Insect-specific viruses and their potential impact on arbovirus transmission

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Abstract

Arthropod-borne viruses (arboviruses) are the causative agents of significant morbidity and mortality among humans and animals globally. In the last few years, the widespread adoption of next generation sequencing and metagenomics has led to a new era of virus discovery, where many novel viruses have been documented, exhibiting a restricted host-range in mosquitoes. They represent a wide-range of insect-specific viruses within the families of *Bunyaviridae, Flaviviridae, Mesoniviridae, Reoviridae, Rhabdoviridae, Togaviridae*, and the newly recognized taxon of *Negeviruses*. Collectively, their discovery has opened new vistas about the extent of viral diversity and evolution, their influence on vector competence and ability of their insect hosts to transmit human pathogens (e.g. arboviruses), and their potential development as biological control agents or novel vaccine platforms.

Introduction

During the past decade, advances in sequencing technology (next generation sequencing and metagenomics) and phylogenetics have identified a wide range of novel RNA viruses associated with hematophagous insects [1–6]. It is now apparent that biting insects harbor a diverse range of RNA viruses, many of which are not commonly known vertebrate pathogens. The RNA viruses of hematophagous insects can be conveniently divided into two broad groups, based on their host range: (1) the insect viruses that infect vertebrates and (2) the insect viruses that do not. RNA viruses that infect both biting insects and vertebrates are generally classified as arthropod-borne viruses (arboviruses) and include agents such as dengue (DENV), yellow fever (YFV), chikungunya (CHIKV) and West Nile (WNV) viruses. These dual-host viruses typically undergo replicative cycles in both their insect and vertebrate hosts and include many important human and veterinary pathogens. The second group of RNA insect viruses are the insect-specific viruses (ISV). ISVs infect insects and

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insect cells, but they do not replicate in vertebrates or vertebrate cells. The focus of this chapter will be on the ISVs, and their interaction with their insect hosts.

Most of the known ISVs that have been described from hematophagous insects are associated with mosquitoes. The predominance of mosquito-associated viruses may simply be a reflection of sampling bias, since most of the known ISVs were initially detected during arbovirus surveillance studies; and these types of studies have generally focused on mosquitoes and mosquito-borne viruses of public health and veterinary importance. A second reason for bias is the availability of a stable and highly susceptible mosquito cell line (*Aedes albopictus* C6/36) for virus isolation. The C6/36 clone has a defective RNAi response after infection to arboviruses infection [7, 8]; and this defect could account for its increased susceptibility to ISVs. There are no comparable cell lines available for culicoid midges, phlebotomine sandflies or other biting flies. It seems likely that some of these latter biting insects also harbor ISVs, but they have been under sampled and relatively few have been tested. Consequently, this review will focus on the ISVs of mosquitoes identified in the last 4 years.

Bunyaviridae

The family *Bunyaviridae* currently encompasses 5 genera (*Hantavirus, Nairovirus, Orthobunyavirus, Phelebovirus* and *Tospovirus*), a classification based on structural, genetic and antigenic characteristics [9]. The first four genera include viruses that infect vertebrates, including humans, whereas the fifth genus contains viruses that are known to cause disease in plants, not animals [9]. Exemplifying the great diversity of species classified within this family, the discovery of Gouléako (GOLV) and Cumuto (CUMV) viruses, during arbovirus surveillance study in Côte d'Ivoire [10] and Trinidad and Tobago [11], suggests a new novel genus evolutionarily related to but distinct from the genus *phlebovirus*. More recently, a unique clade defined by the restricted host range Herbert (HEBV), Tai (TAIV) and Kibale (KIBV) viruses, forms a phylogenetically sister group to the genus *Orthobunyavirus* [6], whereas Jonchet (JONV) and Ferak (FERV) viruses are likely two equally novel sister genera [5].

Flaviviridae

Biologically, viruses in the genus *Flavivirus* can be divided into 3 broad groups, based on their arthropod and vertebrate host associations [12, 13]: (i) dual-host viruses which are maintained in an alternating arthropod/vertebrate cycle [14], which includes most of the flavivirus pathogens; and (ii) insect-specific flaviviruses (ISFs) which naturally infect mosquitoes and replicate in insect cells but do not infect vertebrates or vertebrate cell lines [15]; and (iii) vertebrate-only flaviviruses [also known as 'no known vector' (NKV) group, which naturally infect and are transmitted between vertebrates but have no known arthropod-association [12]. Some of the bat salivary gland viruses (i.e. Rio Bravo) and rodent-associated flaviviruses (i.e. Modoc) are examples of this group [16]. Viruses in the NKV group infect vertebrates (including sometimes humans), but they do not infect arthropods or insect cells [13]. The ISFs form two distinct phylogenetic and antigenic subgroups or clades: (i) one clade includes viruses, such as the cell fusing agent (CFAV)[17, 18], Culex flavivirus (CxFV)[19–22], Aedes flavivirus (AeFV)[23–27], Kamiti River

(KRV)[28, 29], and others (reviewed in [30]), which forms a distinct clade apart from the main flavivirus tree of vertebrate pathogens; and (ii) a second clade, which currently includes Aripo [JA Auguste *et al*., unpublished], Barkedji (BJV) [31], Chaoyang (CHAOV) [32–34], Donggang (DONV) [N Vasilakis and RB Tesh, unpublished], Ilomantsi (ILOV) [35], Lammi (LAMV)[36], La Tina [N Vasilakis and RB Tesh, unpublished], Marisma (MMV)[37, 38], Nanay (NANV) [39], Nhuhirim (NHUV) [40] and Nounané (NOUV)[41] viruses (and reviewed in [30]), which are antigenically and phylogenetically related to and fall within the dual-host group of flavivirus pathogens in close proximity to WNV, DENV and YFV.

Reoviridae

Members of this family are classified into two subfamilies, *Spinareovirinae* and *Sedoreovirinae*, based on their core structures, and both have a wide geographic distribution and wide host range, as they have been isolated from fungi, plants, insects, ticks, arachnids, fish, marine protists, crustaceans, mammals, and birds [42]. Phylogenetic analysis of a single isolate, *Aedes pseudoscutellaris* reovirus (APRV), a host-restricted virus isolated from the AP61 mosquito cell line in 2005, indicates that it represents a distinct genus (genus *Dinovernavirus*) within the subfamily *Spinareovirinae* [43]. A second member of this genus, the host-restricted Fako virus (FAKV), isolated in 2010 during a surveillance study in Cameroon, has the closest genetic and structural relationships are with reoviruses in the genera *Cypovirus* and *Oryzavirus* [44]. Cimodo virus (CMDV), is also a unique ISV that belongs to the family *Reoviridae*. It was isolated in Côte d'Ivoire and likely defines a new genus within the subfamily *Spinareovirinae*, closely related to myco- and coltiviruses [45].

Rhabdoviridae

The family Rhabdoviridae is comprised of a diverse group of non-segmented, negative-sense RNA viruses that infect a wide range of vertebrates, invertebrates and plants [46], distributed across 17 recognized and proposed genera [47]. Vector-borne rhabdoviruses are present in 12 of the 17 groups, and most prevalent in the dimarhabdovirus supergroup. Two host-restricted rhabdoviruses in mosquitoes, Arboretum (ABTV) and Puerto Almendras (PTAMV) viruses, were isolated from pools of female *Ochlerotattus fulvus* and *Psorophora albigenu* mosquitoes, during an arbovirus surveillance in Peru, in 2009 [48]. Experimental infections *in vitro* failed to produce detectable CPE in vertebrate cell lines, and similarly with *in vivo* studies, both viruses failed to produce illness and/or death in newborn mice. Since both viruses have only been detected once in mosquito pools, a comprehensive assessment of host range remains to be determined through surveillance and experimental studies.

Togaviridae

The genus *Alphavirus* within the family *Togaviridae*, consists of 29 species grouped into 10 complexes based on antigenic and/or genetic similarities [49, 50]. Alphaviruses exhibit a wide host range that includes vertebrates such as fish, equids, birds, amphibians, reptiles, rodents, pigs, humans, and nonhuman primates as well as and insect hosts, including mosquito species encompassing at least six genera and also ticks and lice (reviewed in [51]).

While the fish alphaviruses exhibit a restricted host range (replication only in fish), the only known host-restricted alphavirus of mosquitoes is Eilat virus (EILV) [52], which was isolated during a two-year (1982–1984) arbovirus survey of the Negev desert in Israel, from a pool of *Anopheles coustani* mosquitoes [53].

Mesoniviridae

The newly recognized family Mesoniviridae consists of single-stranded RNA, positive-sense RNA viruses belongs within the order Nidovirales [54]. To date, members of this family have only been isolated from various pools of mosquitoes within the family Culicidae [3, 55–60] and appear to have an extensive geographic distribution. The family *Mesoniviridae* includes a single genus *Alphamesonivirus* with seven recognized species: *Alphamesonivirus-1* includes the two closely related viruses, Nam Dinh (NDiV)[55], Cavally (CavV)[56], and the recently characterized NDiV Ngewotan [3] and NDiV Houston [3]; *Alphamesonivirus-2-4* includes the previously described African mesoniviruses, HanaV, MenoV, and NseV, respectively [57]; *Alphamesonivirus-5* includes the four Bontag Baru (BBaV) isolates sampled in the early 1980's in Indonesia [3]; *Alphamesonivirus-6* includes the single isolate of Karang Sari (KSaV) virus, sampled in Indonesia [3]; and *Alphamesonivirus-7* includes the three isolates of Kamphaeng Phet (KPhV), sampled in Thailand in the mid-1980's [3]. Moreover, recent phylogenetic and DEmARC-based analyses involving all these viruses using either all replicase non-structural proteins (nsps) or its large subset (from 3CLpro to OMT domains), reassigns the above viruses in five *Alphamesonivirus* species and two unassigned species (A. Gorbalenya, personal communication).

Negeviruses

The newly recognized taxon of Negeviruses consists of single-stranded RNA of positive polarity viruses consists of six prototype ISVs that have been designated as Negev (NEGV), Ngewotan (NWTV), Piura (PIUV), Loreto (LORV), Dezidougou (DEZV) and Santana (SANV), which were isolated from mosquitoes and phlebotomine sand flies collected in Brazil, Peru, USA, Ivory Coast, Israel and Indonesia [2]. Subsequently, others have reported the isolation and characterization of negeviruses from the Philippines (Tanay virus (TANAV))[61], Trinidad (Wallerfield virus (WALV))[11], Côte d'Ivoire (Goutanap virus (GANV))[62], Brasil (Brajeira and Wallerfield virus (M Nunes, personal communication)), Colombia and the USA (N Vasilakis and RB Tesh, unpublished). Phylogenetic analysis of the prototype negeviruses demonstrated that their closest but still distant relatives are mite-transmitted plant viruses in the genus *Cilevirus*, which occupied a basal position [2]. Moreover, recent analyses that expanded the sequence dataset to include the more recently discovered viruses suggest that negeviruses may exhibit more complex taxonomic relationship on the genus level [11, 61, 62]. Their close relationship with plant viruses of the genera *Cilevirus, Higrevirus* and *Blunervirus*, but heterogenous genome organization and architecture suggests that the Negevirus taxon may eventually be designated as a new virus family [62].

Evolution

To date there is little known about the evolution of ISVs. Confounding this limitation is the lack of available data about their host range, ecology and distribution, which underscores the necessity of further studies to decipher their evolution. What is known is that ISVs belong to viral families where many arboviruses are resident [6, 10, 25, 44, 53] or occupy clades with large phylogenetic distances to established families [2, 55]. A number of recent studies [66, 69, 70] indicate that many of the ISVs in the families *Bunyaviridae* [1, 5], *Flaviviridae* [63] and *Rhabdoviridae* [47] are ancient with highly diverse lineages suggesting that they likely evolved and diversified with their insect hosts. This notion that ISVs have been closely associated with their insect hosts for a long period of time is further supported by the fact that many of these appear to be vertically transmitted, whereas some become integrated within the genomes of their arthropod hosts [64]. Many of the extant arboviruses likely evolved from being ISVs to dual host viruses gaining the ability to infect the vertebrates upon which their insect hosts fed [1], and some may have completely adapted to their vertebrate hosts and lost the need for an insect host [1, 16]. Given the long evolutionary history and genome plasticity of ISVs, it is likely that others will develop the ability to infect vertebrates and thus become new emerging pathogens.

Influence on vector competence

The overwhelming evidence on the influence of ISVs on vector competence is derived from *in vitro* and *in vivo* experiments with ISFs. Initial *in vivo* studies with several *Culex* species suggested that mosquitoes infected with CxFV were less susceptible to secondary WNV infection than control mosquitoes (those without CxFV infection) [65]. Subsequent studies with another ISF, Palm Creek virus (PCV), indicated that it reduced replication of Kunjin (KUNV) and Murray Valley (MVEV) viruses in dually infected C6/36 cells [66]. A recent report indicated that NHUV significantly reduced replication of WNV, Japanese encephalitis (JEV) and St. Louis encephalitis (SLEV) viruses in co-infected C6/36 cells [67]. The mechanism of this reduction is unknown; but competitive inhibition is one possibility. The *in vitro* studies were performed in C6/36 cells, which have a dysfunctional RNAi response [7, 8] so the obvious next step is to determine if inhibition occurs *in vivo* (in live mosquitoes). The data suggest that the inhibition does not occur with all arboviruses, since the most pronounced reduction was observed in co-infection with WNV and SLEV (10^6 - to 10^4, respectively), whereas JEV co-infection exhibited an 80-fold reduction from peak titer [67]. A recent publication [68] indicated that EILV, an insect-specific alphavirus, also induces heterologous interference with several other alphavirus pathogens.

Possible Modes of Transmission

Currently the mechanism of ISV transmission and maintenance in nature is not known. Experimental [65, 69, 70] and field studies [19, 26, 29] suggest that transmission of insect-specific viruses in the genus *Flaviviridae* could be maintained by transovarial transmission (from adult female to progeny). Another possible mechanism may be venereal transmission as recent observations such as tissue tropisms of CxFV in *C. pipiens* [70] and CxFV transmission from naturally infected males to naïve females [65] support this notion.

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Moreover, the extensive mosquito host-range of mesoniviruses and negeviruses within the family Culicidae (*Aedes, Culex, Anopheles, Armigeres, Psorophora, Uranotaenia, Deinocerites, Wyeomyia* and *Trichoprosopan*)(N Vasilakis and RB Tesh, unpublished) and their inability to infect and replicate in laboratory animals and/or various vertebrate cell lines [2, 3] suggest that their hosts do not acquire them from feeding on viremic animals. Based on the negeviruses genomic organization, phylogenetic topology and close relationship to plant viruses of the genera *Cilevirus, Higrevirus* and *Blunervirus* [2, 11, 62], it is possible that their adult insect hosts acquire them by feeding on plant juices (e.g. nectar) or feeding on plant material present in the aquatic environment during their early stages of development [71]. Therefore elucidation of the mechanisms of ISV maintenance and transmission in nature, as well as homeostasis effects on their mosquito hosts, is important for understanding how they could potentially modulate arbovirus transmission.

Conclusion and future directions

The discovery of numerous ISVs in recent years within many virus families that harbor arboviruses of extreme public health importance has provided new insights into the complex nature of vector-borne disease systems. Experimental studies have demonstrated their host-restricted range in various arthropod vectors, in contrast to arboviruses, *sensu strictu*, which exhibit a dual host range in both vertebrate and invertebrate hosts. Phylogenetic analyses have also shown that a large number of these ISVs are closely related to human arboviruses, suggesting they may influence arbovirus transmission. However, many questions remain unanswered. Of highest priority, future studies should explore the ecology and distribution of these viruses in nature as well as their effect on their host homeostasis (e.g. pathogenicity and effect on their vector competence). Understanding microbial diversity in vector hosts is important for control strategies. Given that bacterial endosymbionts have shown promise as control agents in reducing vector competence of mosquitoes to transmit pathogenic arboviruses [72] or modulating their host's immune response to subsequent arbovirus infection [73, 74], it is likely that ISVs may have a similar effect and limited experimental studies [65, 66, 75] support this notion. Thus, further studies are warranted to investigate the potential for using ISVs as an innovative method for vector-borne disease prevention. Lastly, their potential use as platforms for vaccine or diagnostic development should be further exploited given their restrictive host-range facilitated by their defect for vertebrate cell infection at both the entry and RNA replication levels [76]. This inherent safety characteristic should be exploited to use recombinant DNA technology to generate candidate vaccines against emerging arboviruses of public health importance.

Acknowledgements

References

1. Li CX, Shi M, Tian JH, Lin XD, Kang YJ, Chen LJ, Qin XC, Xu J, Holmes EC, Zhang YZ. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-

sense RNA viruses. Elife. 2015; 4 ** Landmark study demonstrating that arthropods are a major reservoir of viral genetic diversity and their central to viral evolution

2. Vasilakis N, Forrester NL, Palacios G, Nasar F, Savji N, Rossi SL, Guzman H, Wood TG, Popov V, Gorchakov R, et al. Negevirus: a proposed new taxon of insect-specific viruses with wide geographic distribution. J Virol. 2013; 87:2475–2488. [PubMed: 23255793] **First description and characterization of the negeviruses as a novel ISV taxon

3. Vasilakis N, Guzman H, Firth C, Forrester NL, Widen SG, Wood TG, Rossi SL, Ghedin E, Popov V, Blasdell KR, et al. Mesoniviruses are mosquito-specific viruses with extensive geographic distribution and host range. Virol J. 2014; 11:97. [PubMed: 24884700]

4. Junglen S, Drosten C. Virus discovery and recent insights into virus diversity in arthropods. Curr Opin Microbiol. 2013; 16:507–513. [PubMed: 23850098]

5. Marklewitz M, Zirkel F, Kurth A, Drosten C, Junglen S. Evolutionary and phenotypic analysis of live virus isolates suggests arthropod origin of a pathogenic RNA virus family. Proc Natl Acad Sci U S A. 2015 **Seminal study suggesting suggest the arthropod origin of bunyaviruses

6. Marklewitz M, Zirkel F, Rwego IB, Heidemann H, Trippner P, Kurth A, Kallies R, Briese T, Lipkin WI, Drosten C, et al. Discovery of a unique novel clade of mosquito-associated bunyaviruses. J Virol. 2013; 87:12850–12865. [PubMed: 24067954]

7. Blair CD. Mosquito RNAi is the major innate immune pathway controlling arbovirus infection and transmission. Future Microbiol. 2011; 6:265–277. [PubMed: 21449839]

8. Brackney DE, Scott JC, Sagawa F, Woodward JE, Miller NA, Schilkey FD, Mudge J, Wilusz J, Olson KE, Blair CD, et al. C6/36 Aedes albopictus cells have a dysfunctional antiviral RNA interference response. PLoS Negl Trop Dis. 2010; 4:e856. [PubMed: 21049065]

9. Plyusnin, A.; Beaty, BJ.; Elliott, RM.; Goldbach, R.; Kormelink, R.; Lundkvist, Å.; Schmaljohn, CS.; Tesh, RB. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Academic Press; 2012. Bunyaviridae; p. 725-741.

10. Marklewitz M, Handrick S, Grasse W, Kurth A, Lukashev A, Drosten C, Ellerbrok H, Leendertz FH, Pauli G, Junglen S. Gouleako virus isolated from West African mosquitoes constitutes a proposed novel genus in the family Bunyaviridae. J Virol. 2011; 85:9227–9234. [PubMed: 21715500]

11. Auguste AJ, Carrington CV, Forrester NL, Popov VL, Guzman H, Widen SG, Wood TG, Weaver SC, Tesh RB. Characterization of a novel Negevirus and a novel Bunyavirus isolated from Culex (Culex) declarator mosquitoes in Trinidad. J Gen Virol. 2014; 95:481–485. [PubMed: 24262627]

12. Kuno G. A survey of the relationships among the viruses not considered arboviruses, vertebrates, and arthropods. Acta Virol. 2004; 48:135–143. [PubMed: 15595206]

13. Kuno G. Host range specificity of flaviviruses: correlation with in vitro replication. J Med Entomol. 2007; 44:93–101. [PubMed: 17294926]

14. Weaver SC, Barrett AD. Transmission cycles, host range, evolution and emergence of arboviral disease. Nat Rev Microbiol. 2004; 2:789–801. [PubMed: 15378043]

15. Cook S, Moureau G, Kitchen A, Gould EA, de Lamballerie X, Holmes EC, Harbach RE. Molecular evolution of the insect-specific flaviviruses. J Gen Virol. 2012; 93:223–234. [PubMed: 22012464]

16. Kuno G, Chang GJ. Biological transmission of arboviruses: reexamination of and new insights into components, mechanisms, and unique traits as well as their evolutionary trends. Clin Microbiol Rev. 2005; 18:608–637. [PubMed: 16223950]

17. Stollar V, Thomas VL. An agent in the Aedes aegypti cell line (Peleg) which causes fusion of Aedes albopictus cells. Virology. 1975; 64:367–377. [PubMed: 806166]

18. Yamanaka A, Thongrungkiat S, Ramasoota P, Konishi E. Genetic and evolutionary analysis of cell-fusing agent virus based on Thai strains isolated in 2008 and 2012. Infect Genet Evol. 2013; 19:188–194. [PubMed: 23871775]

19. Bolling BG, Eisen L, Moore CG, Blair CD. Insect-specific flaviviruses from Culex mosquitoes in Colorado, with evidence of vertical transmission. Am J Trop Med Hyg. 2011; 85:169–177. [PubMed: 21734144]

20. Goenaga S, Fabbri CM, Garcia JB, Rondan JC, Gardenal N, Calderon GE, Enria DA, Levis SM. New strains of Culex flavivirus isolated in Argentina. J Med Entomol. 2014; 51:900–906. [PubMed: 25118428]

21. Huanyu W, Haiyan W, Shihong F, Guifang L, Hong L, Xiaoyan G, Lizhi S, Rayner S, Aiqiang X, Guodong L. Isolation and identification of a distinct strain of Culex Flavivirus from mosquitoes collected in Mainland China. Virol J. 2012; 9:73. [PubMed: 22452813]

22. Kim DY, Guzman H, Bueno R Jr, Dennett JA, Auguste AJ, Carrington CV, Popov VL, Weaver SC, Beasley DW, Tesh RB. Characterization of Culex Flavivirus (Flaviviridae) strains isolated from mosquitoes in the United States and Trinidad. Virology. 2009; 386:154–159. [PubMed: 19193389]

23. Roiz D, Vazquez A, Rosso F, Arnoldi D, Girardi M, Cuevas L, Perez-Pastrana E, Sanchez-Seco MP, Tenorio A, Rizzoli A. Detection of a new insect flavivirus and isolation of Aedes flavivirus in Northern Italy. Parasit Vectors. 2012; 5:223. [PubMed: 23043958]

24. Bolling BG, Vasilakis N, Guzman H, Widen SG, Wood TG, Popov VL, Thangamani S, Tesh RB. Insectspecific viruses detected in laboratory mosquito colonies and their potential implications for experiments evaluating arbovirus vector competence. Am J Trop Med Hyg. 2015; 92:422–428. [PubMed: 25510714] ** First report of ISVs detected in laboratory mosquito colonies used for vector competence studies

25. Calzolari M, Ze-Ze L, Ruzek D, Vazquez A, Jeffries C, Defilippo F, Osorio HC, Kilian P, Ruiz S, Fooks AR, et al. Detection of mosquito-only flaviviruses in Europe. J Gen Virol. 2012; 93:1215–1225. [PubMed: 22377581]

26. Haddow AD, Guzman H, Popov VL, Wood TG, Widen SG, Haddow AD, Tesh RB, Weaver SC. First isolation of Aedes flavivirus in the Western Hemisphere and evidence of vertical transmission in the mosquito Aedes (Stegomyia) albopictus (Diptera: Culicidae). Virology. 2013; 440:134–139. [PubMed: 23582303]

27. Grisenti M, Vazquez A, Herrero L, Cuevas L, Perez-Pastrana E, Arnoldi D, Rosa R, Capelli G, Tenorio A, Sanchez-Seco MP, et al. Wide detection of Aedes flavivirus in north-eastern Italy--a European hotspot of emerging mosquito-borne diseases. J Gen Virol. 2015; 96:420–430. [PubMed: 25326313]

28. Crabtree MB, Sang RC, Stollar V, Dunster LM, Miller BR. Genetic and phenotypic characterization of the newly described insect flavivirus, Kamiti River virus. Arch Virol. 2003; 148:1095–1118. [PubMed: 12756617]

29. Sang RC, Gichogo A, Gachoya J, Dunster MD, Ofula V, Hunt AR, Crabtree MB, Miller BR, Dunster LM. Isolation of a new flavivirus related to cell fusing agent virus (CFAV) from field-collected floodwater Aedes mosquitoes sampled from a dambo in central Kenya. Arch Virol. 2003; 148:1085–1093. [PubMed: 12756616]

30. Blitvich BJ, Firth AE. Insect-specific flaviviruses: a systematic review of their discovery, host range, mode of transmission, superinfection exclusion potential and genomic organization. Viruses. 2015; 7:1927–1959. [PubMed: 25866904] ** Compreghensive review of mosquito-specific flaviviruses covering their discovery, host range, mode of transmission, superinfection exclusion potential

31. Kolodziejek J, Pachler K, Bin H, Mendelson E, Shulman L, Orshan L, Nowotny N. Barkedji virus, a novel mosquito-borne flavivirus identified in Culex perexiguus mosquitoes, Israel, 2011. J Gen Virol. 2013; 94:2449–2457. [PubMed: 23939978]

32. Lee JS, Grubaugh ND, Kondig JP, Turell MJ, Kim HC, Klein TA, O'Guinn ML. Isolation and genomic characterization of Chaoyang virus strain ROK144 from Aedes vexans nipponii from the Republic of Korea. Virology. 2013; 435:220–224. [PubMed: 23127596]

33. Takhampunya R, Kim HC, Tippayachai B, Lee DK, Lee WJ, Chong ST, Kim MS, Lee JS, Klein TA. Distribution and mosquito hosts of Chaoyang virus, a newly reported flavivirus from the Republic of Korea, 2008–2011. J Med Entomol. 2014; 51:464–474. [PubMed: 24724298]

34. Liu H, Gao X, Liang G. Newly recognized mosquito-associated viruses in mainland China, in the last two decades. Virol J. 2011; 8:68. [PubMed: 21314994]

35. Huhtamo E, Cook S, Moureau G, Uzcategui NY, Sironen T, Kuivanen S, Putkuri N, Kurkela S, Harbach RE, Firth AE, et al. Novel flaviviruses from mosquitoes: mosquito-specific evolutionary

lineages within the phylogenetic group of mosquito-borne flaviviruses. Virology. 2014; 464–465:320–329.

36. Huhtamo E, Putkuri N, Kurkela S, Manni T, Vaheri A, Vapalahti O, Uzcategui NY. Characterization of a novel flavivirus from mosquitoes in northern europe that is related to mosquito-borne flaviviruses of the tropics. J Virol. 2009; 83:9532–9540. [PubMed: 19570865]

37. Rizzo F, Cerutti F, Ballardini M, Mosca A, Vitale N, Radaelli MC, Desiato R, Prearo M, Pautasso A, Casalone C, et al. Molecular characterization of flaviviruses from field-collected mosquitoes in northwestern Italy, 2011–2012. Parasit Vectors. 2014; 7:395. [PubMed: 25160565]

38. Vazquez A, Sanchez-Seco MP, Palacios G, Molero F, Reyes N, Ruiz S, Aranda C, Marques E, Escosa R, Moreno J, et al. Novel flaviviruses detected in different species of mosquitoes in Spain. Vector Borne Zoonotic Dis. 2012; 12:223–229. [PubMed: 22022811]

39. Evangelista J, Cruz C, Guevara C, Astete H, Carey C, Kochel TJ, Morrison AC, Williams M, Halsey ES, Forshey BM. Characterization of a novel flavivirus isolated from Culex (Melanoconion) ocossa mosquitoes from Iquitos, Peru. J Gen Virol. 2013; 94:1266–1272. [PubMed: 23515021]

40. Pauvolid-Correa A, Solberg O, Couto-Lima D, Kenney J, Serra-Freire N, Brault A, Nogueira R, Langevin S, Komar N. Nhumirim virus, a novel flavivirus isolated from mosquitoes from the Pantanal, Brazil. Arch Virol. 2015; 160:21–27. [PubMed: 25252815]

41. Junglen S, Kopp A, Kurth A, Pauli G, Ellerbrok H, Leendertz FH. A new flavivirus and a new vector: characterization of a novel flavivirus isolated from uranotaenia mosquitoes from a tropical rain forest. J Virol. 2009; 83:4462–4468. [PubMed: 19224998]

42. Attoui, H.; Mertens, PPC.; Becnel, J.; Belaganahalli, S.; Bergoin, M.; Brussard, CP.; Chappell Ciarlet, JDM.; del Vas, M.; Dermody, TS.; Dormitzer, PR., et al. Reoviridae. In: King, AMQ.; Adams, MJ.; Carstens, EB.; Lefkowitz, EJ., editors. Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Academic Press; 2012. p. 541-637.

43. Attoui H, Mohd Jaafar F, Belhouchet M, Biagini P, Cantaloube JF, de Micco P, de Lamballerie X. Expansion of family Reoviridae to include nine-segmented dsRNA viruses: isolation and characterization of a new virus designated Aedes pseudoscutellaris reovirus assigned to a proposed genus (Dinovernavirus). Virology. 2005; 343:212–223. [PubMed: 16171838]

44. Auguste AJ, Kaelber JT, Fokam EB, Guzman H, Carrington CV, Erasmus JH, Kamgang B, Popov VL, Jakana J, Liu X, et al. A newly isolated reovirus has the simplest genomic and structural organization of any reovirus. J Virol. 2015; 89:676–687. [PubMed: 25355879]

45. Hermanns K, Zirkel F, Kurth A, Drosten C, Junglen S. Cimodo virus belongs to a novel lineage of reoviruses isolated from African mosquitoes. J Gen Virol. 2014; 95:905–909. [PubMed: 24443472]

46. Dietzgen, RG.; Calisher, CH.; Kurath, G.; Kuzmin, IV.; Rodriguez, LL.; Stone, DM.; Tesh, RB.; Tordo, N.; Walker, PJ.; Wetzel, T., et al. Rhabdoviridae. In: King, AMQ.; Adams, MJ.; Carstens, EB.; Lefkowitz, EJ., editors. Virus Taxonomy, Ninth Report of the International Committe on Taxonomy of Viruses. Academic Press; 2012. p. 686-713.

47. Walker PJ, Firth C, Widen SG, Blasdell KR, Guzman H, Wood TG, Paradkar PN, Holmes EC, Tesh RB, Vasilakis N. Evolution of genome size and complexity in the rhabdoviridae. PLoS Pathog. 2015; 11:e1004664. [PubMed: 25679389] ** Seminal study showing rhabdoviral genomic plasticity that may be linked to their discontinuous transcription strategy from the negative-sense single-stranded RNA genome, and propose a model that accounts for the regular occurrence of genome expansion and contraction throughout their evolution

48. Vasilakis N, Castro-Llanos F, Widen SG, Aguilar PV, Guzman H, Guevara C, Fernandez R, Auguste AJ, Wood TG, Popov V, et al. Arboretum and Puerto Almendras viruses: two novel rhabdoviruses isolated from mosquitoes in Peru. J Gen Virol. 2014; 95:787–792. [PubMed: 24421116]

49. Forrester NL, Palacios G, Tesh RB, Savji N, Guzman H, Sherman M, Weaver SC, Lipkin WI. Genomescale phylogeny of the alphavirus genus suggests a marine origin. J Virol. 2012; 86:2729–2738. [PubMed: 22190718] * Seminal phylogenetic study suggesting the aquatic origin of alphaviruses

50. Powers, AM.; Huang, H.; Roehrig, J.; Strauss, E.; Weaver, S. Togaviridae. In: King, AMQ.; Adams, MJ.; Carstens, EB.; Lefkowitz, EJ., editors. Virus Taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Academic Press; 2012. p. 1103-1110.

51. Griffin, DE. Alphaviruses. In: Knipe, DM.; Howley, PM.; Cohen, JI.; Griffin, DE.; Lamb, RA.; Martin, MA.; Racaniello, VR.; Roizman, B., editors. Fields Virology. 6th. Vol. 1. Wolters Kluwer Lippincott Williams & Wilkins; 2013. p. 901-964.

52. Nasar F, Haddow AD, Tesh RB, Weaver SC. Eilat virus displays a narrow mosquito vector range. Parasit Vectors. 2014; 7:595. [PubMed: 25515341]

53. Nasar F, Palacios G, Gorchakov RV, Guzman H, Da Rosa AP, Savji N, Popov VL, Sherman MB, Lipkin WI, Tesh RB, et al. Eilat virus, a unique alphavirus with host range restricted to insects by RNA replication. Proc Natl Acad Sci U S A. 2012; 109:14622–14627. [PubMed: 22908261] **First report desciribing the discovery of a novel host-restricted alphavirus

54. Lauber C, Ziebuhr J, Junglen S, Drosten C, Zirkel F, Nga PT, Morita K, Snijder EJ, Gorbalenya AE. Mesoniviridae: a proposed new family in the order Nidovirales formed by a single species of mosquito-borne viruses. Arch Virol. 2012; 157:1623–1628. [PubMed: 22527862]

55. Nga PT, Parquet Mdel C, Lauber C, Parida M, Nabeshima T, Yu F, Thuy NT, Inoue S, Ito T, Okamoto K, et al. Discovery of the first insect nidovirus, a missing evolutionary link in the emergence of the largest RNA virus genomes. PLoS Pathog. 2011; 7:e1002215. [PubMed: 21931546] **First report of the discovery of a novel nidovirus becoming the prototype member of the newly recognized family of mesoniviridae

56. Zirkel F, Kurth A, Quan PL, Briese T, Ellerbrok H, Pauli G, Leendertz FH, Lipkin WI, Ziebuhr J, Drosten C, et al. An insect nidovirus emerging from a primary tropical rainforest. MBio. 2011; 2:e00077-00011. [PubMed: 21673192]

57. Zirkel F, Roth H, Kurth A, Drosten C, Ziebuhr J, Junglen S. Identification and characterization of genetically divergent members of the newly established family Mesoniviridae. J Virol. 2013; 87:6346–6358. [PubMed: 23536661]

58. Thuy NT, Huy TQ, Nga PT, Morita K, Dunia I, Benedetti L. A new nidovirus (NamDinh virus NDiV): Its ultrastructural characterization in the C6/36 mosquito cell line. Virology. 2013; 444:337–342. [PubMed: 23891522]

59. Kuwata R, Satho T, Isawa H, Yen NT, Phong TV, Nga PT, Kurashige T, Hiramatsu Y, Fukumitsu Y, Hoshino K, et al. Characterization of Dak Nong virus, an insect nidovirus isolated from Culex mosquitoes in Vietnam. Arch Virol. 2013; 158:2273–2284. [PubMed: 23728735]

60. Warrilow D, Watterson D, Hall RA, Davis SS, Weir R, Kurucz N, Whelan P, Allcock R, Hall-Mendelin S, O'Brien CA, et al. A new species of mesonivirus from the Northern Territory, Australia. PLoS One. 2014; 9:e91103. [PubMed: 24670468]

61. Nabeshima T, Inoue S, Okamoto K, Posadas-Herrera G, Yu F, Uchida L, Ichinose A, Sakaguchi M, Sunahara T, Buerano CC, et al. Tanay virus, a new species of virus isolated from mosquitoes in the Philippines. J Gen Virol. 2014; 95:1390–1395. [PubMed: 24646751]

62. Kallies R, Kopp A, Zirkel F, Estrada A, Gillespie TR, Drosten C, Junglen S. Genetic characterization of goutanap virus, a novel virus related to negeviruses, cileviruses and higreviruses. Viruses. 2014; 6:4346–4357. [PubMed: 25398046] **Comphenesive manuscript elucidating the evolutionary relationship of negeviruses

63. Cook S, Chung BY, Bass D, Moureau G, Tang S, McAlister E, Culverwell CL, Glucksman E, Wang H, Brown TD, et al. Novel virus discovery and genome reconstruction from field RNA samples reveals highly divergent viruses in dipteran hosts. PLoS One. 2013; 8:e80720. [PubMed: 24260463]

64. Fort P, Albertini A, Van-Hua A, Berthomieu A, Roche S, Delsuc F, Pasteur N, Capy P, Gaudin Y, Weill M. Fossil rhabdoviral sequences integrated into arthropod genomes: ontogeny, evolution, and potential functionality. Mol Biol Evol. 2012; 29:381–390. [PubMed: 21917725] **Seminal report suggesting Rhabdoviruses likely evolved and diversified with their insect hosts

65. Bolling BG, Olea-Popelka FJ, Eisen L, Moore CG, Blair CD. Transmission dynamics of an insectspecific flavivirus in a naturally infected Culex pipiens laboratory colony and effects of coinfection on vector competence for West Nile virus. Virology. 2012; 427:90–97. [PubMed: 22425062]

66. Hobson-Peters J, Yam AW, Lu JW, Setoh YX, May FJ, Kurucz N, Walsh S, Prow NA, Davis SS, Weir R, et al. A new insect-specific flavivirus from northern Australia suppresses replication of West Nile virus and Murray Valley encephalitis virus in co-infected mosquito cells. PLoS One. 2013; 8:e56534. [PubMed: 23460804]

67. Kenney JL, Solberg OD, Langevin SA, Brault AC. Characterization of a novel insect-specific flavivirus from Brazil: potential for inhibition of infection of arthropod cells with medically important flaviviruses. J Gen Virol. 2014; 95:2796–2808. [PubMed: 25146007] **Seminal study demonstrating the effect of insect specific flaviviruses on the vector competence of mosquitos in vitro

68. Nasar F, Erasmus JH, Haddow AD, Tesh RB, Weaver SC. Eilat virus induces both homologous and heterologous interference. Virology. 2015; 484:51–58. [PubMed: 26068885]

69. Lutomiah JJL, Mwandawiro C, Magambo J, Sang RC. Infection and vertical transmission of Kamiti river virus in laboratory bred Aedes aegypti mosquitoes. J. Insect Sci. 2007; 7:1–7. [PubMed: 20337552]

70. Saiyasombat R, Bolling BG, Brault AC, Bartholomay LC, Blitvich BJ. Evidence of efficient transovarial transmission of Culex flavivirus by Culex pipiens (Diptera: Culicidae). J Med Entomol. 2011; 48:1031–1038. [PubMed: 21936322]

71. Roossinck MJ. Plants, viruses and the environment: Ecology and mutualism. Virology. 2015; 479-480C:271–277. [PubMed: 25858141]

72. Iturbe-Ormaetxe I, Walker T, SL ON. Wolbachia and the biological control of mosquito-borne disease. EMBO Rep. 2011; 12:508–518. [PubMed: 21546911] **Comprehensive review on the use of the bacterial endosymbiont Wolbachia as a biological control agent of mosquito-borne diseases of immense human health significance

73. Ramirez JL, Souza-Neto J, Torres Cosme R, Rovira J, Ortiz A, Pascale JM, Dimopoulos G. Reciprocal tripartite interactions between the Aedes aegypti midgut microbiota, innate immune system and dengue virus influences vector competence. PLoS Negl Trop Dis. 2012; 6:e1561. [PubMed: 22413032] **Seminal study demonstrating the modulating effects of insect microbiota on their immunity and vector competence

74. Pan X, Zhou G, Wu J, Bian G, Lu P, Raikhel AS, Xi Z. Wolbachia induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito Aedes aegypti. Proc Natl Acad Sci U S A. 2012; 109:E23–E31. [PubMed: 22123956]

75. Kent RJ, Crabtree MB, Miller BR. Transmission of West Nile virus by Culex quinquefasciatus say infected with Culex Flavivirus Izabal. PLoS Negl Trop Dis. 2010; 4:e671. [PubMed: 20454569]

76. Nasar F, Gorchakov RV, Tesh RB, Weaver SC. Eilat virus host range restriction is present at multiple levels of the virus life cycle. J Virol. 2015; 89:1404–1418. [PubMed: 25392227] **Seminal manuscript describing the mechanism of the host restriction of the prototype insect-specific alphavirus

Highlights

- ISVs are novel RNA viruses associated with hematophagous insects

- Belong in diverse families such as *Bunyaviridae, Mesoviviridae, and Rhabdoviridae*

- Little is known about the determinants of the ISVs restricted host range

- Little is known about their mode of transmission and maintenance in nature