotide substitutions of the pathogens. Future studies to investigate what factors determine host specificity and how viruses evolve and adapt to new hosts will shed more light on the co-evolution of hosts and pathogens and on factors underlying the emergence of new pathogens.

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