

## THE IMPORTANCE OF INSECT VIROMES: HUMAN HEALTH AND BEYOND

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### SUMMARY

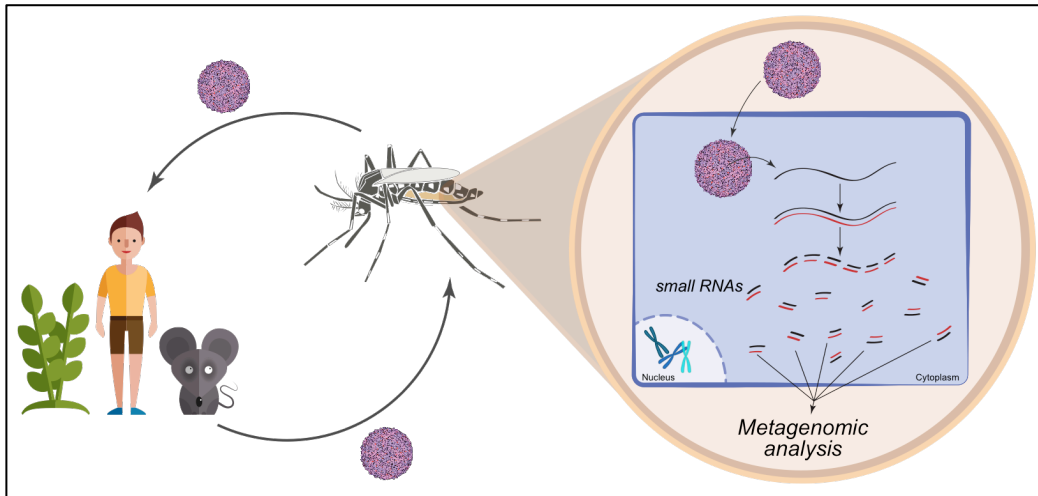
Insects are the most abundant group of multicellular organisms and carry an incredible diversity of viruses. As such, insects can be seen as major reservoirs of viral biodiversity that can also be transmitted to other hosts. Therefore, the collection of viruses (e.g. virome) circulating in insects is of special interest. For example, we have seen many recent worldwide outbreaks by viruses transmitted by mosquitoes, such as Dengue and Zika. In addition, other components of the insect virome might not directly infect other hosts but could affect transmission of arboviruses by modulating vector competence. Finally, characterizing the incredible diversity of insect viruses could lead to the discovery of novel genes that can be tapped for biotechnological applications. Considering the importance of insect viromes, our group has used small RNA-based metagenomic approaches to identify and characterize viruses in different insects. This strategy has important advantages such as the fact that viral sequences are naturally enriched in the small RNA fraction of insects. In addition, the size distribution of small RNAs allows classification of viral sequences independent of homology searches against reference databases. Small RNA based metagenomics has greatly improved our ability to detect and identify novel viruses in insects thus allowing very detailed surveillance of insect viromes.

### INTRODUCTION

Viruses are obligatory intracellular pathogens that are fully dependent on the host machinery to replicate (*Marsh and Helenius, 2006*). These pathogens are found in all branches of the tree of life, from bacteria to higher eukaryotes (*Lauring et al., 2013*). Viruses are remarkably diverse, varying in nucleic acid content, genome structure and organization. Viral genomes can be DNA or RNA, single or double stranded, linear or circular containing one or multiple segments (*Edwards and Rohwer, 2005*).

Insects are reservoirs of an incredible diversity of viruses that is just beginning to be uncovered (*Aguiar et al., 2015; Li et al., 2015; Shi et al., 2016*). From a basic biology point of view, insect viruses help to understand the evolution of animal viruses. From an applied perspective, this immense viral biodiversity could also lead to the discovery of novel genes that could be tapped for biotechnological purposes.

Viruses that circulate in insects also include an important group of arthropod borne viruses (arboviruses) that



**Figure 1:** Insects are major reservoirs of viruses. Insects are the most abundant multicellular organisms. Viruses circulating in insects represent the majority of the viral biodiversity in multicellular organisms at any given point. In addition, insects are able to directly transmit viruses that can infect plants, humans, and other vertebrate animals. Therefore, characterizing viruses in insects is of great importance for basic biology as well as for human health. Metagenomic strategies allow a general overview of the collection of organisms found in any sample. In insects specifically, small RNA based metagenomics is a sensitive approach to identify viruses since virus-derived small RNAs are commonly found in insects. These virus-derived small RNAs are generated by processing of viral RNAs by the host antiviral response and can be used to reconstruct virus sequences.

can be transmitted to other hosts (Figure 1) (Liang et al., 2015). Arboviral infections still represent a major threat to humans, livestock and agriculture (Shepard et al., 2013; Cargnelli et al., 2014; Diez-Domingo et al., 2014; Redinbaugh and Zambrano, 2014). We highlight recent increases in human infections by arboviruses, such as Zika, Dengue and Chikungunya virus (Vijayakumar et al., 2013; Carrington and Simmons, 2014; Diallo et al., 2014). Despite their importance, we still lack effective vaccines or

treatments for diseases caused by most of arboviruses. Monitoring of viruses circulating in vector insects is still an important strategy to prevent outbreaks. Constant surveillance allows early detection of viruses that could eventually cause outbreaks (Gubler, 2001; Parrish et al., 2008).

For reasons highlighted above, characterization of the collection of viruses in insects (e.g. the virome) is extremely important. In this scenario, metagenomics has been an important tool to study viral diversity.

### SURVEILLANCE OF INSECT VIROMES UTILIZING METAGENOMIC STRATEGIES

Metagenomics allow broad analysis of genetic material within a sample without any previous knowledge. Different metagenomic strategies have been suc-

cessfully applied to virus discovery based on both DNA and RNA sequencing with their own advantages and limitations (Kreuze et al., 2009; Yamao et

al., 2009; Webster et al., 2015; Hang et al., 2016). DNA sequencing tends to bias the identification to microorganism that have DNA at some stage of their life cycle, which is not the case of all viruses (Venter et al., 2004; Edwards and Rohwer, 2005). In contrast, RNA sequencing is a more unbiased approach since all viruses generate RNA during their replication cycle. Many studies have taken advantage of RNA sequencing as proxy to infer presence of viruses in diverse organisms (Kreuze et al., 2009; Zhuang et al., 2014; Li et al., 2015; Webster et al., 2015). These studies have relied on sequencing of long or small RNA fractions derived from infected hosts. Interestingly, while long RNAs represent direct products of the viral replication cycle, small RNAs are a result of the antiviral response. Indeed, antiviral mechanisms commonly target exposed viral RNAs that are further degraded to generate virus-derived small RNAs.

Previous work from our group indicated that small RNAs are enriched for viral sequences in comparison to long

RNAs (Figure 1) (Aguiar et al., 2015). We showed that small RNAs also allow identification and classification of viral sequences through pattern-based analyses independent of homology to known references (Aguiar et al., 2016). The requirement for sequence similarity comparisons is a major limitation of metagenomics studies especially considering that viruses have high mutation rates. Identification through sequence homology searches requires considerable similarity to previously identified viruses. In order to overcome this limitation, sequence-independent strategies may increase chances of identifying highly divergent viruses. Since small RNAs are products of specific host antiviral pathways, they show unique molecular signatures that can be used to infer their origin (Aguiar et al., 2016). Unfortunately, small RNA pattern-based analyses are mainly limited to organisms and conditions where there is a functional antiviral RNAi pathway. Absence or inhibition of the RNAi pathway may make it difficult to perform pattern-based analyses.

## THE IMPORTANCE OF STUDYING THE DIVERSITY OF VIRUSES CIRCULATING IN INSECTS

Insects are important hosts for viral infection since they are the most abundant group of multicellular organisms. It is becoming apparent that the collection of viruses found in insects is highly diverse (Li et al., 2015; Shi et al., 2016). The collection of viruses circulating in insects includes most viral families circulating in animals (Shi et al., 2016). Therefore, studies on insect viromes can provide important information about viral biodiversity and evolution. The characterization of this incredible biodiversity in insects could also lead to the discovery of novel

genes with potential biotechnological applications.

In addition, insects are major reservoirs of viral biodiversity that can be transmitted to plants, humans and other vertebrate animals thus threatening agriculture, livestock and public health (Shepard et al., 2013; Cargnelutti et al., 2014; Diez-Domingo et al., 2014; Redinbaugh and Zambrano, 2014). More than 100 different human arboviruses have been described although this is likely not to be the final number (Gubler, 2001). Of note, we have also seen recent worldwide outbreaks by

different mosquito-borne viruses including *Dengue virus* (DENV), *Chikungunya virus* (CHIKV) and *Zika virus* (ZIKV) (Vijayakumar et al. 2013; Carrington and Simmons, 2014; Diallo et al., 2014). In addition to the human and social impact, the economical burden of insect borne viruses is enormous. Only in the Americas, between 2000 and 2007, the estimated economic impact of Dengue was of approximately US\$ 2.1 billion annually (Shepard et al., 2011).

The study of insect viromes can

directly impact public health by providing insights into the circulation of arboviruses and help prevent outbreaks. Nevertheless, it is important to note that insects also carry a large diversity of viruses that are unlikely to directly infect vertebrate hosts (Bolling et al., 2015). These insect specific-viruses are not infectious to other organisms but may indirectly modulate the transmission of arboviruses by affecting vector competence (e.g. the ability of the insect to function as a vector).

## CONCLUSION

In summary, we have highlighted here the importance of characterizing insect viromes and how it can impact human health, biotechnology and evolutionary biology. New strategies such as small RNA based metagenomics have impacted the characterization of viruses circulating in insects. Studies on insect viromes are central to the

understanding of viral evolution since insect viruses are the largest and most diverse group in multicellular organisms. In addition, knowledge on the circulation of viruses in insects would help prevent outbreaks. Finally, these diverse insect viruses could lead to the discovery of novel genes with potential use in the biotechnology industry.

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