

Integration of Picorna-like Viruses in Multiple Insect Taxa

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Abstract

The *Picornaviridae* superfamily consists of over 450 species of positive single stranded RNA viruses. This family is unique in that all members have a protein that is attached to the 5' end which is used as a primer for RNA polymerase during transcription. Picorna viruses infect many different organisms, including mammals, birds, and insects. In this study we provide evidence that picorna-like viruses are present in a range of insect hosts and that this type of virus has integrated into the DNA of various insect species. We provide evidence of picorna-like viruses in the glassy-winged sharpshooter (*Homalodisca vitripennis*) GWSS, the red imported fire ant, RIFA, (*Solenopsis invicta*), and the European honey bee (*Apis mellifera*). Analysis of reverse transcriptase PCR (RT-PCR) demonstrated that viruses in the subgroup *Dicistroviridae* have integrated into the genomes of *S. invicta* and *A. mellifera*. However, integration of HoCV-1 into *H. vitripennis* could not be identified.

Introduction

A fragment of the Israeli acute paralysis virus (IAPV), a picorna-like virus, has reportedly integrated into the genome of the European honeybee, *Apis mellifera* (Fig. 5). Integration of IAPV in the genome prevents infection of the virus in an individual. In addition, individual bees may possess more than one species of virus at one time (Maori *et al.* 2007).

The *Picornaviridae* superfamily of viruses is one of the largest and arguably one of the most important groups to humans and agricultural pathogens (Rueckert 1991). Picornaviruses (Fig. 1) have an icosahedral capsid, where the capsid protein is arranged in 60 promoters tightly packed into four groups of equilateral triangles. All individuals in this superfamily are single-stranded, positive sense RNA, and are between 7.2 and 9.0 kb long (Mettenleiter and Sobrino 2008).

Solenopsis invicta virus (SINV-1) is in the *Dicistroviridae* subgroup, only infectious to *S. invicta* (Fig. 3) (Valles *et al.* 2007). The genome of this SINV-1 is 8026 nucleotides long, has a polyadenylated tail, and includes an RNA genome encoding two large open reading frames (ORF). Currently, four variants of SINV have been described: SINV-1, SINV-1A, SINV-2, and most recently SINV-TX5. All forms of SINV are positive-sense ssRNA, and lack the DNA step involved with viral processing. These viruses easily infect both colony phenotypes and have potential for use as biological control agents on RIFA populations.

Homalodisca coagulata virus-1 (HoCV-1), also in the *Dicistroviridae* subgroup, has shown to increase mortality in the glassy-winged sharpshooter, *H. vitripennis* (Fig. 7) larvae (Hunter *et al.* 2006). This insect is the primary vector of Pierce's disease which affects grapevines and is caused by the bacterial plant pathogen, *Xylella fastidiosa*. Every year the grape industry is severely affected by the reduced fruit yields and premature death caused by *X. fastidiosa* infection, therefore correlation between HoCV-1 and *X. fastidiosa* may prove to be effective in reducing populations of *H. vitripennis*.

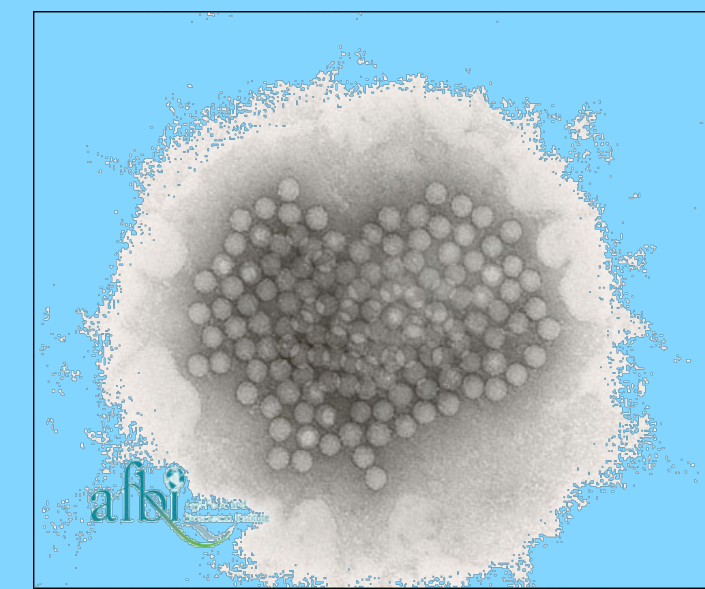


Fig.1. Electron Micrograph of a Picornavirus. (From the Agri-Food and Biosciences Institute)

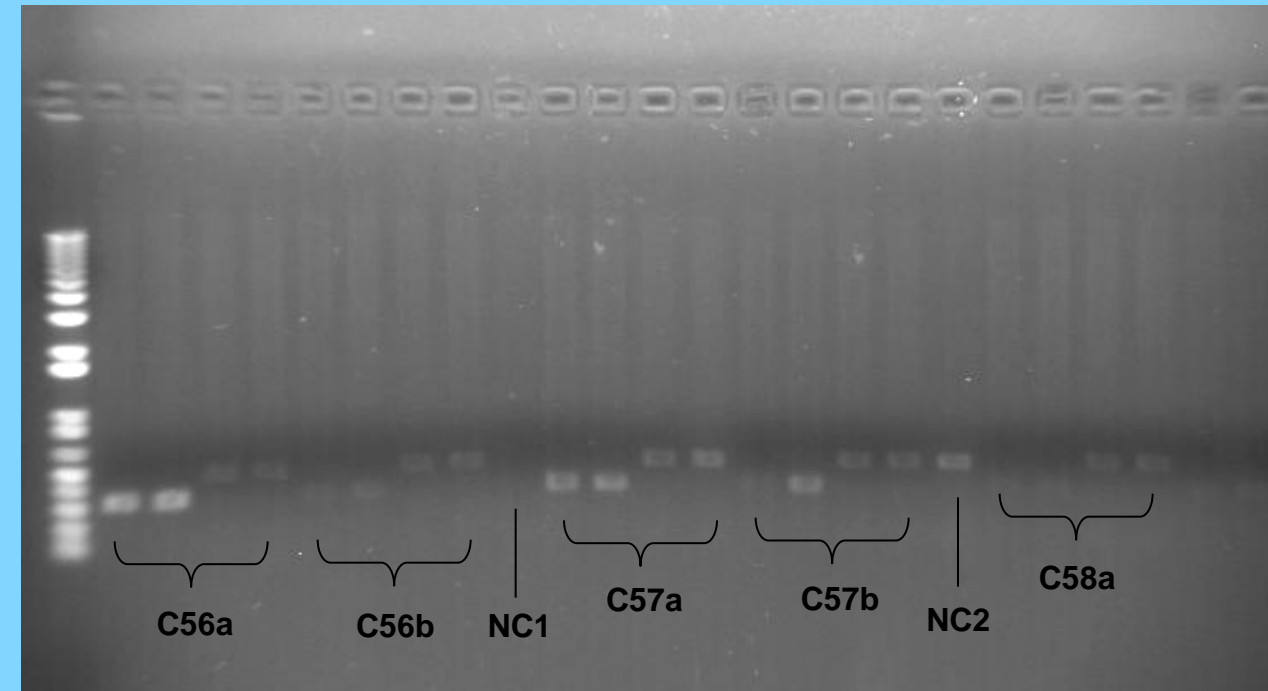


Fig. 2. Gel electrophoresis analysis of three of the four colonies tested for integration of SINV. Each group was tested using two different primer sets, primer set 1 (wells 1 & 2), primer set 2 (wells 3 & 4), and a negative control (NC) for each colony.



Fig. 3. Picture of *S. invicta* the Red Imported Fire Ant RIFA. (From www.ironmountainpest.com)

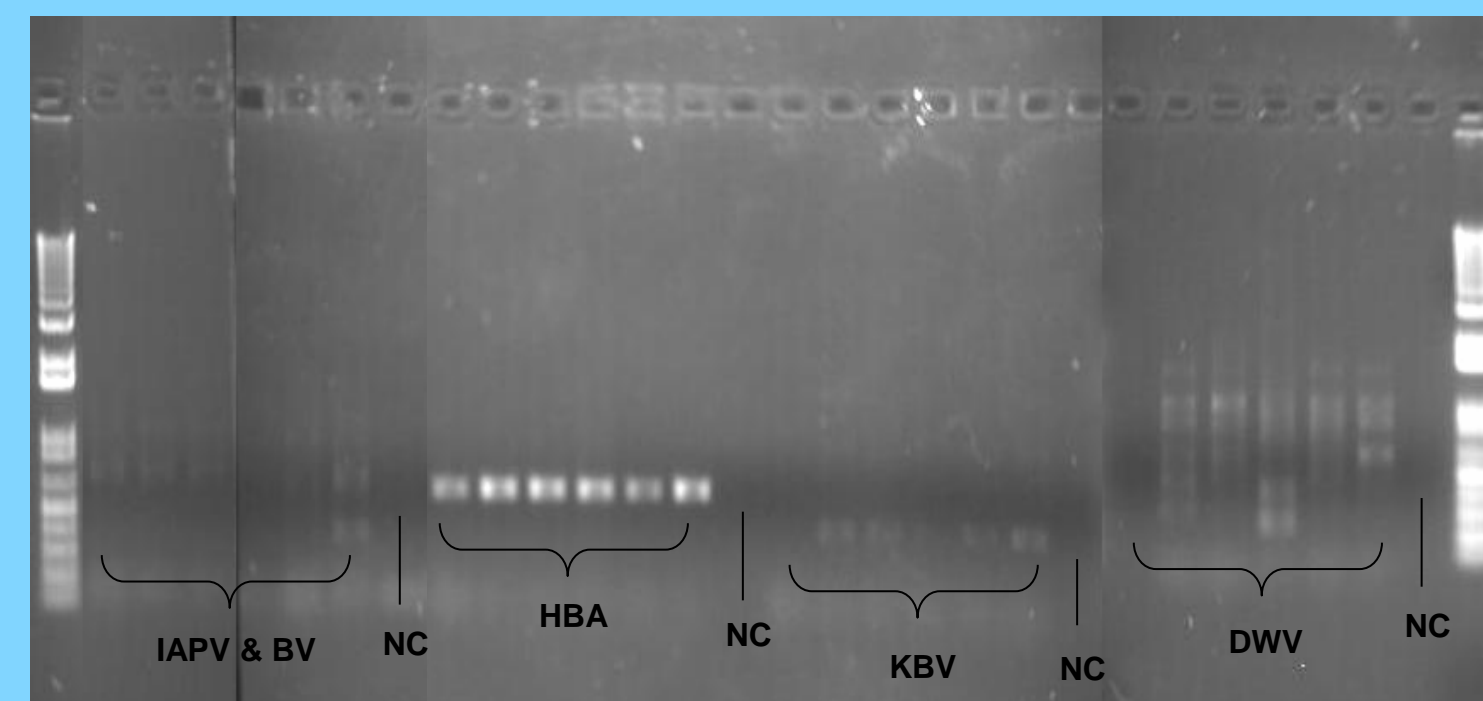


Figure 4. Gel electrophoresis analysis of three bee viruses, Israeli acute paralysis virus (IAPV) (along with a bee virus primer (BV) were used as a positive control for viral infection, honeybee actin (used as positive control), Kashmir bee virus (KBV), and Deformed wing virus (DWV) illustrating integration of these viruses into the DNA of *A. mellifera*. Negative controls (NC) are located at the end of each group. (Multiple Gels shown).



Figure 5. Picture of *A. mellifera* the European honey bee. (From www.termiguardusa.com)

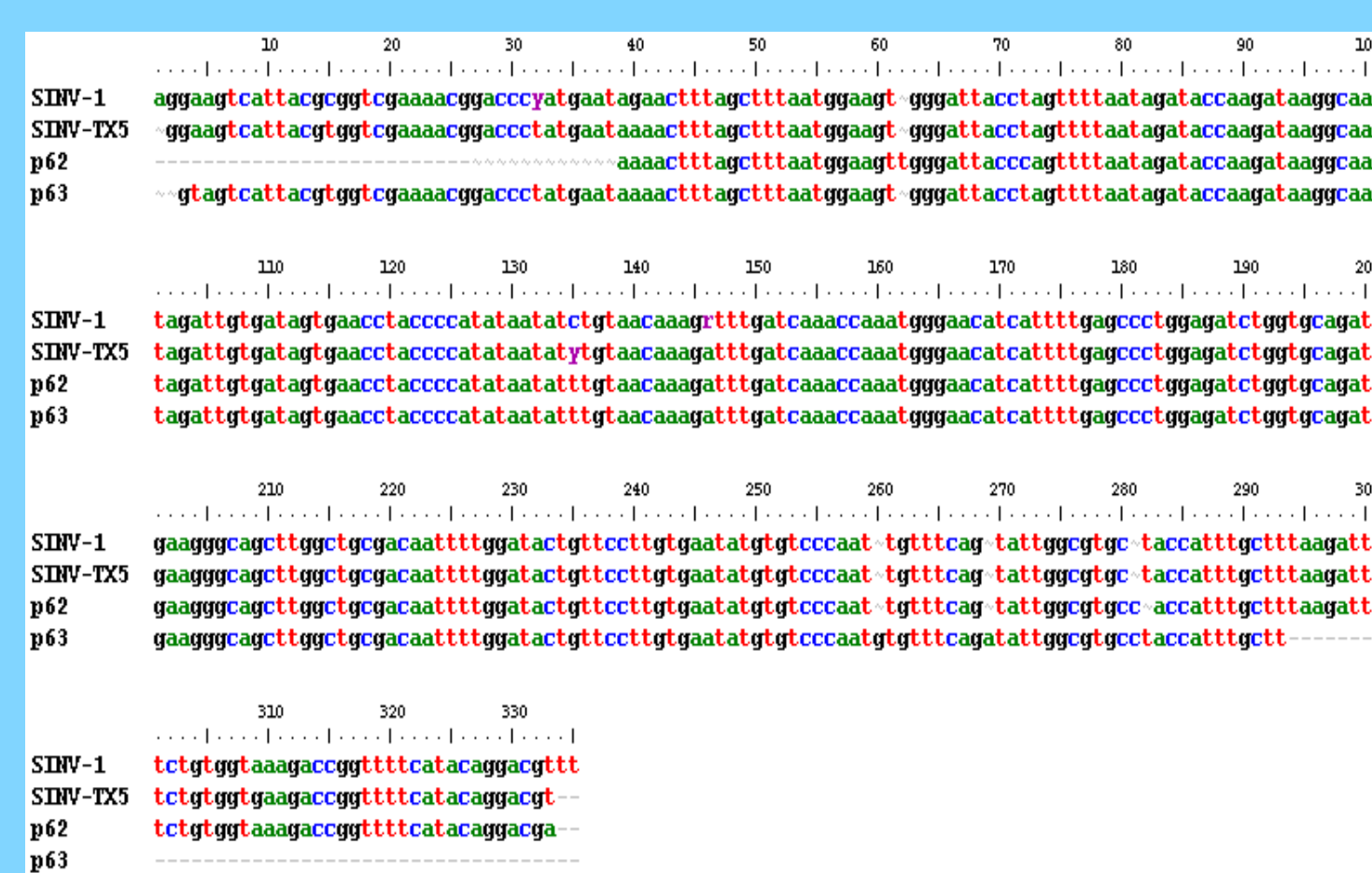


Figure 6. Sequence alignment of SINV-1 and SINV-TX5 to consensus forward (p62) and reverse (p63) sequences of an integrated portion of virus in *S. invicta*.



Figure 7. Picture of *H. vitripennis* the glassy-winged sharpshooter. (From farm2.static.flickr.com)

Materials and Methods

Samples were collected from the field and tested for the presence of virus using multiple species specific primer sets. Multiple extractions for each of the four taxonomic group were performed. All samples were homogenized in 180µl of PBS (phosphate buffer saline) then the samples were divided in half. An RNA extraction using TRIzol reagent (Invitrogen, CA), following the manufacturer's protocol, was performed with one half of the extract, and a DNA extraction using the Qiagen DNeasy tissue extraction kit (Qiagen, CA) was performed with the other half. To ensure no contamination of RNA remained in the DNA extraction, 4µl of RNase A was added to each DNA extraction. Reverse transcription PCR (RT-PCR) was performed using specific primer sets for the RNA extracted samples and a standard PCR was performed using the same specific primer sets for the DNA extracted samples. A 1% agarose gel stained with ethidium bromide was subjected to electrophoresis to determine which samples were positive for the virus integration.

Results

Traditional PCR, gel electrophoresis, and sequencing demonstrate that picorna-like viruses have integrated into the genomes of two of the three experimental insect species used in this study. Three specific primer sets for SINV were tested for integration into the *S. invicta* genome. Traditional PCR and gel electrophoresis confirmed integration (Fig. 2) of SINV into four freshly collected colonies. The negative control (NC2) was determined contaminated, however after replication no contamination of NC2 was observed (data not shown). Bands in the gel depicting positive results were sequenced. Consensus sequences were aligned with SINV-1 and SINV-TX5 (Fig. 6) to confirm integration of SINV into the DNA of *S. invicta*.

Black queen cell virus (BQCV), Deformed wing virus (DWV), Acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), and Israeli acute paralysis virus (IAPV) specific primers were used to test integration into the *A. mellifera* genome. Honeybee actin and a small region indicating viral infection (not found in the bee genome) were used as positive controls (Fig.4). Traditional PCR along with gel electrophoresis for each of these bee viruses showed that DWV and KBV have integrated into a segment of the *A. mellifera* genome.

Seven specific primer sets for various segments of the *H. vitripennis* genome were used to detect HoCV-1 integration. No bands were produced for any of the primer sets (data not shown), therefore integration was not observed.

Conclusions

- Approximately 330bps of SINV was found to have integrated into the *S. invicta* genome, using only one specific primer set.
- Deformed wing virus (DWV) and Kashmir bee virus (KBV) integrated into the *A. mellifera* genome.
- HoCV-1 was not observed to have integrated into the *H. vitripennis* genome.
- Genome walking and inverse PCR will be completed to determine the exact location where SINV, DWV, KBV, and IAPV have integrated into their respective host genomes.

References

- Hunter, WB, CS Katsar, and JX Chaparro. 2006. Molecular analysis of capsid protein of Homalodisca coagulata Virus-1, a new leafhopper-infecting virus from the glassy-winged sharpshooter, Homalodisca coagulata. *J. Insect Science* 28: 1536-2442.
- Maori, E, S Lavi, R Mozes-Koch, Y Gantman, Y Peretz, O Edelbaum, E Tanne, and I Sela. 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. General Virology* 88: 3428-3438.
- Mettenleiter, TC and F Sobrino. 2008. Animal Viruses: Molecular Biology. Caister Academic Press.
- Rueckert, RR. 1991. Picornaviridae and their replication. Fundamental Virology, (2nd ed.) Raven Press Ltd., New York, pp. 409-450.
- Valles, SM, *et al.* 2007. Phenology, distribution, and host specificity in *Solenopsis invicta* virus-1. *J. Invertebrate Pathology* 96:18-27.

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