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Insect-specific viruses: from discovery to potential translational applications

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Over the past decade the scientific community has experienced a new age of virus discovery in arthropods in general, and in insects in particular. Next generation sequencing and advanced bioinformatics tools have provided new insights about insect viromes and viral evolution. In this review, we discuss some high-throughput sequencing technologies used to discover viruses in insects and the challenges raised in data interpretations. Additionally, the discovery of these novel viruses that are considered as insect-specific viruses (ISVs) has gained increasing attention in their potential use as biological agents. As example, we show how the ISV Nhumirim virus was used to reduce West Nile virus transmission when co-infecting the mosquito vector. We also discuss new translational opportunities of using ISVs to limit insect vector competence by using them to interfere with pathogen acquisition, to directly target the insect vector or to confer pathogen resistance by the insect vector.

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Introduction

Viruses are the most abundant microbes on our planet [1] and are found associated with all types of organisms, even other viruses. Insects have long been recognized as vectors of many important viruses that affect humans, animals and plants, but insects also are hosts to many different viruses. In general, viruses belonging to several taxa, including the *Baculoviridae*, *Parvoviridae*, *Flaviviridae*, *Ascoviridae*, *Togaviridae*, *Bunyavirales* (formerly *Bunyaviridae*), and *Rhabdoviridae*, have most commonly been considered as being associated with insects [2]. Most viruses have been discovered because they are pathogens and cause disease in their hosts, but recently many viruses have been discovered from non-diseased plants and animals (including insects) suggesting that viruses are much more abundant than previously recognized. Recent estimates suggest that currently less than 1% of the virosphere — the total universe of viruses — has been discovered and classified [3], and traditional virus detection and discovery methods, which require prior knowledge of the viral genome sequences, are considered as one of the causes of this restricted view of the virosphere. These limitations indicate a need for new, improved and unbiased virus discovery approaches. With the introduction of high-throughput next-generation sequencing (NGS) in 2005 [4], a new age of virus discovery has commenced and novel viruses/viral sequences have been identified in many organisms including insects [5–25].

Many of the newly discovered insect viruses are referred to as insect-specific viruses (ISVs). ISVs replicate only in their insect hosts, give persistent infections and are likely transmitted vertically (transovarially) within the population [24,26]. Phylogenetic studies indicate that many of the newly discovered ISVs belong to several different virus families, as well as many which are as yet unclassified [8,24]. These newly discovered viruses/viral sequences provide novel opportunities for gaining a greater understanding of virus evolution and virus-host interactions, but also many may have potential for translational applications for manipulating traits in insects. Whether ISVs have potential to be used for novel translational applications as wild-type or even recombinant viruses is a new chapter of research in virology, and offer new opportunities for efforts to target insects and the pathogens they transmit.

In this review, we focus on ISVs discovered among different insects. We discuss the different methods used to collect metagenomics data, and the challenges in interpretation of viral metagenomes. We also discuss/propose possible translational applications of ISVs; although, we are just at the beginning of this interesting but challenging research area.

NGS technologies for insect virus discovery

NGS technologies are high throughput and highly sensitive non-Sanger-based methodologies that generate

millions to billions of nucleic acid sequences in a single run [27,28]. These massively parallel sequencing platforms are capable of sequencing complex mixtures of genetic material [28] and have been extremely efficient in detecting DNA and RNA viruses, even when their titers are low in the infected host/tissue [29]. Furthermore, NGS is comparatively inexpensive, allows for accurate sequencing and is currently considered the fastest available approach for virus discovery [27,28,30].

Given the high diversity of viruses in both animal and plant hosts and their different replication strategies, several NGS sample and library preparation methods and types of bioinformatics analyses have been used to identify viral sequences in diverse types of host tissues. Some have used different types of templates in order to enrich the libraries for viral sequences [31], and the most commonly used are: total RNA or DNA; virus-derived small interfering RNAs (siRNAs); double-stranded RNAs (dsRNAs); polyadenylated RNAs (poly(A) RNAs); ribosomal RNA depleted total RNA, and RNA from purified or partially purified viral particles [31,32]. All of these have shown advantages and disadvantages and are reviewed in Roossinck *et al.* [32]. Different sample and library preparation methods and currently available NGS platforms have also been reviewed in detail [27,28,32–37]. In general, transcriptome and small RNA (sRNA) libraries appear to be the most suitable for virus discovery [7,8,27], and in this section, we compare these two types of NGS technologies for detecting/discovering insect viruses.

Transcriptome sequencing

The transcriptome is the complete set of transcripts in cells [38,39]. In a virus-infected sample, this will include host and viral RNAs (genomic or mRNAs). Transcriptome sequencing or RNA sequencing (RNA-Seq) by using NGS technology has allowed characterization and quantification of the total transcriptome from different organisms and has shown clear advantages over other approaches in terms of detection of viral nucleic acids in host cells, providing also insights into host responses to virus infection (virus-host interactions) [31]. In general, transcriptome libraries are constructed from total or fractionated RNAs, which are converted to a library of cDNA fragments that have adaptors linked to one or both ends [40]. cDNA fragments, PCR-amplified or not, are then sequenced by using NGS techniques, which can generate short sequences (30–400 nucleotides) from one end (single-end sequencing) or both ends (paired-end sequencing) [40]. Several bioinformatics tools are available for NGS data analysis/processing (see below). Basically, the adaptor sequences and low-quality reads are removed from the libraries and the high-quality reads are generally assembled by two different assembly methods: reference-based (transcriptome is assembled by mapping reads to previously known sequences) and *de novo* assembly (transcriptome is reconstructed without the aid of a reference genome) [39,40]. These methods, combined with Basic Local Alignment Search Tool (BLAST) searches against non-redundant databases or viral databases, have successfully led to virus detection and the discovery of new viruses, respectively [27].

In insects, the first application of NGS technology for transcriptome sequencing was in 2007, when a metagenomics survey of the honey bee (*Apis mellifera* L.) was conducted in a search for a causal agent of colony collapse disorder [21]. Several viruses were identified, which indicated the potential of using this approach [27]. Since then, several groups have sequenced the transcriptomes of different insects and detected not only the known viruses and their variants, but also, in some cases, discovered novel viruses [8,17,30,41–46]. However, an important shortcoming of sequencing transcriptome libraries for detecting viral sequences is that the majority of the generated reads are derived from the host. The percentage of viral RNA genomes within the host RNA background can be very low (less than 1%) and viruses present at low titers may not be detected by this approach [31,32]. In order to reduce the host RNA background and enrich the transcriptome libraries with viral sequences, methods for removing the abundant host ribosomal RNA from the total RNA, or for removing RNA derived from a control (or healthy) host by subtractive hybridization, have been explored [31,32,34]. Other strategies, such as using dsRNA and/or RNA from partially purified viral particles for transcriptome sequencing, have also been used [31,32].

After sequences are obtained, an important challenge is how to manage the large amount of data generated by transcriptome sequencing. There is no standardized pipeline to analyze the data, and viral sequences assemble differently depending on the program and parameter settings used [31]. The challenges of bioinformatics have demanded the development of efficient methods mainly to overcome concerns including storage, quality control, mapping, error correction, single nucleotide variant calling, haplotype reconstruction and data integration [47].

Small RNA sequencing

Deep sequencing of sRNAs has also been used for discovering DNA and RNA viruses in both animal and plant hosts [8,9,48–50]. This approach takes advantage of the natural host antiviral defense system found in most eukaryotes as a component of a more general sequence-specific mechanism for gene expression regulation, which is known as RNA interference (RNAi) [32,50,51]. In arthropods, RNAi consists of at least three different pathways: the small interfering (si), the micro- (mi) and the piwi-interacting (pi) RNA pathways [53]. These three pathways generate sRNAs that have distinct molecular characteristics, including their biogenesis, length, targets and modes of action, and also reflect the virus from which they originated [51].

The most important and well known defense response to viral infection in arthropods occurs through the siRNA pathway, which is triggered by the presence of dsRNAs often produced during RNA virus replication [49]. The dsRNAs are recognized and cleaved by Dicer, a dsRNA-specific endoribonuclease (RNase), into 20–23 nt long duplex siRNAs [51,53]. One of the siRNA strands is then loaded onto the RNA induced silencing complex (RISC) containing Argonaute protein (AGO), the core protein of RISC [49]. RISC directs the siRNA molecules to their respective complementary target viral RNA, which is cleaved by the endonuclease activity of the AGO protein [49,54].

The miRNAs and piRNAs have central roles in regulating gene expression and maintaining genome integrity by suppressing transposable element (TE) activity in eukaryotes, respectively [53,55]. However, many efforts have shown that miRNAs and piRNAs, especially in insects, are also involved with host-virus interaction or antiviral defense [49,57–61]. The miRNAs are derived from short RNA hairpins (~65 to 70 nt) containing a precursor miRNA, which are processed by Drosha and Dicer endoribonucleases to generate miRNA duplexes (~20 to 25 nt long) [61,62]. One of the strands of the duplex, which is also called the guide strand or mature miRNA, is incorporated into the RISC, forming the miRISC complex that binds to complementary regions in the RNA transcript, and affects gene expression by inhibiting the translation or destabilizing the mRNA [61,62]. Unlike siRNAs and miRNAs, the piRNAs are processed in a Dicer-independent manner from long single-stranded RNA precursors that are transcribed from genomic loci called piRNA clusters [31,51], which consist mainly of remnants of TE sequences [57]. The RNA precursor is cleaved by an endonuclease into ~24 to 30 nt long primary piRNAs, which are incorporated into RISC containing Piwi proteins, a subclass of the AGO family, resulting in the piRISC complex that binds and mediates degradation of complementary RNA sequences [49,55,56].

Virus infection triggers the production of distinct species of sRNAs reflecting the virus from which they originated. With NGS, the sRNA sequences can be enriched and sequenced in order to identify and characterize known and novel viruses using different bioinformatics approaches. In general, sRNA library preparation consists of adapter ligation at both ends (3′ and 5′) of the total RNA, followed by a primer-based RT-PCR [27,63,64]. Amplicons are then gel-purified and used for deep sequencing [64]. The sRNA reads are analyzed similarly to the transcriptome sequencing analysis described above. Due to the short size of sRNA reads, they must be *de novo* assembled. The optimal overlapping length (*k*-mer) required to assemble two sRNAs into a contig can range from ~15 to 19 nucleotides depending on the quality of the sRNA library [9,65]. The *de novo* assembled sequences can then be subjected to BLAST searches against non-redundant databases or viral database to identify novel viruses [27]. The first report of using deep sequencing of sRNAs for virus detection/discovery in arthropods was in 2010, when several novel viruses were identified in sRNA libraries, constructed from *Drosophila*, mosquito and nematode RNAs [9]. Since then, this approach has been extensively used to identify known and novel arthropod viruses, especially insect viruses [7,8,22,27].

Deep sequencing of sRNA pools from either animal or plant hosts, followed by bioinformatics analysis, has shown to favor assembly of viral contigs in less processing time, since the background of long RNAs from the host is lower in those libraries, compared with transcriptome libraries [51]. Furthermore, sRNA libraries require small amounts of RNA input for sample preparation [51]. However, due to short reads lengths obtained in sRNA libraries, the available *de novo* assembly tools have not been highly effective in assembling longer viral contigs. This requires further RT-PCR and sequencing steps to fill gaps in the viral sequences [27,31].

Challenges of using NGS for virus discovery: EVEs and virus validation

NGS-based virus discovery is very powerful, but care must be taken when interpreting the results. One problem encountered by performing NGS approaches for virus discovery is that the identification of viral sequences is normally based on their similarities to known viral sequences available in databases. Hence, new viruses representing new taxa without common motifs or domains (usually RNA-dependent RNA polymerase (RdRP) and structural proteins) [7] shared by different viruses and/or known but very diverse viral genome/segment sequences showing no similarity to available sequences are not detectable by this approach. Also during BLAST analysis, many assembled non-viral contigs often hit available viral genomes because of some sequence similarities, which could give either ambiguous or false-positive viral sequences. Therefore, it is essential to validate the viral sequences identified in BLAST analysis by other experiments.

Another problem is that NGS also may detect sequences from endogenous viral elements (EVEs). Both DNA and RNA viruses occasionally leave viral imprints called EVEs within their host genomes [66–80]. Although most insect EVEs have been identified and studied in mosquitoes [52], recent comprehensive studies have characterized large numbers of integrated ISVs in other insects and other arthropods [8,17,42,81]. The process of endogenization can occur either during replication, in case of endogenous retroviruses (ERVs) [79,80], or for non-retroviral RNA viruses as Non-Retroviral Integrated RNA Virus Sequences (NIRVS) [52,75,82]. The details of

possible routes of integration of NIRVS have been nicely reviewed elsewhere [67,75]. From an evolutionary standpoint, EVEs are extremely valuable pieces of an evolutionary puzzle guiding researchers like a map to the past history of viruses and hosts, and allowing the study of viral evolutionary timescales and dynamics in more detail. However, for virus discovery using NGS approaches care must be taken since some of the EVEs could be transcribed to give long and even sRNAs [83]. Furthermore, recent studies have shown that in some insects viral RNA can be reverse transcribed to viral DNA (vDNA) by cellular reverse transcriptase (RT) from long-terminal repeat (LTR) retrotransposons [84,86]. These vDNAs can occur in both linear and circular non-integrated forms [84,86], and circular vDNAs can be a source of siRNAs which offer partial immunity in future challenge with a related virus(es) [84–86]. Thus, when NGS and bioinformatics analyses identify a viral sequence, further analysis must be done to determine if it represents a replicating virus, an EVE, or even non-integrated vDNA. This could include identification and demonstration of virions and infectivity, or at least demonstrating replication.

Insect-specific virus applications to target insect vectors and pathogens

Many microbes, including viruses, have been and still are evaluated as potential agents for use in controlling specific pests or pathogens. Traditionally, wild-type pathogenic viruses have been considered as agents for potential biological control approaches in insects, and the most widely studied and some are even used commercially are baculoviruses [87]. These will not be discussed here, but it is important to mention that the use of baculoviruses as bio-pesticides has limitations because of their slow killing action, narrow host range and technical challenges for *in vitro* commercial production [88].

In addition to killing their insect hosts, microbes might be used for other goals, particularly for interfering with insect vector transmission of pathogens. There have been and continue to be many efforts in this area with insect vector borne-pathogens of animals [89,90], but with insect vector-borne plant pathogens, this is in its infancy. An important factor to consider when comparing insect vector-borne pathogens of animals vs. plants is that almost all of the animal pathogens also infect their insect vector, and while insect vector-borne bacteria, phytoplasmas and some viruses affecting plants do infect their vectors, a great many of the plant viruses do not. But insect vector-borne plant pathogens still specifically interact with their vectors, and the determinants of the interaction, whether internal in the body or external, offer targets for disrupting vector transmission. Furthermore, the insect vectors of plant pathogens also harbor microbial communities that can be considered as potential tools for targeting specific traits in the vector. With our increased knowledge of the insect virosphere, it is important to consider whether the newly discovered ISVs offer additional opportunities to target insects and/or the pathogens they transmit. ISVs do not replicate in vertebrates and, many have not shown pathogenicity to their insect hosts, thus, ISVs can be considered as members of insect viral communities (viromes). This may suggest that they are well adapted to their insect hosts. Therefore, ISVs might be useful, either as wild-type or engineered viruses, to induce desirable traits in their insect hosts. Examples of desired traits could be: (1) negatively impacting the competence of insect vectors of pathogens[91]; (2) using virus-induced gene silencing (VIGS) to target specific insect RNAs and negatively impact traits such as insect survival, fecundity and behavior [92]; or (3) using recombinant ISVs to induce RNAi defenses against other pathogens vectored by the insect [93,94]. Such efforts are being explored in particular for mosquito vectors of human pathogens [24,90,94,96], but for insect vectors of plant pathogens these represent an unexplored opportunity. We explore and expand on some possible scenarios below.

Choosing and then being able to manipulate ISVs to give positive traits is not trivial. There is not one ISV to fit all approaches and due to the different types of transmission relationships for different insect vector-borne viruses, no one strategy is applicable to all types of vector-borne viruses. Wild-type ISVs offer potential opportunities, particularly for viruses that have circulative relationships with their vectors. One recent example showing the potential for using a wild-type ISV for inducing a desirable trait is with Nhumirim virus (NHUV) and mosquitoes [91]. NHUV is a member of the *Flaviviridae*, but is an ISV that is phylogenetically related to many mosquito-borne flaviviruses that can also infect vertebrates, including West Nile virus (WNV). Co-infection of adult mosquitoes with NHUV and WNV showed interference towards WNV and significantly reduced mosquito vector competence [91]. The authors suggested that the genetic relatedness of the ISV NHUV with WNV allowed for super-virus exclusion (SIE) although how and where this occurred was not shown. Still, NHUV was able to induce a desired trait, and resulted in reduced vector competence in mosquito vectors of WNV.

The above example shows that it is possible to find wild-type ISVs that can be used for specific applications, but it is also very likely that recombinant ISVs could be precisely engineered for specific effects. By definition, if one is to use a recombinant ISV, most likely the recombinant virus will first be produced in plasmids as cloned DNA copies of the virus genome. Specific sequences to give the desired trait are introduced into the cloned DNA. Then either DNA or RNA derived from the plasmid(s) is used to transfect cultured cells to produce virions since infecting whole insects directly with the recombinant DNA or RNA is much more difficult. The virions will then be delivered to the whole insect, either orally or via

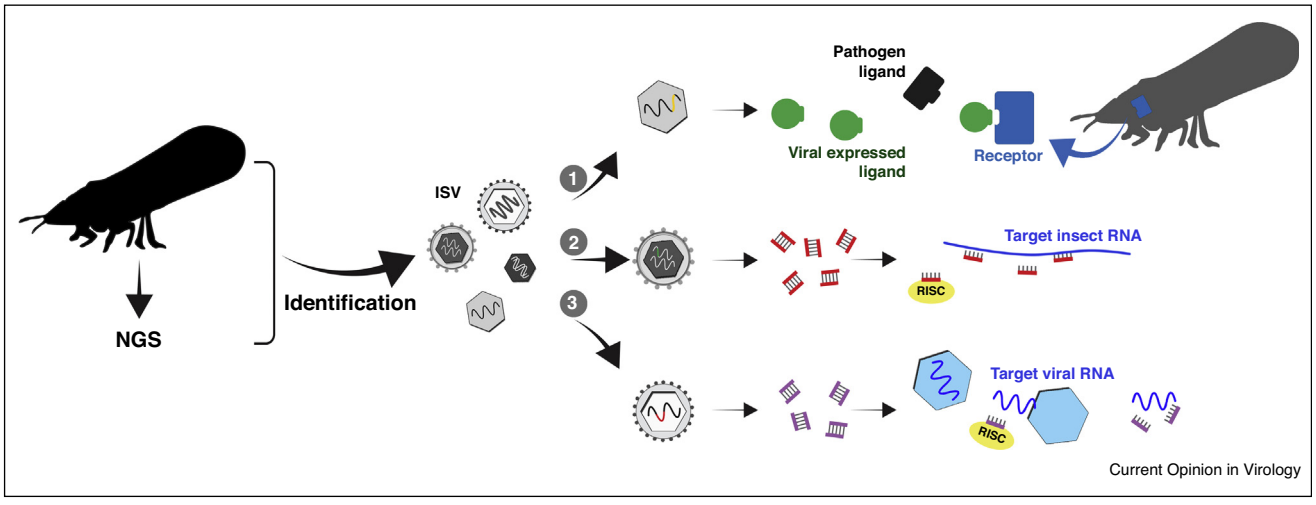
microinjection. This overall approach is conceptually straightforward and applicable to growing engineered ISVs for which insect cell lines are available. However, many ISVs appear to have relatively narrow host ranges. Thus, the common insect cells lines such as *Drosophila* S2, Sf9, or mosquito cells may not be suitable for many ISVs, including those for insect vectors of plant pathogens. For example, we have discovered several ISVs of the Asian citrus psyllid, *Diaphorina citri* [8], but the lack of a suitable cell line makes it very difficult to assess infectivity of *D. citri* recombinant viruses.

It is also not necessarily straightforward to choose which traits might be desirable and which would not lead to strong selection pressure to negate the efficacy of the approach. One example of a desired trait might be to confer vector incompetence to insect vectors, making them unable to, or at least severely debilitated for transmitting a specific pathogen, as was shown for NHUV and WNV above [91]. So wildtype ISVs can confer this trait, however one has to find the appropriate ISV. Conceptually, we can envision approaches whereby one might achieve this goal by using recombinant engineered ISVs.

One approach could be to block receptors within the insect vector that are required for uptake and/or transmission of the target pathogen. If the ligands involved in receptor binding are known, then it might be possible to engineer an ISV to express the ligand which could bind to and saturate receptors thereby interfering with the pathogen gaining entry into cells of the insect vector. The ISV-expressed ligand might then prevent the pathogen from infecting the insect vector rendering it to be non-competent for subsequent virus transmission (Figure 1(1)). Blocking ligand:receptor interactions to disrupt vector transmission of plant viruses has been studied and recently reviewed [96], but using ISVs to achieve this goal represents an unexplored approach. It is worth mentioning that the size of the insertion (encoding the ligand) and its retention by the recombinant ISV are two key factors playing important roles in this approach.

VIGS also could be used to directly target insect RNAs [97]. This could be achieved by using recombinant ISVs engineered to contain and express insect RNA sequences within the infected insect. In this case, the insect RNAi response against the recombinant virus carrying the insect RNA would also target and regulate specific insect mRNAs. If targets were known, then the recombinant ISV could be used to disrupt processes including insect development, fecundity, survival, and also pathogen replication and transmission [92] (Figure 1(2)). However, the corresponding target mRNAs must first be identified, but there is a precedent for this strategy. RNAi was used in plants to target a plant mRNA encoding a host susceptibility factor that was essential for translation of the target virus mRNA, resulting in virus resistance [98]. In addition to targeting insect-mediated virus transmission, this type

Figure 1



Overview of ISV discovery by NGS and potential translational applications of recombinant viruses engineered using the ISVs. Insect at left indicates wild collected sample. RNA (sRNAs or transcriptome) is purified and used for NGS and bioinformatics to identify the insect virome. Potentially many types of ISVs will be discovered and are indicated by the virus particle cartoons. Genetic engineering approaches can then be used to introduce desired sequences (indicated as yellow, green and red segments) into the ISV genome. The engineered ISVs can be inoculated back into the target insect host/vector in order to induce desirable traits upon infection. Three possibilities are indicated: **(1)** negatively impacting the competence of insect vectors of pathogens by using ISV-expressed ligands to saturate receptors important for virus acquisition; **(2)** using VIGS to target specific insect RNAs and negatively impact traits such as insect survival, fecundity, behavior or even virus susceptibility; and **(3)** using the recombinant ISV to induce RNAi defenses against other pathogens vectored by the insect.

of approach could also be used towards transmission of prokaryotic plant pathogens.

Another approach to interfere with vector competence is to make the insect vector resistant to the target virus. This could be done by using a recombinant ISV engineered to express a sequence of the target virus (Figure 1(3)), and conceptually could be used towards viruses that have propagative transmission relationships with their insect vectors. When the insect responds by RNAi to the recombinant ISV, this would induce RNAi activity also targeting the inserted sequence which could confer immunity against the target virus. This has been achieved experimentally in mosquitoes, using recombinant Sindbis virus [94], but not yet with ISVs.

The above discussion presents conceptual approaches, but obviously there are many other considerations and technical difficulties to overcome. For example, how to deliver the recombinant virus, how effectively will it spread among the population, and will it maintain the desired trait long enough to be effective? These are important questions that need to be addressed. ISVs already are widespread in natural insect populations. If wild-type forms of the recombinant virus to be used are already widespread among the insect host population, then it may not be possible to introduce and spread the recombinant ISV in the population due to superinfection exclusion. Superinfection exclusion means that if a host is already virus infected, it cannot be infected by closely related variants of the same virus. However, in our own analysis of worldwide populations of the Asian citrus psyllid, *Diaphorina citri*, and the incidence of *D. citri* ISVs [8], we found that most of the viruses were not universally distributed (Table 1). Some viruses were more widespread than others, thus, choosing and using the correct ISV will be critical.

Table 1

Insect-specific viruses[a] of *Diaphorina citri* detected from worldwide sampling areas. The plus sign indicates the presence of indicated viruses in the respective samples.

Virus[b]	China	Taiwan	Hawaii[d]	Brazil	California	Florida	Texas	Pakistan
DcRV	+	+	+			+	+	
DcPLV	+	+		+				
DcDNV	+	+						+
DcBV	+	+						
DcACV	+				+	+	+	
DcFLV						+		
CLas[c]	+	+	–	+	+	+	+	+

[a] These data are taken from Nouri *et al.* [8].
[b] DcRV, *Diaphorina citri* reovirus; DcPL, *D. citri* picorna-like virus; DcDNV, *D. citri* densovirus; DcBV, *D. citri* bunyavirus; DcACV, *D. citri*-associated C virus; DcFLV, *D. citri* flavi-like virus.
[c] *Candidatus* Liberibacter asiaticus is a bacterium that also infects *D. citri*, and is the causal agent of Huanglongbing disease of citrus.
[d] The incidence of DcRV in Hawaiian *D. citri* analyzed by us is almost 100%.

Another potential problem is the mode of transmission and maintenance of ISVs in nature. Little is known but many ISVs appear to not efficiently spread horizontally to naïve individuals in the natural population [90]. Rather, they appear to be primarily spread vertically via transovarial transmission from infected females to progeny. However, this is not true for all ISVs, for example some such as the *Rhopalosiphum padi* virus (RhPV; *Dicistrovirus*) can be spread from one aphid to another while they are feeding on separate parts of the same plant [99]. Thus, RhPV moves through, but does not replicate within the plant and the plant then can be a source of the ISV.

Another valid concern is whether or not it is a sound strategy to use recombinant viruses in the environment. Surely not all viruses should be considered for this strategy, and care must be taken to ensure host range specificity among other things. But this also must be considered in the context of virus prevalence and what is already being done in other systems. Using recombinant viruses in the environment is not novel, recombinant viruses are already used in nature for inducing desirable traits in target vertebrates. Recombinant vaccinia virus engineered to express the Rabies virus (RV) G protein is widely used in baits to infect and immunize wild RV carriers such as raccoons, skunks, and foxes. By 1995, millions of doses have been distributed in the environment in parts of Europe and in the United States [100]. This very effective strategy continues to be used even in national parks such as Yellowstone, and therefore the concept of using recombinant viruses to induce desired traits is not without precedent. The last, but not least concern with using recombinant viruses is that over time they often lose the inserted sequence [101]. If this were to happen, the virus would only return to the wild-type form, but its application would be lost.

Conclusions

By taking advantage of NGS and advanced bioinformatics tools, our understanding about the diverse nature of insect-associated viruses (including ISVs and viruses vectored by insects to other hosts) has changed dramatically. Interestingly, many of these viruses lead to persistent infections without obvious pathogenic effects on the insect host, and these ISVs may be considered as natural components of the insect virome. Describing new ISVs from different insect taxa not only gives us new insight into the insect virome in general, but also provides new potential biological candidates which might be useful in combating insect vectors or the pathogens they transmit. Herein we present concepts and in some cases evidence as to how ISVs might be used for targeting of insect vectors and their ability to transmit viruses and other pathogens to plants and animals. Admittedly, we speculate on how some of this may be achieved, but some experimental approaches are already underway with mosquito-transmitted viruses. Our intent is to stimulate new

research efforts, particularly among plant insect vector biologists, which take advantage of ISVs and contemporary technologies. Such investigations will for sure provide valuable new scientific information, but may also offer opportunities to take advantage of the still unknown properties of ISVs for insect vector and pathogen control.

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