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Trends in Parasitology

Opinion

Translating mosquito viromes into vector management strategies

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Mosquitoes are best known for transmitting human and animal viruses. However, they also harbour mosquito-specific viruses (MSVs) as part of their microbiota. These are a group of viruses whose diversity and prevalence overshadow their medically relevant counterparts. Although metagenomics sequencing has remarkably accelerated the discovery of these viruses, what we know about them is often limited to sequence information, leaving much of their fundamental biology to be explored. Understanding the biology and ecology of MSVs can enlighten our knowledge of virus–virus interactions and lead to new innovations in the management of mosquito-borne viral diseases. We retrace the history of their discovery and discuss research milestones that would line the path from mosquito virome knowledge to vector management strategies.

Highlights

The mosquito virosphere comprises not only mosquito-borne viruses, but also mosquito-specific viruses (MSVs) and viruses of the mosquito bacterial and fungal microbiota.

As they can influence host vector competence for viral pathogens, MSVs may be key factors shaping arbovirus epidemiology and can provide valuable insights into mosquito antiviral immunity and virus evolution.

MSV-based vector control and arbovirus outbreak risk mapping can be achieved through advances in our knowledge of the biology and ecology of MSVs.

Mosquitoes and their viruses

Mosquitoes participate in natural ecosystems as plant pollinators and food sources for a wide range of animals. From an anthropocentric point of view, they are also fearsome vectors of pathogens, including **arboviruses** (see Glossary), due to the blood-feeding behaviour of (most) female mosquitoes. The mosquito life cycle spans aquatic and terrestrial habitats, which brings them into contact with a wide range of microorganisms that then constitute their microbiota. In particular, the viral community of the mosquito microbiota has garnered significant research interest due to its potential impact on mosquito **vector competence** [1–3]. Prior to this, the notion of mosquito viruses was strongly tinted by the human- or animal-pathogenic viruses they transmit, such as dengue, chikungunya, Zika, West Nile, yellow fever viruses, and many others [4].

In 1975, the first indication of a mosquito virus not pathogenic to vertebrates was reported in an *Aedes aegypti* mosquito cell line. The cell fusing agent virus (CFAV) induced **syncytial cytopathic effects** following inoculation into *Aedes albopictus* cells, as did some flaviviruses, though it did not replicate in vertebrate cell lines [5]. Its taxonomy as a flavivirus was later confirmed by shared genome homology [6]. Due to its restricted host range, it was termed a **mosquito-specific virus (MSV)**. Since then, the mosquito virosphere as we know it has expanded considerably through discoveries of new MSVs and their distributions, facilitated by next-generation sequencing (NGS) technologies. Newly discovered mosquito viruses span many families of positive- and negative-sense single-stranded RNA viruses (except *Retroviridae*), double-stranded RNA viruses, and single-stranded DNA viruses. As a significant proportion of them remain unclassified, they are poised to reshape current virus taxonomies [7]. Notably, most mosquito viruses discovered by NGS in the past two decades are classified as MSVs based on genetic relatedness, or rather, genetic distance from known arboviruses.

MSVs that belong to the same genera as arboviruses make attractive platforms for new recombinant vaccines and antigens for arbovirus diagnostics [8]. In parallel, the ubiquity of MSVs in almost every mosquito population investigated has raised many questions regarding their

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implications for the transmission of mosquito-transmitted diseases. Indeed, experimental studies have revealed the effects of MSV infections on host fitness and susceptibility to arboviruses, indicating a potential for developing MSV-based vector management strategies [2,8,9]. Here, we propose the research milestones leading from mosquito **virome** knowledge towards concrete entomological measures to prevent or manage arbovirus outbreaks.

Virus hunting

Since the Stollar and Thomas report in 1975, another CFAV isolate has been subsequently obtained from wild mosquito populations in Puerto Rico [10]. This was followed by the detection and isolation of other flaviviral MSVs: Kamiti River virus [11,12], Culex flavivirus [13], and Aedes flavivirus [14], to name a few. Notably, the earliest MSVs discovered were flaviviruses owing to the method of detection at the time – using pan-flavivirus RT-PCR primers targeting the NS5 gene [15]. Similarly, the use of pan-alphavirus primers led to the discovery of mosquito-specific alphaviruses, such as Eilat virus (EILV) and Agua Salud alphavirus [16–18].

Often, the detection of MSVs was a serendipitous result of studies aiming to uncover new potential arboviruses. Pools of mosquito samples were either tested directly or first cultured on highly permissive *Ae. albopictus* C6/36 cells, followed by testing of cell culture media when cells displayed cytopathic effects from viral infection.

When studies began performing NGS on cultured virus isolates, full virus genome sequences became more readily obtainable [19–21]. Later, the application of NGS approaches on mosquito samples without prior culture (i.e., **metagenomics**) sharply accelerated the rate and diversity of mosquito virus discovery [22–24]. NGS has since become a mainstay in characterising and profiling mosquito viromes. The gradual shift from PCR-based to NGS-based virus discovery approaches reflects a broadened expectation of the diversity of mosquito viromes to be uncovered.

Studies on mosquito viromes typically follow one of the four pipelines depicted in Figure 1, each providing different potential insights (Table 1). Methodology choice is often a balance between cost and obtainable information. For example, sample species identification may be performed either through morphological or molecular methods [e.g., mitochondrial cytochrome oxidase subunit 1 (*COI*) gene or rRNA sequences]. Morphological identification is time-consuming and skill-intensive, whereas molecular identification requires laboratory equipment and reagents. Samples may be processed as pools to maximise the probability of detecting new viruses, or as individuals to allow the analyses of prevalence rates and diversity measures at a finer resolution. As viral loads vary considerably between individuals, it may be useful to store biological material of individuals prior to pooling under careful conditions for later qPCR. While extracted viral RNA may be stable at –80°C, storing homogenised or intact mosquitoes in appropriate stabilisation buffers such as RNA*later*™ (Invitrogen) or others is essential for long-term viability without compromising viral infectivity for isolation purposes [25–27].

Although whole bodies of adult mosquitoes are the most common sample type, studies may also choose to focus on immature stages (larvae or pupae) or on specific tissues (e.g., salivary glands and midgut, which are key infection barriers to arbovirus transmission [28]) to gain information on tissue tropism, **transstadial** stability, and potential impact on vector competence [29,30]. However, it should be noted that MSVs detected in larval samples include those acquired from the aquatic habitat and those vertically transmitted.

Isolating potential viruses by cell culture prior to molecular analyses ensures the detection of only viruses able to infect a mosquito host, while testing non-cultured samples would capture viruses

Glossary

Arboviruses (arthropod-borne viruses): sometimes called mosquito-borne viruses, these are viruses that cause disease in humans and vertebrate animals. They are referred to as dual-host viruses for their ability to infect both insects and vertebrate hosts.
Endogenous viral elements (EVEs): fragments of viral genome sequences integrated into host genomes. In mosquitoes, where RNA viruses dominate the discovered virome [7,67], EVEs are thought to arise due to retrotransposon reverse transcriptase activity and are often found flanked by transposable elements.
Horizontal transmission: the transmission of infectious agents from the environment, from food sources, or from other individuals in the population, excluding progenitors.
Metagenomics: a study approach seeking to sequence all genomes within a given system, including those of the host, and of associated microbiota and symbionts.
Metatranscriptomics: a metagenomics approach where the total RNA within a given system is sequenced, capturing gene transcripts and viral RNA genomes or replication intermediates.
Mosquito-associated viruses (MAVs): also called the mosquito virosphere, this refers to all viruses found in mosquitoes. These include arboviruses, MSVs, prokaryotic viruses, viruses of fungi, and viruses with undetermined hosts.
Mosquito-specific viruses (MSVs): also called insect-specific viruses, these are viruses that infect and replicate in mosquitoes without the involvement of vertebrates in their transmission cycle. Their host range is limited to mosquitoes or insects, unlike dual-host mosquito-borne viruses.
Population modification: a type of vector control strategy seeking to modify, rather than eliminate, natural vector populations to render them less efficient at transmitting disease.
Sentinel animals: susceptible animals screened for infection of known diseases for the purpose of monitoring disease circulation and outbreak risk within a specific area.
Syncytial cytopathic effects: morphological changes in cells induced by viral infection where multiple cells fuse to form a large multinucleated cell.

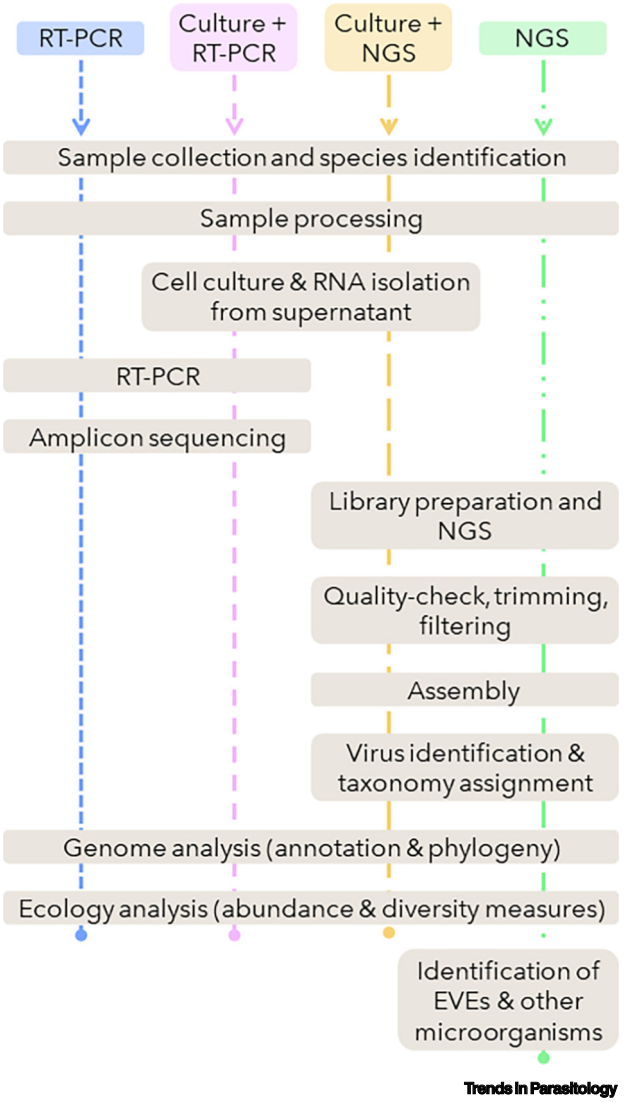

Figure 1. Mosquito virome discovery pipelines. Molecular detection and discovery of viruses are achieved by reverse-transcription PCR (RT-PCR) or by next-generation sequencing (NGS). Mosquito samples are either directly tested or first cultured in cells. Abbreviation: EVE, endogenous viral element.

Transstadial: the maintenance of an infectious agent in an individual across life stages.
Truncated open reading frames (ORFs): a series of nucleotides coding for amino acids ending in a stop codon. A truncated ORF is shortened or incomplete in such a way that it is unlikely to produce a functional protein.
Vector competence: the capacity of an insect vector to acquire, propagate, and transmit a pathogen to a vertebrate host.
Vector control strategy: measures to limit the spread of vector-borne diseases that are directed towards the vector population.
Vertical transmission: the transmission of an infectious agent from progenitors (maternal or paternal) to the offspring.
Virome: the collection of viral genomes within a given system.

of mosquitoes as well as viruses of ingested biological material and viruses of the bacterial and fungal communities of the mosquito microbiota. The latter is particularly the case for **metatranscriptomics** where NGS is performed on total RNA. Although such analysis provides important information on the mosquito's ecological context, further investigations are necessary to ascertain the natural host of detected mosquito viruses. Alternatively, small RNA sequencing, in which RNAs 15–30 nucleotides long are selectively sequenced, allows the detection of mosquito-infecting viruses based on the activity of the mosquito RNA interference (RNAi) antiviral immune response (Box 1). Given that actively replicating viruses produce small RNAs of a distinct size, putative viruses may be detected based on their small RNA profiles instead of on sequence similarity to records in public virus databases [31–33].

Limitations and caveats in virus discovery

Because unbiased virus metagenomics captures all viruses associated with the mosquito sample, viruses of fungi or plants may be erroneously classified as mosquito viruses in pipelines

Table 1. Potential insights and biological resources obtainable from virus discovery pipelines[a]

Obtainable insight/resource	RT-PCR	Culture + RT-PCR	Culture + NGS	NGS
Virus discovery	Within a known taxon	Culturable viruses within a known taxon	Culturable viruses	All mosquito viruses
Virus isolates	Not obtainable	Yes	Yes	Not obtainable
Host range	May be detected in multiple species	Testable	Testable	May be detected in multiple species
Phylogeny	Yes	Yes	Yes	Yes
Abundance quantification	With qRT-PCR	With qRT-PCR	Reads-based or with qRT-PCR	Reads-based or with qRT-PCR
Full virus genome	With targeted RT-PCR and and 5' and 3' RACE-PCR	With targeted RT-PCR and and 5' and 3' RACE-PCR	Yes	Yes
Prevalence and distribution	Yes	Yes	Yes	Yes
Diversity factors	Yes	Yes	Yes	Yes
EVEs and bacterial or fungal metagenomics	Not obtainable	Not obtainable	Not obtainable	Yes

[a]Abbreviations: NGS, next-generation sequencing; RACE, rapid amplification of cDNA ends PCR; RT-PCR, reverse-transcription PCR; qRT-PCR, quantitative real-time PCR.

without isolation by cell culture. For example, several viruses from the family *Totiviridae* have been recently reported in *Anopheles*, *Aedes*, and *Culex* samples [32–35]. Members of this virus family are known to infect fungal, plant, animal, and protist hosts. Although some mosquito-associated totiviruses phylogenetically cluster apart from totiviruses of other hosts [34], those that are placed alongside fungal totiviruses require closer scrutiny to determine their true natural hosts. In this case, a metagenomics approach that also characterises the fungal microbiota could reveal co-occurrences with certain fungi, providing evidence that these viruses have fungal hosts.

Along the same vein, the status 'mosquito-specific' is often conferred on novel viruses based on phylogenetic relatedness to another purported MSV with no empirical evidence for the inability to proliferate in animal cells. However, the majority of new entries to virus databases are taxonomically unclassified [7]. Without phylogeny to indicate their natural host, there is a possibility that some of these viruses represent unknown arboviruses. Metagenomics studies on **sentinel** and suspected reservoir animal hosts or experimental infection of culturable viral isolates could later identify these new viruses as arboviruses [35]. Hence, the term '**mosquito-associated virus**' **(MAV)** is preferable until mosquito specificity has been proven.

Both RT-PCR and NGS approaches produce virus genome data and rely on sequence similarity searches against public databases to identify viruses or their nearest known relative. As such, care should be taken on several fronts. First, the quality and completeness of submitted virus genome records must be strictly controlled for accurate virus identification and taxonomic assignment in future virus discovery [36]. To facilitate this, a guideline for the minimum required information of an uncultured virus genome submission has been proposed [37]. Second, unregulated nomenclature of 'newly discovered' viruses can be problematic as it results in multiple virus

Box 1. The small RNA pathways in mosquitoes

RNAi is a conserved antiviral defence mechanism in insects. It is one of the three small RNA-based pathways defined by their functions, the protein actors, and the sources of RNA substrate involved in their biogenesis: the miRNA pathway, the siRNA pathway, and the PIWI-interacting RNA (piRNA) pathway [78].

The miRNA pathway functions in gene expression regulation and is activated by endogenous premature miRNA hairpins encoded within the host genome. The siRNA pathway is one of the major antiviral immune responses in insects and uses virus-derived double-stranded RNA as a pathogen-associated molecular pattern (PAMP). Activation of this pathway results in the production of 21-nucleotide single-stranded siRNAs that serve as sequence-specific guides within an RNA-induced silencing complex (RISC), which targets and cleaves complementary virus genome molecules. As all groups of viruses produce a double-stranded RNA intermediate during genome replication, the siRNA response is broad-acting yet specific to actively replicating viruses. The piRNA pathway was thought to function primarily in genome defence against transposable elements, using single-stranded transcripts from genomic piRNA clusters as precursors. However, virus-derived piRNAs have been reported for several infecting viruses [79]. In both cases, this pathway generates single-stranded primary and secondary piRNAs between 25 and 31 nucleotides long. The two populations respectively show U1 and A10 nucleotide biases and a ten-nucleotide sequence overlap characteristic of a ping-pong amplification cycle [78].

The viral targets of RNAi activity can be discerned through small RNA sequencing, characterised by an abundance of 21-nucleotide small RNAs, which can be assembled to reconstruct full-length virus genomes [31–33]. Distinct from replicating viruses that may trigger both the siRNA and piRNA pathways, EVE transcripts would only induce the production of larger piRNAs [32,33].

names, often based on their geographic associations, given to the same viral species [38]. In such cases, virus database querying would return several matches, and manual curation is needed to ascertain whether the query virus is related to a single virus with multiple names or to two or more viruses equidistantly. Third, in most cases the taxonomy of a new virus cannot be determined due to the unassigned taxonomy of its closest relative [7]. New viruses are being discovered at a pace that exceeds that at which the International Committee on Taxonomy of Viruses (ICTV) receives and ratifies new taxon proposals. This may naturally be resolved with time if deliberate efforts are made to classify reported viruses. Fourth, sequence-based virus identification is inherently limited to within a certain divergence range from existing database records. Currently, there are undoubtedly putative viral genomes below an arbitrary sequence similarity threshold that end up as 'dark matter' in metagenomic datasets. These will eventually be illuminated by reanalysis of published sequencing libraries as the virus catalogue expands [39].

It is worth mentioning that the expansion in known virus diversity owing to metagenomics has sparked discussions on how to define virus species based on their genome sequences. Historically, arbovirologists, clinicians, and epidemiologists found it useful to classify mosquito viruses based on their ecology, biological properties, host association, or disease phenotype. By contrast, virus taxonomy is principally based on evolutionary histories and is regularly revised. These two distinct approaches to virus classification could cause issues that are succinctly outlined by Blitvich and colleagues following the abolishment of the viral family *Bunyaviridae* [40]. A consensus roadmap for a universal and long-term virus taxonomy was recently reached which affirms evolutionary relationships as the foundation of virus taxonomy as well as the complementary value of phenotypic properties [36].

Finally, **endogenous viral elements (EVEs)** pose an additional challenge in virus metagenomics as they are ubiquitous in the genomes of *Ae. aegypti* and *Ae. albopictus*. They are likely to also be present in other mosquito species [41]. Distinguishing EVE-derived transcripts from viral genomic RNA is a critical step in NGS metagenomics analysis. Characterising EVEs within mosquito genomes is therefore important as EVEs provide insights into virus–host co-evolution histories as markers of ancient viral infections. Furthermore, unidentified EVEs in reference host genomes can result in the removal of cognate but genuine viral transcripts during the host read filtering step. Within metagenomic data, EVE contigs can be recognised by certain characteristics: having

regions that share homology to viral and host sequences within a single contig, having **truncated open reading frames (ORFs)**, or containing fragments of known transposable elements. Small RNA sequencing can also be beneficial here, as EVE-derived small RNAs range from 24 to 30 nucleotides in length whereas replicating viruses characteristically produce small RNAs of 21 nucleotides (Box 1) [31–33].

The path to vector management strategies

MSVs may serve as cornerstones for new clinical and entomological innovations to tackle the spread of arboviruses [8,42]. On the clinical side, several promising vaccine candidates against flaviviral arboviruses – West Nile, yellow fever, Zika, and dengue viruses – have emerged using the mosquito-specific flaviviruses Binjari virus (BinJV) or Aripo virus as genomic backbones [43–47]. Similarly, among alphaviruses, the mosquito-specific EILV has given rise to vaccine candidates against the chikungunya virus, and the eastern and Venezuelan equine encephalitis viruses [48,49]. These technologies were preceded by experimental studies on the biological properties of the candidate MSVs [17,50]. Due to their host-restricted nature, these recombinant virions can also serve as immunologically relevant antigens for diagnostics development under lower biosafety conditions [51,52].

On the entomological side, modifying the mosquito viral microbiota to reduce vector susceptibility to arbovirus infections is an intriguing avenue of biological **vector control** in endemic regions. In parallel, understanding the impact of the mosquito viral microbiota on vector competence could improve arbovirus outbreak risk mapping and inform public health decisions in deploying preventative vaccine campaigns or short-term vector control measures. However, for the majority of discovered MSVs, our knowledge stops at the level of genomic sequence information. Some open questions on MSV biology, ecology, and evolution have been put forward by Altinli *et al*. [9]. Here, we outline the essential attributes and required knowledge that would pave the way towards entomological applications (Figure 2, Key figure).

Virus isolation

The number of cultured MAV isolates is a small proportion of the number of detected viruses based on genomic data. This may be largely due to a lack of suitable mosquito cell lines – MAV isolation protocols principally rely on the use of *Ae. albopictus* C6/36, which are ideal for virus production given the loss-of-function mutations in a key protein within the antiviral RNAi machinery [53]. Although there have been instances where MSVs from *Culex*, *Anopheles*, and even *Coquillettidia* mosquitoes were able to propagate in C6/36 cells [16,17,54], the use of this cell line imposes a fine sieve in regard to which MSVs will go on to shape our knowledge of mosquito virus biology. While C6/36 are naive, samples of commonly used mosquito cell lines of *Aedes* (Aag2, U4.4, Aa23) and *Culex* (Hsu, CT) origins have been found persistently infected with MSVs [55–57]. As such, naive samples of these cell lines or new cell lines from different mosquito genera would be valuable tools for virus isolation.

Host range

As mentioned, it is crucial to test the host range of isolated MAVs to ascertain their mosquito-specific status in a repertoire of invertebrate and vertebrate cell lines, including mammalian, birds, reptiles, amphibians, and fish [16,17,54]. In addition, conducting *in vivo* assays in live mosquitoes would permit insights into infection dynamics and tissue tropism – special attention should be given to the salivary glands. A caveat for these experiments is that, although infection by intrathoracic injection is an efficient method to produce infection and is commonly performed, it does not recapitulate natural transmission routes and may not give information on whether the mosquito can orally acquire the virus.

Key figure

Roadmap from mosquito virus discovery to entomological applications

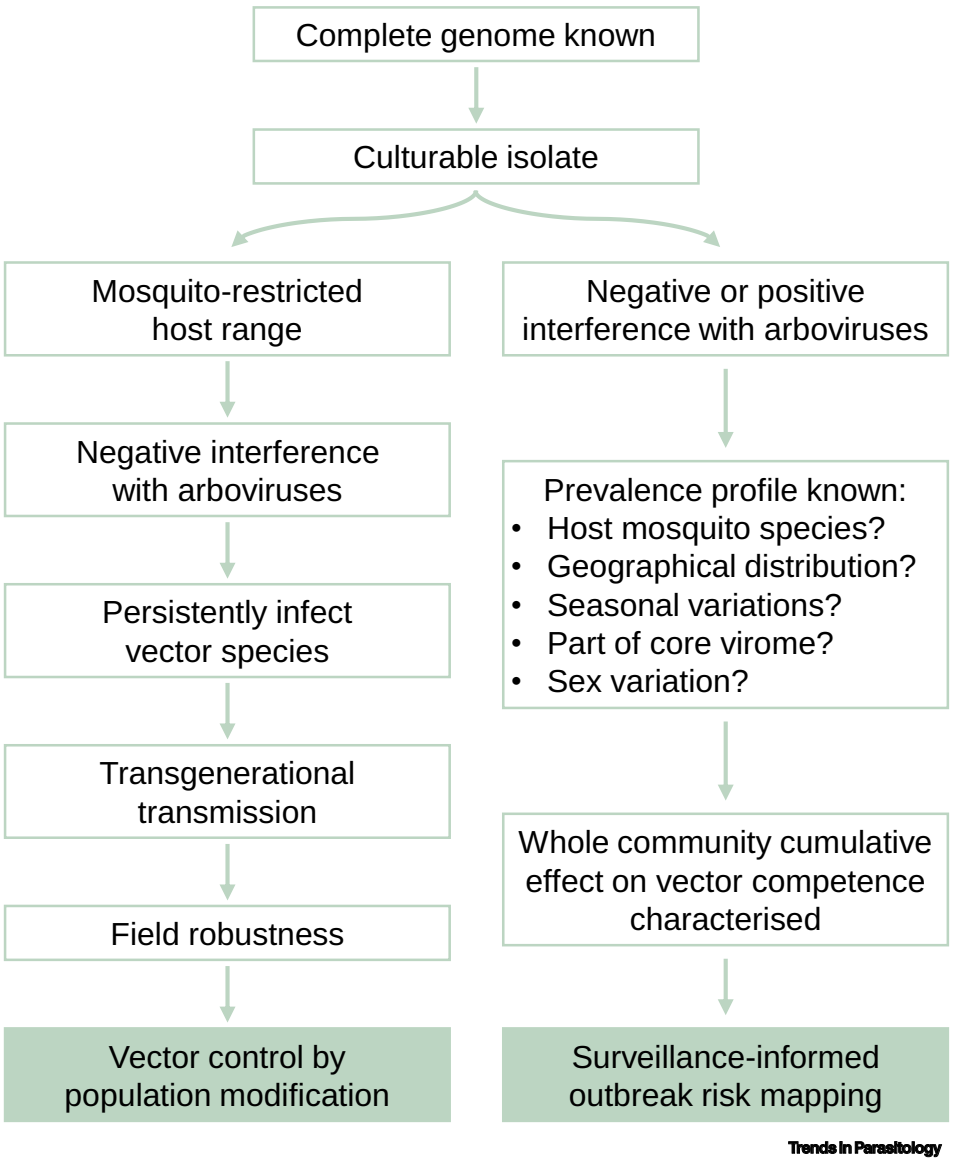

Figure 2. Incredibly prevalent and diverse, mosquito viruses play an important role in the disease ecology of arboviruses. Utilising the mosquito viral microbiota to control the spread of mosquito-borne viruses is a promising prospect, but key biological properties and attributes of mosquito-specific viruses (MSVs) of interest remain to be established through experimental research. MSVs can serve as a tool for biological vector control or as important variables in arbovirus outbreak risk maps.

Interference with arboviruses

A multitude of experimental studies have reported negative or positive effects or interferences by certain MSVs on the replication and dissemination of arboviruses within a shared mosquito host

[56,58–62]. Naturally, this fundamental attribute determines the epidemiological importance of an MSV. Thus, MSV–arbovirus interactions should be tested in different vector–arbovirus systems. The mechanism behind these interference phenotypes is currently a subject of highly active research. The challenge of 'curing' a mosquito or a cell line from an infecting MSV hinders mechanistic understanding of MSV interactions. To date, only two studies have demonstrated methods to clear MSVs from persistently infected *Ae. aegypti* Aag2 cell lines, permitting insights into MSV impact on arbovirus interactions and host antiviral immune responses [63,64].

Inevitably, in assessing arbovirus interference, some experiments will yield no-effect outcomes. Such results may be under-reported in traditional scientific dissemination channels because they are deemed to be of lesser impact. We caution that this could lead to inefficient allocation of resources where the same research questions are investigated repeatedly. No-interference virus–virus interactions following rigorous investigations are therefore valuable knowledge advances.

Persistent infection and transgenerational transmission

The capacity to establish persistent infection and to disseminate into host reproductive tissues are critical for transgenerational transmission of artificially transinfected symbionts. The latter would allow self-propagation of any MSVs of interest within target mosquito populations as part of a **population modification** vector control strategy. It would be beneficial to know how MSVs of interest are maintained in their natural ecosystems, which may involve both **horizontal transmission** and **vertical transmission** modes. Only a small number of experimental studies have sought to characterise the transmission modes of flaviviral and alphaviral MSVs [65,66]. Virome studies on individual mosquitoes revealed the existence of mosquito core viromes, shaped primarily by host species [34,67]. Notably, these core viromes are maintained by vertical transmission, displaying transgenerational and transstadial stability [29,68].

Field robustness

Infection, transmission, and arbovirus interference phenotypes observed under carefully controlled laboratory settings may not extrapolate well into field conditions. As with any vector control strategy, a deciding factor on feasibility is whether the desired phenotypes from viral microbiota modulations persist in the field without fitness and reproductive costs.

Prevalence profile

Prevalence profile information is largely derived from virome studies, but this is rarely comprehensive due to differences in study scopes and aims, resulting in differences in collection methods, seasonality, sampling sites, and other variables. Known vector species circulating in urban or periurban environments tend to be the focus, whereas arbovirus outbreaks often begin from a zoonotic spillover event and are vectored by forest-dwelling mosquito species in close proximity to animal hosts. Longitudinal observations are rare, and female mosquitoes are prioritised given their blood-feeding behaviour, obscuring variations driven by season and sex. It is also possible that important ecological metadata or high-risk localities are inadvertently missed.

Perhaps the most comprehensive vector virome diversity maps available are of the *Ae. aegypti* and *Ae. albopictus* mosquitoes, considered the most important arbovirus vectors given the global public health burden presented by the diseases they transmit [2,7]. The collective sample numbers and geographical breadth in sampling locations have shed light on the factors shaping mosquito virus diversities [29,34,60,67]. Comparatively, there have been fewer investigations into *Culex* and *Anopheles* viromes, and even fewer still in forest-dwelling genera such as *Haemagogus* or *Sabethes*.

Taken together, virome data are often too fragmented in spatiotemporal metadata to make meaningful associations between the virus ecology of the local mosquito population and arboviral disease incidence. To accurately generate outbreak risk maps that factor in the viromes of specific mosquito populations, continued and routine surveillance on vector species and epidemiologically relevant animal hosts, in combination with human serological data, must be conducted by relevant stakeholders, such as the local public or veterinary health authorities. Significant resources and cohesive political support are needed to achieve this.

Whole community interactions

The viral microbiota of individual mosquitoes comprises between four and 25 viruses and most commonly include members of the *Phenuiviridae*, *Totiviridae*, *Orthomyxoviridae*, *Rhabdoviridae*, and *Tymoviridae* virus families [7,34]. A vector control strategy based on MSV modification would have to consider the interactions between desirable arbovirus-suppressing MSVs and other members of the viral microbiota.

Certain bacterial and fungal microbiota of mosquitoes also have profound effects on mosquito vector competence [3,69]. For example, a gut bacterial symbiont of Anopheline mosquitoes, *Delftia tsuruhatensis* TC1, inhibits malarial *Plasmodium* development through the action of a secreted biomolecule [70]. These microorganisms have been proposed as biocontrol agents against mosquito-borne diseases [56,57]. The endobacterium *Wolbachia* is of particular importance here as it is currently deployed as a biological vector control strategy against dengue virus in 14 countries[i]. However, *Wolbachia* has been shown to influence the titres of certain MSVs [71–74]. Hence, it is imperative to understand the impact of whole community interactions on overall vector competence as interactions between MSVs and other biocontrol agents could alter the efficacy of these interventions. In the case of *Anopheles gambiae*, which is a natural host for *Wolbachia* as well as a vector of the O'nyong-nyong virus and *Plasmodium falciparum*, these multi-microorganismal interactions reach a new level of complexity to be untangled [75–77].

Concluding remarks

Virome studies have expanded our knowledge of the mosquito virosphere to great depths. Although we have barely scratched the surface of the biology and ecology of MSVs, these viruses may significantly change our perspective of arbovirus epidemiology and bring about innovations in entomological approaches towards arboviral disease management. To achieve this, transdisciplinary advances beyond the laboratory are needed, combining virus–virus interactions, medical entomology, vector-borne disease epidemiology, and risk modelling.

While we have focused on the immediate research milestones to bridge the gap between virus genome discovery and vector management strategies, there are other fascinating questions about mosquito antiviral immunity and virus evolution to explore (see Outstanding questions). Our favourite ones are: can the genomes of MSVs give insight into how dual-host replication evolved? What determines virus specificity for mosquito hosts? Are viruses with broad mosquito host ranges more likely to emerge as vertebrate pathogens? Can mosquito-associated prokaryotic viruses modulate vector competence through dysbiosis? These inquiries could lead us to novel insights into the complex interactions between mosquitoes and their viruses.

Outstanding questions

Can we predict the nature of interactions between mosquito-borne viruses and MSVs?

Can the mosquito core virome serve as a platform for paratransgenesis?

What are the titres of MSVs in the field? Do viral titres vary with acquisition mode, that is, via horizontal, venereal, or vertical transmission?

Can the mosquito viral microbiota alter the evolutionary trajectory of arboviruses?

How does the virome of polar mosquitoes compare with that of the rest of the globe?

Do the two *Ae. aegypti* subspecies (*formosus* and *aegypti*) differ in their viromes?

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Declaration of interests

The authors declare no competing interests.

Resources

[i]www.worldmosquitoprogram.org/

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