The Insect Virome: Opportunities and Challenges

Bryony C. Bonning*

Department of Entomology and Nematology, University of Florida, Gainesville, FL, USA.

*Correspondence: bbonning@ufl.edu

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Abstract

The insect virome is composed of a myriad of viruses. Both field populations and laboratory colonies of insects harbour diverse viruses, including viruses that infect the insect itself, viruses of microbes associated with the insect, and viruses associated with ingested materials. Metagenomics analysis for identification of virus-derived sequences has allowed for new appreciation of the extent and diversity of the insect virome. The complex interactions between insect viruses and host antiviral immune pathways (RNA interference and apoptosis), and between viruses and other members of the microbiome (e.g. *Wolbachia*) are becoming apparent. In this chapter, an overview of the diversity of viruses in insects and recent virus discovery research for specific insects and insect-derived cell lines is provided. The opportunities and challenges associated with the insect virome, including the potential impacts of viruses on both research and insect management programmes are also addressed.

Introduction

The ubiquity and abundance of viruses in the environment have long been recognized. In recent years, the analyses of virus sequences identified from metagenomics data are changing our perception of virus evolution and the roles played by viruses in organismal biology (Zhang *et al.*, 2018). Our rapidly expanding knowledge of the insect virome is no exception. While the foundational knowledge of the insect virome was based on characterization of viruses that caused distinct phenotypes using conventional approaches (Liu *et al.*, 2011), high throughput sequencing has allowed for the identification of sequences from viruses that are asymptomatic, covert or latent, and of virus sequences incorporated into the host genome (Varghese and van Rij, 2018). In this new era of virus discovery, genomic technologies are used for identification of virus-derived sequences, with examination of virus phenotype only conducted for viruses of particular interest.

While virus sequences can provide valuable insight into evolutionary processes, additional experimentation is essential to confirm the presence of an actual virus, rather than just virus-derived sequence. Such validation would include filling of sequence gaps and sequence confirmation for a putative virus genome, testing for virus replication (dsRNA detection for RNA viruses, small RNA sequencing), visualization of virus particles, and virus isolation for infectivity tests in host insects or cell lines. Many of the viruses from metagenomics analyses described below are based on sequence data alone and therefore represent putative viruses. While a case has been made for the International Committee on Taxonomy of Viruses to include the sequences of putative viruses in their classification for the purposes of sequence-based taxonomy (Simmonds *et al.*, 2017), maintenance of a clear distinction between validated viruses, virus-derived sequence and putative viruses is important for the integrity of the field.

Transcriptome sequencing can be used for initial characterization of an insect virome for detection of virus-derived genome- (for RNA viruses) or transcript-sequences (for RNA and DNA viruses). The use of a single round of polyA purification (rather than the more typical two rounds) increases the likelihood of detection of RNA viruses that lack polyA tails (Liu *et al.*, 2016). Virus-derived sequences incorporated into the genome of the host insect (which may or may not represent an active virus) can be identified from insect genome sequences. Sequencing of small RNA (sRNA) libraries provides greater sensitivity for identification of virus-derived sRNA against the host RNA background (Wu *et al.*, 2010), as does virus-particle purification followed by deep sequencing. No single method will provide an exhaustive overview of the insect virome.

The invertebrate virome

The greatest increase in our knowledge of virus diversity as a result of metagenomics has been in the invertebrates (Webster *et al.*, 2015; Shi *et al.*, 2016a), with arthropods, representing 80% of all known animal species, being a primary component (Shi *et al.*, 2016b). The abundance of RNA viruses is consistently higher in invertebrates than in vertebrates, with apparent tolerance by invertebrates of significant virus loads. Analysis of viral sequences identified from 220 invertebrate species highlighted the dynamic nature of the virus genome with frequent gene loss or gain, genomic rearrangement, recombination and lateral gene transfer between viruses and hosts (Shi *et al.*, 2016a). Some RNA viruses of invertebrates were found to be ancestral to those of vertebrates (Marklewitz *et al.*, 2015). Similarly, in a study focused on viruses with negative-sense RNA genomes, sequencing of 70 arthropod species resulted in identification of sequences derived from 112 novel viruses including those ancestral to viruses that cause disease in plants and vertebrates (Li *et al.*, 2015). This study highlighted the key role of arthropods as reservoirs for virus recombination and genetic exchange resulting in virus genome evolution.

Abundance of RNA viruses

In addition to allowing for identification of virus-derived sequence, metagenomics provides a measure of relative virus abundance based on the proportion of virus-derived transcripts (excluding rRNA) that map to a given virus. This measure can provide an indication of whether the host organism from which the virus sequence was isolated is likely to be a host for the virus, rather than being associated with food material or host associated microorganisms. The validation of a given virus, and assignment to a particular host requires careful additional analysis as described previously however (Carrillo-Tripp *et al.*, 2015).

The diversity of viruses in insects

For any insect population, whether field or laboratory colony-derived, a transcriptomic analysis will reveal a plethora of virus-derived sequences. These sequences will include those derived from *bona fide* insect viruses (Table 1.1), viruses of microorganisms associated with the insect of interest, and viruses of ingested materials including plants, microorganisms, and those found in animal blood for haematophagous insects. The greatest diversity of virus types in the insects shown in Table 1.1 is for drosophilids, which may simply reflect extensive study of this group. Even this list is likely to be incomplete however with some viruses underrepresented due to limitations in methodology (see below). Overall, insect virus discovery results support the observation of a preponderance of positive-sense RNA viruses in eukaryotes (Koonin *et al.*, 2015).

In addition to viruses present as distinct entities, virus-derived sequences may originate from the host genome itself. Virus-derived sequences are abundant in arthropod genomes originating either from endogenized DNA virus genomes (see Chapter 8), or from partial genome sequences (Suzuki *et al.*, 2017; see also Chapter 2). Some endogenized virus-derived sequences function in antiviral immunity (Goic *et al.*, 2013, 2016; Poirier *et al.*, 2018) (Fig. 1.1). The presence of virus-derived sequences in the host genome (endogenous viral elements, EVEs) may contribute to the variation in susceptibility to infection between populations [e.g. Israeli acute paralysis virus of honey bees (Maori *et al.*, 2007)], and has important implications for arbovirus transmission (Schultz *et al.*, 2018).

The discovery of virus-derived sequences from insects provides opportunities for (1) insight into both virus diversity and virus evolution, (2) knowledge of the pervasive presence and dynamic nature

Table 1.1 Diversity of viruses associated with insects

Genome	Virus	*Drosophila* spp.	Mosquito	Honey bee
dsDNA	*Baculoviridae*		×	
	Entomopoxvirinae			
	Iridoviridae		×	
	Nudiviridae	×		
	Polydnaviridae			
	Ascoviridae			
	Hytrosaviridae		×	×
ssDNA	*Parvoviridae*		×	
	(*Densovirinae*)			
	Bidnaviridae	×	×	
	Circoviridae		×	
dsRNA	*Retroviridae*	×		
	Cypovirus			
	Reoviridae	×		
	Partitiviridae	×	×	
	Birnaviridae	×	×	
(-)ssRNA	*Plasmaviridae*	×		
	Bunyavirales		×	×
	Rhabdoviridae	×	×	×
(+)ssRNA	*Dicistroviridae*	×	×	×
	Iflaviridae	×	×	×
	Caliciviridae			
	Permutotetraviridae	×		
	Flaviviridae	×	×	×
	Negevirus	×		
	Nodaviridae	×		
	Nora virus	×		
	Unclassified RNA viruses	20		
Various	Unclassified DNA viruses	1		

Viruses associated with insects include viruses with single-stranded (ss) or double-stranded (ds) genomes comprised of either DNA or RNA. ssRNA viruses have positive (+) or negative (–) sense genomes. Shown is an indication of the breadth of virus diversity found in *Drosophila* spp., mosquito species and in the honey bee, *Apis mellifera*, at the time of writing. Not represented in the table are viruses associated with these insects that are known to infect plants, vertebrates or microorganisms.

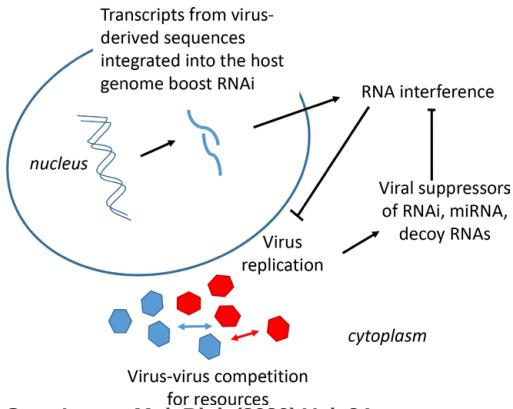

Figure 1.1 Multiple forces drive the insect virome. The outcome of infection by a given virus depends on multiple factors including competition with other viruses present in the host, and interaction with the RNAi pathway. Antiviral immunity may be boosted as shown by endogenous viral elements (EVEs) derived from the virus. An infecting virus may benefit from a strong suppressor of RNAi produced by other resident viruses. Not depicted are the potential impacts of the presence of other microbes, and other components of the immune system.

of virus incidence, (3) information on virus–virus and virus–microbe interactions. Distinguishing sequences derived from viruses that infect the insect under study, from those derived from associated organisms or ingested materials, and from virus-derived EVEs, presents a challenge. The abundance of a given virus-derived sequence is not sufficient to confirm the presence of a replicating insect virus, and additional analyses and experimentation are commonly required for this. Consequently, appropriate naming of viruses identified from metagenomics analysis can be confounded by the lack of knowledge of the host of a given virus.

In the sections below, an overview of the virus diversity for specific insects is provided, for (1) *Drosophila* spp., representing an important model organism in addition to economically important pest species, (2) mosquitoes including species of significant public health importance, and (3) the honey bee, *Apis mellifera*, a beneficial insect of particular importance for pollination services.

The *Drosophila* virome

As a model organism for numerous fundamental physiological systems including antiviral immunity (Huszar and Imler, 2008), *D. melanogaster* is likely the most closely studied of all insects. The power of metagenomics was underscored by the discovery of more than 20 new putative RNA viruses and a new DNA virus (nudivirus) of *Drosophila melanogaster* (Webster *et al.*, 2015, 2016). Analysis of more than 2000 individual wild-caught adult flies showed that more than one third carried detectable virus, with 6% of individuals testing positive for multiple viruses (Webster *et al.*, 2015). This study demonstrated the abundance of viruses in field populations and highlighted the disparity between the virome of field versus laboratory populations of insects: Only a sub-set of the newly discovered viruses was widespread in laboratory colonies, and notably also ubiquitous in cell culture. Characterization of the *D. melanogaster* virome provides an important basis providing an ecological and evolutionary context for use of this species as a model for virus research.

For this work, small RNA was sequenced as a complementary approach for virus sequence discovery. During active virus replication, dsRNA produced as a replication intermediate for RNA viruses, or resulting from RNA secondary structure, is processed by the enzyme Dicer-2 (Dcr2) into small interfering RNA (siRNA). These siRNA are bound by Argonaute (Ago) proteins for use in the RNA interference (RNAi)-mediated, antiviral immune pathway (Ding, 2010; Vijayendran *et al.*, 2013; Mongelli and Saleh, 2016). Research in *Drosophila* demonstrated that transcripts from virus-derived DNA can feed into the RNAi pathway and be processed into siRNA as a means to boost antiviral immunity (Goic *et al.*, 2013; Poirier *et al.*, 2018).

The presence of siRNA that spans the virus genome provides evidence for replication of viruses identified by metagenomics analysis, on the basis that only actively replicating viruses would be processed into siRNA derived from across the viral genome (Wu *et al.*, 2010; Webster *et al.*, 2015). Assembly of siRNAs resulted in identification of novel 'siRNA candidate' viruses although their classification remains to be determined.

Interestingly, the siRNA derived from *Twyford virus*, an iflavirus with a positive-sense ssRNA genome, differed from those of other positive-sense ssRNA virus-derived siRNA, in being negative strand biased, and 22 to 23 nt rather than 21 nt in length. Most of these siRNA also had uridine at the 5′ end, a feature more typical of piRNA. These factors suggest that *Twyford virus* RNA is not processed by *Drosophila* Ago2-Dcr2. The authors suggest that the virus may infect a eukaryotic commensal of *Drosophila* rather than the fly itself, or that a novel pathway is involved in siRNA production (Webster *et al.*, 2015). This case highlights the potential use of siRNA profiles to discriminate between viruses of the target insect versus associated organisms or ingested material.

Combined with a metagenomic analysis of various CO_2-sensitive *Drosophila* spp. for identification of rhabdoviruses, some 85 viruses have now been identified from Drosophilidae (Longdon *et al.*, 2015; Webster *et al.*, 2016). By far the majority of these are RNA viruses. Viruses with positive-sense (+)ssRNA, genomes predominate (50%), followed by those with dsRNA (28%) and negative-sense (−)ssRNA (21%) genomes (Webster *et al.*, 2016). Despite this abundance of drosophilid viruses, some viruses may not be represented due to low abundance (below detection levels), the use of polyA purification of mRNA with associated bias against viral RNAs (such as those from *Nodaviridae*)

that lack a polyA tail, and the potential impact of virulent viruses on flight resulting in underrepresentation in collection traps.

It is interesting that only three DNA viruses have been identified from *Drosophila* spp. Given that transcripts derived from DNA viruses can readily be identified in the transcriptomes and small RNA libraries from other insects, it is possible that DNA viruses are underrepresented in the *Drosophila* virome, rather than underrepresentation due to technological limitations.

From these studies, several observations were made including (1) more closely related host insects share more viruses, (2) many viruses infect more than one host and are widely distributed, (3) few viruses are common, many are rare. Only three of 16 viruses surveyed by PCR exceeded 50% prevalence, with most only exceeding 10% prevalence in a few of the populations surveyed.

The mosquito virome

The viruses of mosquitoes have also received considerable attention on account of the role of mosquitoes in disease transmission. In addition to the arthropod-borne viruses (arboviruses) transmitted by mosquitoes to humans and animals, many other viruses are associated with the mosquito vector, including insect-specific viruses that are also members of the arbovirus families (Halbach *et al*., 2017; Roundy *et al*., 2017). A primary question has been whether the presence of other viruses in the mosquito vector impacts the ability of the vector to transmit disease (Parry and Asgari, 2018). Given the wide variation in virus infection levels, dependent in part on the efficacy of the viral suppressor of RNAi (VSR) (Nayak *et al*., 2010; Guo *et al*., 2018), and direct competition for resources between closely related viruses (Carrillo-Tripp *et al*., 2016) (Fig. 1.1), the biological impact of resident mosquito viruses on arbovirus transmission has been difficult to pin down. Practical applications of mosquito-specific viruses include their potential use as biological control agents for suppression of mosquito populations, or as novel vaccine agents (Bolling *et al*., 2015).

As mosquitoes are both haematophagous and nectar feeding, they are potentially exposed to a greater diversity of viruses than is typical (i.e. from both animals and plants), and surveillance is important to monitor for emerging zoonotic disease. Of viral sequences identified from four mosquito species in Hubei, China, the majority (88%) were attributed on the basis of sequence homology as insect viruses, with vertebrate (3.6%), plant (0.8%), bacteriophage (1.87%) and mycovirus (0.03%) also represented (Shi *et al*., 2015). Sequences derived from insect viruses represented the vast majority of those identified in *Armigeres subalbatus*, about half of those identified from *Culex tritaeniorhynchus*, while the majority from *Anopheles sinensis* were derived from vertebrate viruses (*Anelloviridae* and Parvovirinae).

Mosquitoes in the genus *Culex* include some of the most important vectors of human pathogens. Analysis of culicine species from California, USA, resulted in the identification of 32 novel virus genomes (Sadeghi *et al*., 2018). The total estimated number of virus families represented in *Culex* in California is 21, with several additional unclassified DNA and RNA viruses. These included six viruses with (−)ssRNA genomes (*Bunyavirales* and *Rhabdoviridae*), and 12 with (+)ssRNA genomes (*Dicistroviridae*, *Iflaviridae*, *Flaviviridae*). In addition, sequences derived from four virus families only known to infect plants were also identified, again highlighting the potential for misinterpretation of host species based on metagenomics data, without careful analysis.

The honey bee virome

Given the economic importance of the honey bee, *Apis mellifera*, which provides both pollination services for diverse crops, in addition to hive-derived products, honey bee pathogens have been extensively studied. One of the earliest studies to reveal the extent of microorganisms associated with a given insect was a metagenomic analysis conducted to identify pathogens associated with the so-called colony collapse disorder of the honey bee, *Apis mellifera* (Cox-Foster *et al*., 2007). Seven viruses with (+)ssRNA genomes were identified in this study with *Israeli acute paralysis virus* (IAPV) identified as a significant marker of CCD. Subsequent research highlighted that multiple factors are involved with colony collapse, and also the dynamic nature of the honey bee virome with weekly and monthly variation in viruses detected (Runckel *et al*., 2011). Although the situation among social insects such as

the honey bee is rather unique, with frequent contact and opportunity for virus exchange both within and between hives, the number of viruses identified that are associated with the well-studied honey bee is likely to be representative. Around 30 viruses infect or are associated with honey bees (Chen and Siede, 2007; Remnant *et al.*, 2017), most of which are positive-sense RNA viruses in the families *Dicistroviridae* and *Iflaviridae* (de Miranda *et al.*, 2010; de Miranda and Genersch, 2010).

The spread of the *Varroa* mite, which serves as a vector for several viruses of the honey bee has exacerbated colony losses (Wilfert *et al.*, 2016; Ryabov *et al.*, 2017). In particular, the *Varroa* mite alters the honey bee virome increasing the prevalence of a specific strain of *Deformed wing virus* (DWV) (Martin *et al.*, 2012), and decreasing the abundance of other viruses (Roberts *et al.*, 2018). Recent analysis of honey bee colonies from Europe, Africa and the Pacific resulted in genomic evidence for seven viruses associated with honey bees (Remnant *et al.*, 2017). Notably, sequences derived from four viruses with negative-sense ssRNA genomes were identified, although based on sequence similarity, one of these (ABV-1) is likely to infect protozoan parasites of the honey bee rather than the honey bee itself. This analysis included *Apis mellifera capensis* from South Africa and the Pacific islands of Tonga that are resistant to the *Varroa* mite on the basis that high abundance of *Varroa*-associated viruses in colonies elsewhere may have outcompeted other viruses resulting in reduced virus diversity. This study reported the first sequence-based evidence for a flavivirus of honey bees (Remnant *et al.*, 2017). Along these same lines, sequences derived from 42 putative new viruses were identified from honey bees in Australia, which is *Varroa*-free (Roberts *et al.*, 2017, 2018). Virus-derived sequences included those from 11 putative new dicistroviruses, 11 putative new iflaviruses and 20 other putative new small RNA viruses (Roberts *et al.*, 2018). Sequences from viruses associated with drosophilids and hemipterans were also identified, although no validation was presented.

Sequencing of siRNA derived from the honey bee provided support for replication of two newly identified rhabdoviruses in the honey bee, with the typical 21 or 22 nt siRNA derived from both positive and negative-sense RNA strands (Remnant *et al.*, 2017). In contrast, siRNA derived from these same viruses in the mite were predominantly 24 nt from the negative-sense RNA, with a range of siRNA sizes derived from the less abundant positive strand (Remnant *et al.*, 2017). This study provides another example of the potential use of siRNA to address the host organism for a given virus, although whether these rhabdoviruses replicate in the mite remains to be confirmed.

Implications of virus abundance in field populations

The use of viruses for biological control

The examples above provide an indication of both the diversity and abundance of viruses in insect populations in the field. Some viruses naturally regulate insect populations, in some cases resulting in dramatic epizootics under either field or colony-based conditions (Szelei *et al.*, 2011; Myers and Cory, 2016). Indeed, some of these viruses have been employed for pest management purposes with lepidopteran-specific nucleopolyhedroviruses (*Alphabaculovirus* spp.) and granuloviruses (*Betabaculovirus* spp.) being produced commercially, along with a few additional viruses registered for small scale production in China (Lacey *et al.*, 2015). In these cases, the level of exposure to the viral control agents was sufficient to overcome any potential for competition by viruses resident in the targeted pest insect. In broad terms, viruses use different ecological strategies: Those that are relatively virulent (e.g. the paralytic dicistroviruses – *Cricket paralysis virus*, *Israeli acute paralysis virus*) infect multiple hosts and rely primarily on horizontal transmission (i.e. among individuals of the same generation). In contrast, viruses with low virulence without obvious negative fitness consequences, tend to be restricted to a single or few closely related host species, with a greater propensity for vertical transmission (i.e. from mother to offspring; e.g. *Drosophila C virus*) (Bonning and Miller, 2010). This latter strategy is consistent with long-term adaptation a given host.

The use of dsRNA for pest suppression

The use of dsRNA for pest suppression through RNAi-mediated silencing of essential genes in a

target insect has been adopted as an alternative strategy for pest management (Baum *et al.*, 2007; Zhang *et al.*, 2017). A pending question is whether the presence of myriad RNA viruses within a given insect pest will have any impact on the efficacy of dsRNA used for suppression of damaging populations, given that RNA viruses commonly encode suppressors of the host RNAi pathway. As these suppressors target different components of the RNAi pathway (dsRNA, Dcr2, Ago2) (van Mierlo *et al.*, 2014), suppression mediated by one virus will impact the RNAi-mediated antiviral defence against other viruses present in the host. For viruses that result in chronic infection, an equilibrium is maintained between the VSR and the host RNAi pathway. Viruses with highly efficient VSR cause acute infection (Nayak *et al.*, 2010). Based on published reports, the expression levels of dsRNA by transgenic crop plants are sufficient to overcome the impact of VSR from viruses resident in the targeted pest (e.g. Baum *et al.*, 2007), which vary considerably in diversity and abundance.

In parallel with the adoption of RNAi for insect pest management, attention has turned to the use of small RNA viruses as potential delivery systems for silencing RNAs to specific crop pests. As these viruses have evolved to overcome the enzymatic challenges faced by dsRNA on exposure to the saliva or gut milieu, there is potential for use of the viral particle as a protective dsRNA delivery system. To this end, viruses have been identified in soybean aphid (Liu *et al.*, 2016; Feng *et al.*, 2017), western corn rootworm (Liu *et al.*, 2017a–c), stink bugs (Liu *et al.*, 2015) and leafhoppers (Chen *et al.*, 2015) among others. The availability of suitable, virus-free cell lines for development of infectious clones of viruses of such crop pests presents a significant limitation however. Along these same lines, a proof of concept study demonstrated the utility of *Flock House virus* as a virus-induced gene silencing (VIGS) vector for delivery of dsRNA to insect cells (Taning *et al.*, 2018).

Alteration of immune response

An additional implication of abundant viruses in field populations of insects is the potential for alteration of the immune response to other pathogens. Conversely, the presence of other microorganisms may impact antiviral immunity. *Wolbachia* has been shown under laboratory conditions to negatively impact RNA viruses of *Drosophila* (Hedges *et al.*, 2008; Teixeira *et al.*, 2008; Martinez *et al.*, 2017). However, no correlation was detected between the presence of any virus or infection level with *Wolbachia* infection in field caught populations (Webster *et al.*, 2015). This result may reflect the limits of relatively small sample sizes or may reflect more complex interactions when multiple viruses are present in a given host (Webster *et al.*, 2015). A more recent study of *D. melanogaster* field populations with or without *Wolbachia* (*w*Mel) in Australia supported this idea, with no impact of *w*Mel on viral abundance for any of the nine families and floating genera present (Shi *et al.*, 2018). However, the viruses in which *w*Mel-associated reduction is the greatest, were absent (*Drosophila C virus*), or at low abundance (Nora virus) in only one dataset.

The presence of diverse viruses in wild populations may have influenced the antiviral impact of *Wolbachia* in field populations of *Drosophila* that was expected based on laboratory studies (Webster *et al.*, 2015). Indeed, the detailed molecular interactions between resident viruses and bacteria is an area of study ripe for investigation with our increased awareness of the complexity of the microbiome. Knowledge of such interactions in relation to arboviruses will be particularly important for development of potential disease-mitigating strategies.

Similarly, the complexity of the interactions between the many viruses that infect honey bees combined with the propensity of investigators to consider a single pathogen in isolation, has confounded clear elucidation of the potential role of any pathogen in colony collapse. Studies in this area highlight the need for a comprehensive systems approach, i.e. considering individual organisms in the context of their environment and observing the relationships between them, rather than focusing on a single component.

Viruses in insect cell lines

The challenge associated with the presence of cryptic viruses in cell lines used for laboratory research is exemplified by the analysis of 26 commonly used *Drosophila* cell lines and detection of virus-derived reads from 37 different viruses (Fig. S9 in Webster *et al.*, 2015). The numbers of reads detected ranged from < 1% to 50% viral reads in

the cell line DmBG1-c1. Table 1.2 lists some of the viruses that have been identified in insect-derived cell lines.

Implications of adventitious viruses in cell lines

For many applications, the presence of cryptic viruses in a cell line is of little consequence, but there are exceptions to this. First, as viruses encode suppressors of antiviral immune systems [both RNA interference (Guo *et al*., 2018) and apoptosis (Clem, 2015)], resident viruses are likely to alter the immune responsiveness of cultured cells. Second, for virological studies, the presence of an adventitious virus in a cell line may alter the behaviour of viruses used to inoculate the cell line, through competition or complementation. The outcome of infection will depend on the particular virus present: IAPV outcompeted *Sacbrood virus* in the honey bee cell line, AmE-711, unless *Kashmir bee virus* (KBV) was present (Carrillo-Tripp *et al*., 2016), while the titre of baculovirus is reduced by the presence of rhabdovirus in Sf9 and Sf21 cell lines (Maghodia *et al*., 2016). Complementation or competition between resident viruses and test viruses may result in false positives or false negatives respectively for the ability of cell lines to support replication of the test virus, which can be particularly problematic for generation of infectious clones of insect RNA viruses (Carrillo-Tripp *et al*., 2015). Third, virus replication may impact the expression of housekeeping genes commonly used as reference for RNA quantification. For quantification of RNA viruses, virus genome equivalents in relation to total RNA are typically used as a result of virus-mediated disruption of the transcription of housekeeping genes. Fourth, cryptic viruses in cell lines used for baculovirus expression of antigens for use in vaccines, may result in the presence of virus in the antigen sample. In the case of rhabdovirus contamination of *Spodoptera frugiperda* (Sf) cell lines, the virus host range was found to be narrow thereby minimizing risk (Maghodia and Jarvis, 2017), but rhabdovirus-free cell lines were subsequently generated to resolve the problem (Maghodia *et al*., 2016).

Table 1.2 Adventitious viruses identified in insect-derived cell lines

Insect cell line	Species	Virus	Reference
Lepidoptera			
Sf9, Sf21	*Spodoptera frugiperda*	Rhabdovirus (Sf)	Ma *et al*. (2014)
SL221	*Spodoptera litura*	Rhabdovirus (Sf)	Geisler and Jarvis (2018)
BCIRL-HS-AM1	*Heliothis subflexa*	Rhabdovirus* (Sf)	McIntosh (1989)
Bm-N	*Bombyx mori*	Rhabdovirus* (Sf) *Maculavirus* (plant)	Katsuma *et al*. (2005); Geisler and Jarvis (2018)
High Five™, Tn368, Tn Pro™	*Trichoplusia ni*	*Alphanodavirus* (TnCLV)	Li *et al*. (2007)
Tn368	*Trichoplusia ni*	Nudivirus (HzNV-1)	Lin *et al*. (1999)
Ld652Y	*Lymantria dispar*	*Iflavirus*	Carrillo-Tripp *et al*. (2014)
Diptera			
Drosophila 26 cell lines	*Drosophila melanogaster*	Multiple	Webster *et al*. (2015)
68 K S2-GMR	*Drosophila melanogaster*	*Birnavirus*, *Totivirus*, *Tetravirus*	Teninges *et al*. (1979); Wu *et al*. (2010)
S1	*Drosophila melanogaster*	*Alphanodavirus*	Friesen *et al*. (1980)
Aag2	*Aedes aegypti*	*Anphevirus* *Bunyavirus* *Flavivirus*	Zhang *et al*. (2016); Di Giallonardo *et al*. (2018); Schultz *et al*. (2018)
Hymenoptera			
AmE-711	*Apis mellifera*	*Iflavirus**	Carrillo-Tripp *et al*. (2016)

While some viruses are likely to be derived from source material, others are known to result from contamination*. After Geisler and Jarvis (2018).

Viral suppressors of RNAi can be used to indicate the presence of adventitious viruses in cell lines as shown in Fig. 1.2. However, sequencing of the cell line transcriptome or small RNA is recommended for the identification of covert viruses. Establishment of virus-free cell lines is particularly important for production of cell line-derived commercial products, and for immunological and virological studies as described above. Given the high potential for cell line contamination within the laboratory setting (Table 1.2), clean cell lines should be maintained separately from both infected cells and from insect colonies.

Future directions

The exponential increase in novel viral sequence discovery presents a challenge for virus nomenclature. Few of the discovered sequences receive further attention, and some viruses have been named without the necessary validation (Carrillo-Tripp *et al.*, 2015) and without knowledge of host range. Viruses derived from bat faecal material have been named bat viruses for example, even though they were known to include insect and plant viruses (Li *et al.*, 2010). A recent appeal for the International Committee on Taxonomy of Viruses (ICTV) to include metagenomics sequence data in sequence-based virus taxonomy to benefit analysis of virus evolution in particular, has been made (Simmonds *et al.*, 2017).

Several avenues present themselves for future research, building on our expanding knowledge of the insect virome. While the increase in discovery of novel insect viruses provides opportunity for further understanding of the insect virome and for practical applications, the lack of appropriate cell lines for *in vitro* virological studies is limiting. There is a significant need for the establishment of virus-free cell lines for further study of viruses of interest (particularly for pest species) and for production of infectious virus clones for potential use as dsRNA delivery vehicles against pests that are recalcitrant to the impact of dsRNA (Terenius *et al.*, 2011; Nandety *et al.*, 2015). Similarly, the establishment of honey bee cell lines will allow for continued analysis of interactions between honey bee viruses. Knowledge of insect virus interactions with other viruses and other microbes within the host will be of paramount importance for more comprehensive understanding of disease and has potentially important implications for arbovirus transmission.

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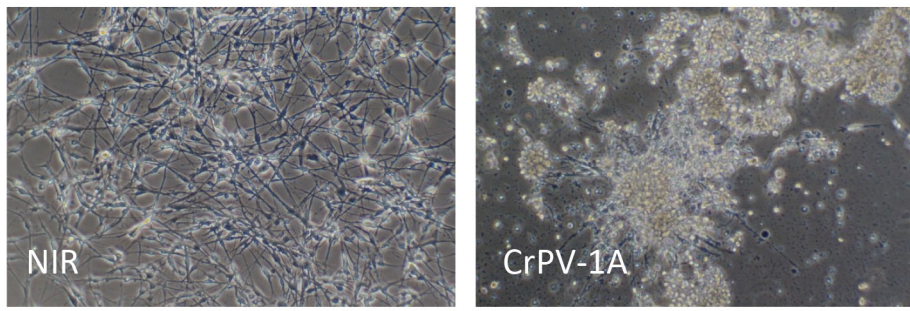

Figure 1.2 Infection of a honey bee-derived cell line was detected following transfection of the cells with the *Cricket paralysis virus* (CrPV)-derived 1A suppressor of RNAi. Cells transfected with non-infective RNA (NIR) retained the typical fibroblast-like structure, while cells transfected with CrPV-1A (Nayak *et al*., 2010) rounded and clumped in a manner typical of virus infection. Analysis of relative titres of *Deformed wing virus* (DWV) by RT-qPCR confirmed partial release of DWV from suppression by the cell RNAi pathway. For further details, see Carrillo-Tripp *et al*. (2016). Infection with DWV ultimately contributed to the loss of this honey bee-derived cell line.

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