Historical presence of Israeli Acute Paralysis Virus in the United States

Yanping Chen and Jay D. Evans*
USDA-ARS, Bee Research Laboratory, Beltsville, MD 20705

*Corresponding Author:
Jay D. Evans
Bee Research Laboratory
USDA-ARS
Bldg. 476, BARC-East
Beltsville, MD 20705 USA
Phone: 301-504-5143
Fax: 301-504-8736
email: jay.evans@ars.usda.gov

Abstract
High bee colony losses in the United States this past year can be attributed in part to an unresolved syndrome termed Colony Collapse Disorder (CCD). An extensive genetic survey found one virus, Israeli Acute Paralysis Virus (IAPV), to be strongly associated with CCD. Using DNA sequencing and phylogenetic analyses, we provide evidence that IAPV was present in U.S. bees collected several years prior to CCD, and prior to the recent importation into the U.S. of honey bees from Australia and New Zealand. While downplaying the importance of bee importation for the appearance of CCD, these results indicate an urgent need to test specific strains of IAPV for their disease impacts.

Honey bees are of great agricultural importance in the U.S. and worldwide (Morse and Calderone, 2000), and are continually threatened by parasites and pathogens. During the winter of 2006-2007, a rare and extreme syndrome of honey bee losses was observed. This syndrome, labeled Colony Collapse Disorder (CCD), is defined by a rapid depopulation of adult bees in colonies, often leaving a substantial standing brood of healthy larvae (http://www.ento.psu.edu/MAAREC/ColonyCollapseDisorder.html). Survey evidence suggests that roughly 25% of beekeepers have suffered the effects of CCD, as defined by characteristic traits and colony losses of >50% (van Engelsdorp *et al.*, 2007). Many beekeepers lost substantially more than 50% of their operations. While events similar to CCD have occurred in past decades (Wilson and Menapace, 1979), the severity of this event has caused appropriate concern nationally and internationally.

Recently, an unprecedented 'metagenomic' approach was used to detect parasites and pathogens in bees associated with CCD and controls (Cox-Foster et al., 2007). This study described numerous microbes from bees, some known as pathogens and others that had not been seen prior in honey bees. One striking result was the tight correlation

between Israeli Acute Paralysis Virus (IAPV), an unclassified Dicistroviridae virus, and CCD. IAPV was detected in 25 of 30 (83 %) CCD-affected honey bee colonies but only once in 21 healthy colonies (Cox-Foster et al., 2007). This virus was also found in package bees imported from Australia and isolates of royal jelly imported from China. The identification of IAPV as a newly described virus for the U.S., it's association with an important disease, and implications for both bee management and trade issues, have all led to intensive efforts to study this virus. These efforts are focused on past and present worldwide distributions IAPV, on determining mechanisms by which this and related viruses can cause disease, and on determining whether IAPV strains differ substantially in their impacts on bees.

To help address these questions, we screened honey bee samples collected in California, Maryland and Pennsylvania from 2002 to 2007 for the presence of IAPV. The genetic relationships of different IAPV strains were studied by sequencing one section (the 5'UTR) from the IAPV genome for isolates collected from 33 U.S. honey bees (out of several hundred screened bees). The results from this survey indicate that IAPV has been circulating in U.S. bee populations since at least 2002, and forms a worldwide species that is greatly diverged from the related Kashmir Bee Virus (Figure 1). Specifically, IAPV isolates from this study can be split into four distinct clusters supported with bootstrap statistical values > 55%. These clusters reflect collections from California, Maryland, Pennsylvania, and Israel. CA, and PA isolates each formed separate lineages with strong bootstrap support, while the Maryland and Israeli lineages were less well defined. Israeli samples, including the strain first named as IAPV, are not distinct from the U.S. isolates as a group.

We also sequenced the entire genomes of IAPV isolates from California, Maryland and a Pennsylvania apiary with a history of Australian importation and CCD symptoms, using a combination of long-template RT-PCR, primer walking, and Rapid Amplification of cDNA Ends (RACE) methods (protocols available from Y. Chen). These three complete genomes, when compared to the definitive (Israeli) IAPV genome sequence, show 4.2 – 4.7% divergence at the RNA level, while all IAPV strains showed >25% divergence from Kashmir Bee Virus. Genetic heterogeneity across the studied 5' region is interesting in that this region is involved in the initiation of protein translation, and genetic variability of this region may lead to different pathogenicities. Further analyses are needed to explore the implications of these and other genome sequences for virulence traits of IAPV.

Our results show that IAPV in the U.S. predates both the latest incarnation of CCD and the importation of Australian package bees. Nevertheless, we caution that much work is still needed to absolve or implicate this virus, or specific imports, in CCD. Most importantly, experimental studies are ongoing to determine the relative virulence of imported or domestic IAPV strains, and such studies will provide the best evidence for making importation and management choices. Viruses with minimal genome sequence differences can show greatly different levels of virulence, and all isolates of IAPV we studied showed at least some sequence variation. Given its observed association with CCD, this virus remains an important candidate for honey bee disease.

Figure Legend. Phylogenetic tree based on 450 nt of the 5'-UTR region of 33 IAPV isolates from CA, MD, PA, and Israel. Sequences were aligned with MegAlign (DNASTAR Lasergene) and the tree was generated using a heuristic Maximum Parsimony algorithm (PAUP 4.03; Sinauer Associates, Sunderland, MA). The strength of branch relationships was assessed by bootstrap replication (N=1000 replicates). Sequences deposited in Genbank by the senior author.

References
Cox-Foster, D.L., Conlan, S., Holmes, E., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., van Engelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S.K., Simons, J. F., Egholm, M., Pettis, J. S., Lipkin W. I. (2007) A metagenomic survey of microbes in honey bee colony collapse disorder. Science. 318(5848): 283-287.

Morse, R. A. and Calderone, N. W. (2000) The value of honey bee pollination in the United States. Bee Culture 128: 1-15.

vanEngelsdorp, D., Underwood, R., Caron, D., Hayes, Jr., J (2007) An estimate of managed colony losses in the winter of 2006 - 2007: A report commissioned by the Apiary Inspectors of America, American Bee Journal 147: 599-603.

Wilson, W.T., and D.M. Menapace, 1979. Disappearing disease of honey bees: a survey of the United States. American Bee Journal 119: 118-119, 184-186, 217.