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Cross-species transmission of honey bee viruses in associated arthropods☆

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☆ The deformed wing viruses sequenced here have been submitted to GenBank and assigned accession numbers ArthropodDWV.sqn Apis_mellifera-BGS1-L1-2010, KF314827; ArthropodDWV.sqn Apis_mellifera-BGS2-L1-2010, KF314828; ArthropodDWV.sqn Apis_mellifera-BGS3-L1-2010, KF314829; ArthropodDWV.sqn Apis_mellifera-BGS6-L1-2010, KF314830; ArthropodDWV.sqn Bombus_impatiens-BGS7-L1-2010, KF314831; ArthropodDWV.sqn Apis_mellifera-BGS8-L1-2010, KF314832; ArthropodDWV.sqn Apis_mellifera-BGS11-L1-2010, KF314833; ArthropodDWV.sqn Apis_mellifera-BGS12-L1-2010, KF314834; ArthropodDWV.sqn Apis_mellifera-BGS16-L1-2010, KF314835; ArthropodDWV.sqn Apis_mellifera-BGS17-L1-2010, KF314836; ArthropodDWV.sqn Apis_mellifera-BGS18-L1-2010, KF314837; ArthropodDWV.sqn Apis_mellifera-BGS21-L1-2010, KF314838; ArthropodDWV.sqn Apis_mellifera-BGS23-L1-2010, KF314839; ArthropodDWV.sqn Apis_mellifera-BGS27-L1-2010, KF314840; ArthropodDWV.sqn Apis_mellifera-BGS28-L1-2010, KF314841; ArthropodDWV.sqn Apis_mellifera-BGS29-L1-2010, KF314842; 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ABSTRACT

There are a number of RNA virus pathogens that represent a serious threat to the health of managed honey bees (*Apis mellifera*). That some of these viruses are also found in the broader pollinator community suggests the wider environmental spread of these viruses, with the potential for a broader impact on ecosystems. Studies on the ecology and evolution of these viruses in the arthropod community as a whole may therefore provide important insights into these potential impacts. We examined managed *A. mellifera* colonies, nearby non-*Apis* hymenopteran pollinators, and other associated arthropods for the presence of five commonly occurring picorna-like RNA viruses of honey bees – black queen cell virus, deformed wing virus, Israeli acute paralysis virus, Kashmir bee virus and sacbrood virus. Notably, we observed their presence in several arthropod species. Additionally, detection of negative-strand RNA using strand-specific RT-PCR assays for deformed wing virus and Israeli acute paralysis virus suggests active replication of deformed wing virus in at least six non-*Apis* species and active replication of Israeli acute paralysis virus in one non-*Apis* species. Phylogenetic analysis of deformed wing virus also revealed that this virus is freely disseminating across the species sampled in this study. In sum, our study indicates that these viruses are not specific to the pollinator community and that other arthropod species have the potential to be involved in disease transmission in pollinator populations.

1. Introduction

Many pathogens can infect more than one host species, including the approximately 60% of human pathogens that are zoonotic (Taylor et al., 2001) and the more than 80% of pathogens that infect domesticated animals (Cleaveland et al., 2001; Woolhouse et al., 2001). How pathogens jump species boundaries, and the factors that shape their species distribution, are therefore key issues in disease ecology and evolution. How readily pathogens are able to emerge in host species has recently become a pressing concern with honey bees (*Apis mellifera*), particularly since the dramatic losses of thousands of honey bee colonies due to colony collapse disorder (CCD) and other causes (Cox-Foster et al., 2007; vanEngelsdorp et al., 2008; Cornman et al., 2012). Indeed, since the appearance of CCD there has been increased concern that honey bee viruses can infect other arthropod species, and vice versa, in the same ecosystems.

In a previous study we reported the detection of deformed wing virus (DWV), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), and sacbrood virus (SBV) in 11 non-*Apis* hymenopteran species and in pollen pellets from forager bees (Singh et al., 2010). Other studies have also identified DWV and BQCV infections in some species of bumble bees, including *Bombus terrestris, Bombus pascuorum*, and *Bombus huntii* (Genersch et al., 2006; Li et al., 2011; Meeus et al., 2010; Morkeski and Averill, 2010; Peng et al., 2011). Other viruses normally found in *A. mellifera*, including acute bee paralysis virus (ABPV) and KBV, have also been identified in bumble bees (Anderson, 1991; Bailey and Gibbs, 1964). Detection of these viruses in other pollinator species suggests that they may play a larger role in the ecosystem than originally thought.

The four most common viruses found in *A. mellifera* in the United States are DWV, BQCV, KBV and SBV (Chen and Siede, 2007; Welch et al., 2009). In addition, although it is less commonly found, IAPV was strongly associated with CCD (Cox-Foster et al., 2007). All these viruses are single stranded, positive-sense RNA (ssRNA(+)) viruses in the order *Picornavirales* (Le Gall et al., 2008). Both DWV and SBV have been assigned to the genus *Iflavirus*, while BQCV belongs to the genus *Cripavirus*, and KBV and IAPV are members of the genus *Aparavirus* (King et al., 2012). Previous studies show that cross-host species transmission occurs more commonly with RNA viruses than other microbial parasites (Cleaveland et al., 2001; Elena & Sanjuan, 2005; Holmes, 2006; Woolhouse, 2002). This likely reflects an elevated rate of adaptive evolution mediated by highly error-prone and frequent replication, as well as large population sizes (Holmes, 2009). Accordingly, the most common honey bee RNA viruses are also candidates for cross-species transmission.

A number of factors dictate whether a virus will be able to emerge in a new host species, and particularly whether the virus can efficiently infect the relevant cell types. Restrictions impeding this process include receptor binding, entry or fusion, trafficking within the cell, genome replication, and gene expression (Parrish et al., 2008). At the host level the frequency and extent of interspecific contact mediated by population density and host behavior are likely to play a critical role in successful cross-species transmission. Since honey bees are part of a larger ecosystem and interact with other arthropods, it is important to determine the distribution of relevant viruses in that ecosystem as well as the proportion of virus species infecting non-honey bee species to understand disease dynamics in pollinator communities.

During replication of RNA viruses with a single-stranded, positive-sense genome, a full-length, complementary, negative-sense RNA is synthesized that serves as the template for replication to form new virions. As a consequence, strong evidence of active infection is the detection of nucleic acid species that are only produced during virus replication, such as the negative-strand RNA (Ongus et al., 2004; Yue and Genersch, 2005). This approach has increased in popularity, particularly the use of strand-specific RT-PCR assays, which demonstrated active DWV infections in *Varroa destructor* and *Aethina tumida* (Dainat et al., 2009; Eyer et al., 2009; Gisder et al., 2009; Ongus et al., 2004; Yue and Genersch, 2005). In addition this method has been used to show that IAPV can replicate in the varroa mite (*V. destructor*) (Di Prisco et al., 2011) and chronic bee paralysis in a species of carnivore ant (*Camponotus vagus*) and in *V. destructor* (Celle et al., 2008).

Our study examines the extent to which non-*Apis* pollinators and other arthropods associated with honey bee apiaries carry viral RNAs of the five most common and/or important viruses known to infect *A. mellifera*. We also determined which of these host species showed evidence of replication of DWV or IAPV, making them potential reservoirs for honey bee viruses.

2. Materials and methods

2.1. Origin of the sample

Samples of *Apis* and non-*Apis* hymenopteran pollinators along with other associated arthropods were collected from several locations in Pennsylvania from 2006–2011. All non-*Apis* specimens collected for this study were found within approximately 800 m of established honey bee apiaries and were identified to species whenever possible. All samples were identified at least to order. Table 1 shows the taxon collected, how many of each taxon was collected, and the percentage of samples that tested positive for

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