Nanopore Direct RNA Sequencing revealed the diverse defective viral genome species produced by insect-specific and arthropod-borne alphaviruses

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Abstract

Defective viral genomes (DVGs) are viral replication products that cannot complete a full replication cycle without coinfection with a standard virus. Accumulating evidence demonstrates the key role played by DVGs in virus-host interactions. Historically, only one or a few DVGs were identified for specific viruses relying on a high multiplicity of infection (MOI) passaging condition. The advent of Nanopore Direct RNA Sequencing (DRS) facilitates the identification of DVGs due to its capacity to read through full-length RNAs. Here, we applied this technology to characterize the diverse defective RNA species generated by alphaviruses, a group of positive-strand RNA viruses. The viruses studied include insect-specific Eilat virus (EILV) and its chimeric viruses and three arthropod-borne alphaviruses, Sindbis virus (SINV), Chikungunya virus (CHIKV) and Venezuelan equine encephalitis virus (VEEV). It was found that EILV produced numerous deletion-type DVGs spanning the viral genome in mosquito cells even at a low MOI. On the other hand, SINV produced relatively few defective RNAs in mosquito and mammalian cells, with most originating from sgRNAs. Many of the EILV DVGs were replicable and packageable. Certain DVGs were found to serve as templates for synthesizing viral DNAs, speculated to play an essential role in modulating insect antiviral immunity and persistent infection. We postulate that the proneness to produce DVGs is under high selective pressure, and insect-specific EILV acquires this characteristic to facilitate its adaption in mosquitoes.

Methods

- ★ Nanopore DRS to recover all species of viral RNAs in the infected cells.
- ★ RT-PCR to verify the presence of DVGs.

Conclusions

- ★ We found insect-specific alphaviruses formed numerous deletion-type DVGs during infection.
- ★ We showed arthropod-borne alphaviruses tended to produce defective sgRNAs.

Results

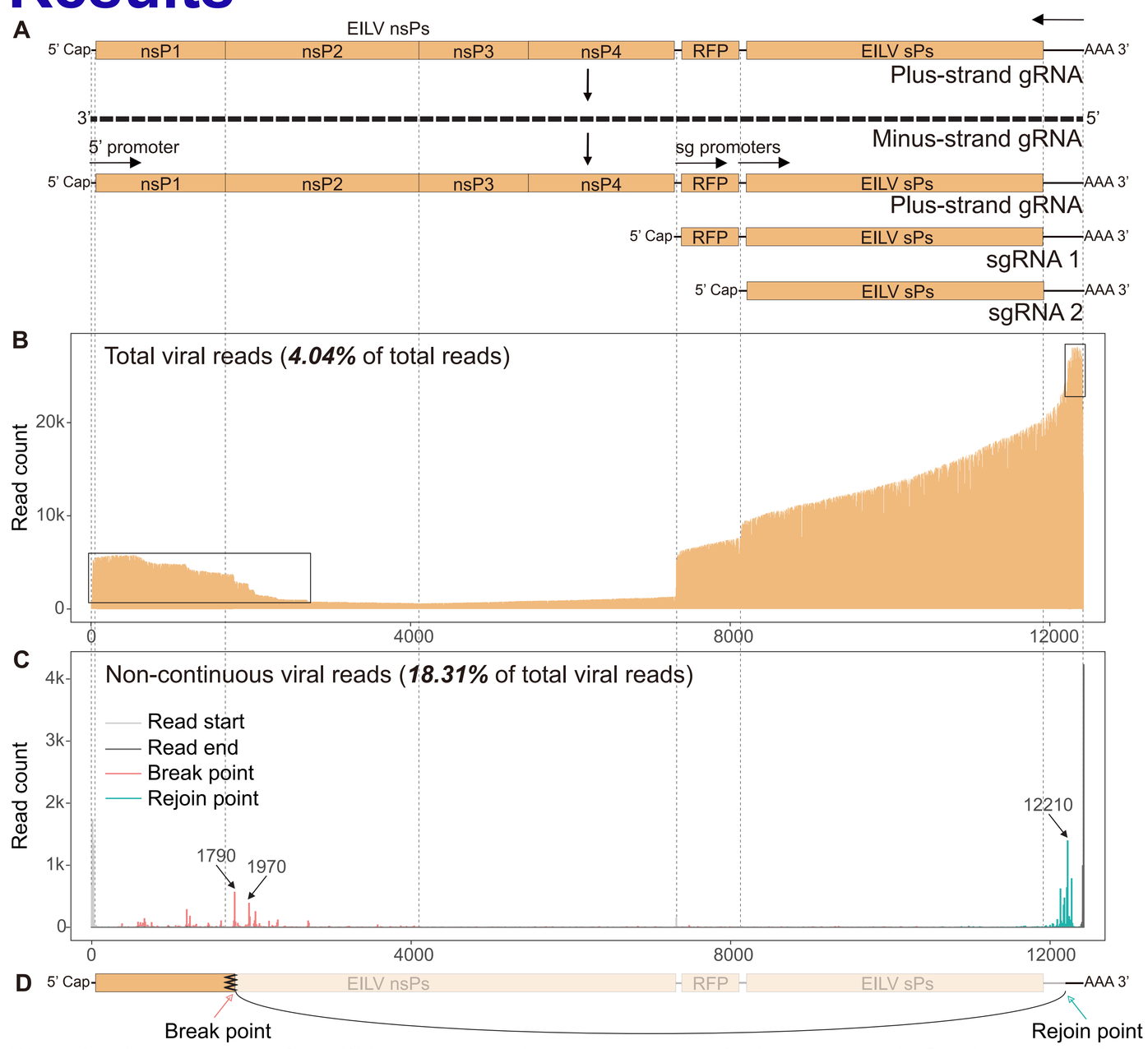

Fig. 1 Insect-specific EILV produced numerous deletion-type DVGs in mosquito cells. (A) Genome organization and replication diagram of EILV containing a RFP reporter gene. (B) Sequencing coverage of viral reads. Polyadenylated RNAs of the infected cells were sequenced using the Nanopore DRS kit on a MinION device. Note, the two boxes highlight the aberrantly high coverage at the 5' and 3' ends of the viral genome, indicating the presence of deletion-type DVGs. (C) Junction boundary counts for non-continuous viral reads. (D) Schematic of a deletion-type DVG spanning the EILV-RFP genome. The shaded region indicates the deleted sequence in the DVG.

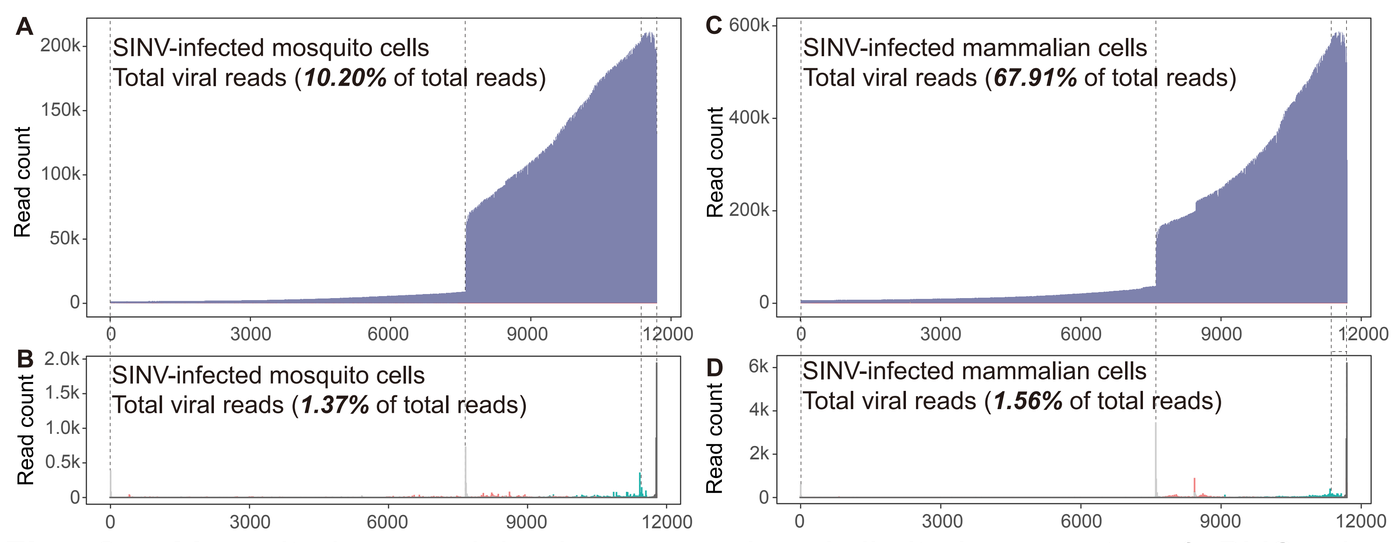

Fig. 2 Mosquito-borne alphaviruses produced limited amounts of DVGs in mosquito and mammalian cells. (A) and (C) Sequencing coverage of reads aligned to the SINV genome. (B) and (D) Junction boundary counts for non-continuous viral reads. Note, most SINV non-continuous reads were derived from sgRNAs, and the junction sites were mainly located at the subgenomic region.

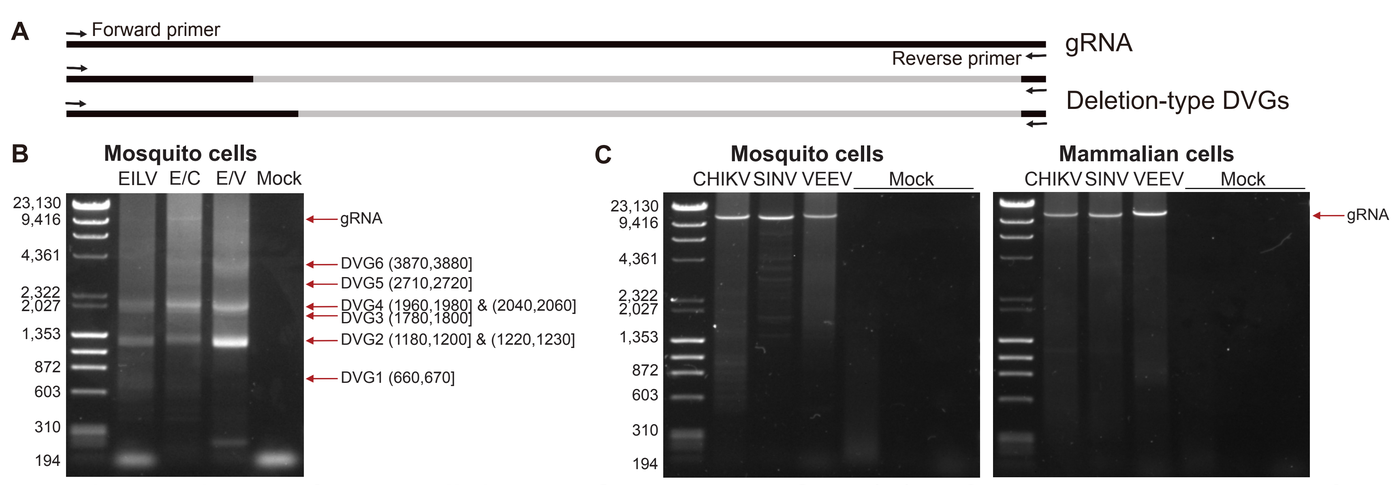

Fig. 3 Analysis of intracellular DVGs by RT-PCR. (A) PCR primers designed for DVG detection. (B) RT-PCR analysis of EILV and its chimeras in mosquito cells. (C) RT-PCR analysis of CHIKV, SINV, and VEEV in mosquito and mammalian cells. Note, mosquito-only EILV and its chimeras generated a large population of deletion-type DVGs, a phenomenon not commonly seen in arthropod-borne alphaviruses.

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