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A scientific note on Israeli acute paralysis virus infection of Eastern honeybee *Apis cerana* and vespine predator *Vespa velutina*

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The Israeli acute paralysis virus (IAPV; Maori et al. 2007; de Miranda et al. 2010) is widespread in Western honeybees (*Apis mellifera*) for whom it can be a marker of colony losses (Cox-Foster et al. 2007). It has also been found in other Hymenoptera (e.g., *Vespula vulgaris*; Singh et al. 2010), but the ability of the virus to replicate in such alternative hosts still has to be confirmed, as has been shown recently for the ectoparasite *Varroa destructor* (Di Prisco et al. 2011). We investigated whether IAPV can be found in Western and Eastern (*Apis cerana*) honeybees and in their hornet predator *Vespa velutina* in China and whether this virus is able to replicate in these organisms. Positive-strand RNA virus replication requires the production of negative-strand RNA replicative intermediates. Detection of negative-strand RNA of IAPV in *A. cerana* and *V. velutina* would strongly support their function as alternative hosts of this virus.

Eastern honeybee workers (N=180) were sampled from six healthy colonies in Jinhua, Zhejiang Province, China. Western honeybee workers (N=90) were obtained from three healthy colonies at Zhejiang University in Hangzhou subject to predation by *V. velutina* wasps, which were caught in flight (N=10). Samples were stored immediately at −20°C until RNA extraction.

RNA was extracted from 30 pooled honeybees per colony of each species and from the whole bodies of five *V. velutina* hornets. Extractions were done with the total RNA Kit II (E.Z.N.A™) following the manufacturer's recommendations. The same kit was used for the separate extraction of the head, thorax and abdomen of the remaining five *V. velutina* hornets in order to locate in which body parts these viruses occurred. The extracted RNA was eluted in 60 μL of RNAse-free water. Reverse transcription was performed using M-MLV RT Thermoscript® RT-PCR (Invitrogen) with 9 μL of extracted RNA using random hexamer oligonucleotides as primers in 20 μL of reaction final volume, following the manufacturer recommendations. Tenfold dilutions of the resulting cDNA were used for the PCR assays. IAPV detection was performed using MyTaq™ reagents in 50 μL solutions containing 2 μL template cDNA, 10 μL of 5X reaction buffer, 2 μL of each forward and reverse primers (10 μM) and 0.25 μL of *Taq* polymerase (1.25 U). The primers used are described in Di Prisco et al. (2011) (sense: 5′-

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GCGGAGAATATAAGGCTCAG-3′; antisense: 5′-CTTGCAAGATAAGAAAGGGGG-3′ generating a PCR product of 586 bp). The PCR assays consisted of 2 min incubation at 95°C and 35 cycles of 20 s at 95 °C for denaturation, 20 s at 57°C for annealing, 30 s at 72°C extension and then a final step of 7 min at 72°C. The specificity of individual PCR bands was confirmed by sequencing. Moreover, we analysed the samples for Kashmir bee virus (KBV) and acute bee paralysis virus (ABPV) (primers described in Shen et al. 2005 and Gauthier et al. 2007, respectively) in order to verify the occurrence of related viruses (de Miranda et al. 2010).

For the detection of IAPV-negative-strand RNA, reverse transcription was performed using M-MLV RT Thermoscript® RT-PCR (Invitrogen) at 55°C for 50 min followed by 70°C for 10 min for inactivation in 20-μL volume. The tagged primer (5′-agcctgcgcacgtggGCGGAGAATA TAAGGCTCAG-3′; Di Prisco et al. 2011) was used for the reverse transcription. The resulting cDNAs were purified for the excess of primers (Boncristiani et al. 2009) using the Kit Nucleo-Spin Extract II and eluted in 30 μL elution buffer. PCR was performed using MyTaq™ reagents. The amplifications were performed under the same conditions as for the diagnostic PCR assays using the Tag oligonucleotide (5′-AGCCTGCGCACCGTGG-3′; Yue and Genersch 2005) as forward primer. PCR reactions of purified samples without Tag primer were included as negative control.

IAPV was detected in all three *A. mellifera* colonies and in all ten *V. velutina* hornets, but in only one *A. cerana* colony out of the six sampled (Figure 1). The sequences were compared with that in accession number EF219380 (Maori et al. 2007) confirming the identity of IAPV. KBV and ABPV were not detected in our samples. IAPV-negative-strand RNA was not detected in *A. cerana*, but could be found in *A. mellifera* and in nine out of the ten *V. velutina* individuals, including their heads, thoraces and abdomens.

Our data show that, in China, both Western and Eastern honeybees as well as the hornet predator *V. velutina* are carriers of IAPV, the latter probably due to foodborne contamination via infected honeybee prey. The virus was rarely found in *A. cerana*, which could be due to the absence of IAPV in nearby *A. mellifera* colonies that may have acted as reservoirs (Singh et al. 2010). The detection of IAPV-negative-strand replicative intermediates in *V. velutina* strongly suggests that the virus replicates in the wasp. Given the presence of negative-strand RNA in the heads and thoraces of the predator, it is unlikely that these replicative intermediates are of honeybee origin. If this were the case, replicative intermediates from the prey would only be expected in the abdomen of the hornet. We detected evidence of IAPV replication in *A. mellifera*, but not in *A. cerana*. The virus may not be able to replicate in *A. cerana* although these bees were carriers. We consider this unlikely given the closer phylogenetic proximity between the two honeybee species than between *A. mellifera* and *V. velutina* or varroa in which evidence of replication was found (this study; Di Prisco et al. 2011).

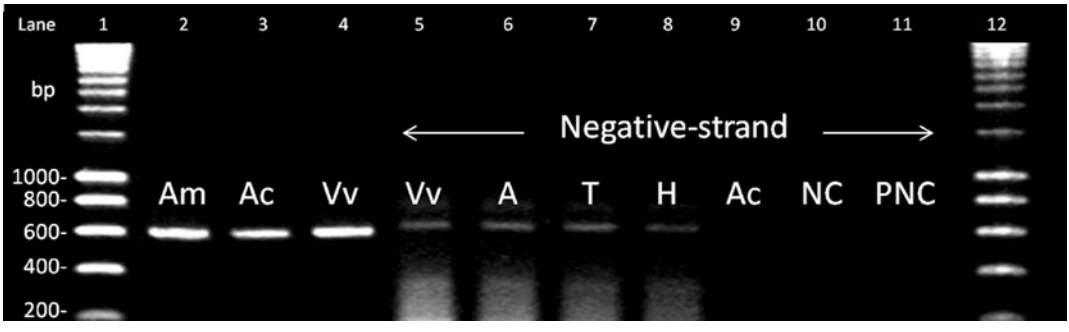

Figure 1. IAPV detection and negative-strand detection. *Lanes 1 and 12*: size scales; *lanes 2 to 4*: detection of IAPV in representative individuals of *A. mellifera* (*Am*), *A. cerana* (*Ac*) and *V. velutina* (*Vv*); *lanes 5 to 8*: detection of negative-strand RNA of IAPV in a representative individual of *V. velutina* (*Vv*) as well as heads (*H*), thoraces (*T*) and abdomens (*A*) of a pool of five *V. velutina* individuals. No negative-strand RNA of IAPV was detected in *A. cerana* (*lane 9*). *NC* PCR negative control (*lane 10*); *PNC* purification negative control (*lane 11*).

The detection of IAPV in *A. cerana* and *V. velutina*, together with that of various other honeybee viruses in predatory or non-predatory Hymenoptera (Anderson 1991; Celle et al. 2008; Dainat et al. 2009; Genersch et al. 2006; Singh et al. 2010), suggests that honeybee viruses can expand to multiple non-*Apis* species. With the detection of signs of replication of IAPV in a wasp, our results indicate that a non-*Apis* Hymenoptera could not only be a carrier (Singh et al. 2010), but also an additional host for this honeybee virus. Our results and those of others suggest that the number of pathogens shared between social insects and their associated arthropods has been considerably underestimated. Their role could be relevant for the health of honeybees and other insects given the potential increase of pathogen virulence following host shifts (Woolhouse et al. 2001).

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Note scientifique sur l'infection par le virus israélien de la paralysie aiguë (IAPV) de l'abeille orientale *Apis cerana* et de son prédateur *Vespa velutina*

Eine wissenschaftliche Notiz zur Infektion der östlichen Honigbiene *Apis cerana* und der predatorischen Wespe *Vespa velutina* mit dem israelischen aktuten Paralyse Virus (IAPV)

REFERENCES

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

Boncristiani, H.F., Di Prisco, G., Pettis, J.S., Hamilton, M., Chen, Y.P. (2009) Molecular approaches to the analysis of deformed wing virus replication and pathogenesis in the honey bee, *Apis mellifera*. Virol. J. **6**, 221

Celle, O., Blanchard, P., Olivier, V., Schurr, F., Cougoule, N., Faucon, J.P., Ribière, M. (2008) Detection of chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread. Virus Res. **133**, 280–284

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., Vanengelsdorp, 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**, 283–287

Dainat, B., Ken, T., Berthoud, H., Neumann, P. (2009) The ectoparasitic mite *Tropilaelaps mercedesae* (Acari: Laelapidae) as a vector of honeybee viruses. Insect Soc. **56**, 40–43

de Miranda, J.R., Cordoni, G., Budge, G. (2010) The acute bee paralysis virus–Kashmir bee virus–Israeli acute paralysis virus complex. J. Invertebr. Pathol. **103**, 30–47

Di Prisco, G., Pennacchio, F., Emilio, C., Boncristiani, H., Evans, J.D., Chen, Y.P. (2011) *Varroa* destructor is an effective vector of Israeli acute paralysis virus in honey bees, *Apis mellifera*. J. Gen. Virol. **92**, 151–155

Gauthier, L., Tentcheva, D., Tournaire, M., Dainat, B., Cousserans, F., Colin, M.E., Bergoin, M. (2007) Viral load estimation in asymptomatic honey bee colonies using the quantitative RT-PCR technique. Apidologie **38**, 426–435

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

Maori, E., Lavi, S., Mozes-Koch, R., Gantman, Y., Peretz, Y., Edelbaum, O., Tanne, E., Sela, I. (2007) Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: evidence for diversity due to intra- and inter-species recombination. J. Gen. Virol. **88**, 3428–3438

Shen, M., Cui, L., Ostiguy, N., Cox-Foster, D. (2005) 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

Singh, R., Levitt, A.L., Rajotte, E.G., Holmes, E.C., Ostiguy, N., Vanengelsdorp, D., Lipkin, W.I., Depamphilis, C. W., Toth, A.L., Cox-Foster, D.L. (2010) RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-*Apis* hymenopteran species. PLoS ONE **5**, e14357. doi:10.1371/journal.pone.0014357

Woolhouse, M.E.J., Taylor, L.H., Haydon, D.T. (2001) Population biology of multihost pathogens. Science **292**, 1109–1112

Yue, C., Genersch, E. (2005) RT-PCR analysis of *Deformed wing virus* (DWV) in honey bees (*Apis mellifera*) and mites (*Varroa destructor*). J. Gen. Virol. **86**, 3419–3424