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Promiscuous viruses – how do viruses survive multiple unrelated hosts?

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Abstract

Arthropod-borne viruses (arboviruses) require efficient replication in taxonomically divergent hosts in order to perpetuate in nature. This review discusses recent advances in our understanding of the phylogenetic position of arthropod-borne viruses relative to insect-specific viruses, which appear to be more common and ecological requirements for successful adoption of the “arbovirus phenotype.” Several molecular and other mechanisms that permit replication in divergent hosts are also discussed.

Many viruses appear to be host-specific, confining replication to a single type of organism, and frequently to a subset of cells within that organism. The mechanisms that confer host specificity are varied, but include several mechanisms that are well understood and have been extensively investigated. For example, HIV-1 requires the presence of the main receptor, the cluster of differentiation 4 (CD4) and a co-receptor, either chemokine receptor 5 (CCR5) or the alpha-chemokine receptor CXCR4. Specific combinations of these receptors are required in order for efficient virus infection to occur within humans. Notably, human immunodeficiency viruses arose from simian immunodeficiency viruses that acquired the ability to infect humans at some point in the not-too-distant past. This type of “species jump” has occurred frequently in history. The most dramatic recent example of this type of shift involved Ebolavirus jumping from (apparently) bats to humans, resulting in the extensive Ebola outbreak of 2013–2016¹. Viruses clearly have the ability to jump from one host species to another, often with devastating consequences.

Viruses that require more than one species for perpetuation in nature, however, pose a different challenge to science and public health. The most burdensome of these are the arthropod-borne viruses (arboviruses). These agents inherently, and by definition, possess the ability to productively infect hosts that are highly taxonomically divergent: arthropods and another host, typically vertebrates or plants. Arboviruses pose some of the most persistent and difficult problems facing humanity. The last two decades have witnessed the emergence and resurgence of several of these viruses at a global level. In addition to the

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emergence of several agricultural arboviruses such as bluetongue virus², Yellow Fever recently emerged in Angola and Brazil despite the existence of an extremely safe and effective vaccine^{3, 4}. West Nile virus, first introduced into the western hemisphere in 1999, continues to cause seasonal epidemics of neuroinvasive disease in the Americas⁵. Chikungunya virus has recently spread throughout much of the world's tropics, causing massive epidemics of debilitating arthritis⁶. Zika virus, of course, has now similarly spread in the tropical Americas, with devastating consequences⁷. Based on recent history, it seems likely that arboviruses will continue to emerge as global pathogens at an increasing rate as globalization, climate change, and international commerce increase.

Adopting a multi-host lifestyle seems to be inherently problematic for a virus. The body temperatures, cell types, and immune systems of arthropods are all profoundly different from vertebrates. Arbovirus infections in vertebrates may be pathogenic and tend to result in lifelong sterilizing immunity, ensuring that the virus only resides in the host for a short time (i.e. are acute). By contrast, arbovirus infections of invertebrates tend to be relatively benign and although immune pathways are activated in response to infection, the virus tends to persist for the life of the arthropods.

The mechanisms that permit arboviruses to successfully replicate in taxonomically distinct hosts are comparatively poorly understood. This review highlights recent advances that shed light on how multi-host viruses such as arboviruses retain the ability to productively infect hosts as taxonomically divergent as human beings and mosquitoes, with a particular focus on the flaviviruses and related, flavi-like viruses.

The advent of next-generation sequencing (NGS) and renewed interest in metagenomics has resulted in a rapid expansion in knowledge regarding the virome of many organisms. As part of these studies, it has become clear that most arboviruses are closely related to several non-arboviruses, including both vertebrate- and insect-specific viruses. These discoveries have spanned important arbovirus genera. For example, within the Flavivirus, Alphavirus and Bunyavirus genera, several insect-specific viruses have recently been discovered^{8, 9, 10, 11, 12}. Strikingly, the family *Flaviviridae*, including flavi-like viruses, encompasses viruses that replicate in arthropods only, arthropods and vertebrates, vertebrates only, plants only, and other non-arthropod invertebrates¹³. The number of non-arbovirus members of the *Flaviviridae* exceeds that of arboviruses, suggesting that invertebrates are the likely evolutionary reservoir of this group of viruses^{13, 14}. This, combined with the recent discovery of novel arthropod-specific genera of viruses with no known related arboviruses^{11, 15}, suggests that successful adoption of the arbovirus phenotype may be relatively uncommon. Interestingly, single-host viruses are interspersed within arbovirus taxa, suggesting that the promiscuity of a multi-host lifestyle, once gained, may also be lost¹⁶. Nonetheless, the presence of the arbovirus phenotype in multiple viral taxa suggests that opportunities for viruses to adopt it may be relatively common on an evolutionary timescale.

Whether these evolutionary opportunities ultimately result in the generation of a bona-fide arbovirus depends on two critical factors. The first is *vector competence*. Vector competence refers to the ability of a given virus to infect and replicate within a few key arthropod tissues,

including the arthropod midgut and salivary glands. Competent vectors acquire arboviruses during bloodfeeding, support replication with gut tissue and dissemination to secondary sites of replication, ultimately including the salivary glands. During subsequent feeding episodes, virus that has been secreted into salivary acini is inoculated into a new host.

The second critical factor is eco/epidemiological in nature and is referred to as *vectorial capacity* (reviewed extensively by Kramer et al. (2003)¹⁷). Vectorial capacity is the entomological restatement of the basic reproductive rate of a pathogen (R_0), and incorporates information on the abundance of *competent* mosquitoes, the degree to which they focus their feeding on susceptible vertebrate hosts, the probability that a mosquito survives one day, and the number of days required for a newly infected mosquito to become infectious to a new host (i.e. the *extrinsic incubation period*). Moreover, in order for a virus to be an arbovirus, it must be able to infect the right tissues of hematophagous arthropods, and those arthropods must have the right constellation of reproductive and feeding patterns to support movement of a virus between vertebrates. Arthropods are extraordinarily efficient at spreading viruses, as is clear from recent worldwide outbreaks of arbovirus-induced disease. The mechanistic underpinnings that allow arboviruses to replicate in widely divergent hosts are only partially understood.

Attachment and entry of arboviruses into host cells generally does not rely on a single host receptor molecule that binds a given virus surface protein. Supporting this, structural analyses of mature flavivirus and alphavirus particles reveal a relatively smooth topology with few obvious targets for receptor-ligand interactions. The current view of host cell entry by arboviruses involves attachment to host cells through interactions between viral envelope glycoproteins and various host cell molecules including C-type lectins in vertebrates^{18, 19} and invertebrates²⁰, Fc-gamma receptors in the case of Dengue virus, Axl^{21, 22}, and others, followed by clathrin-mediated entry into host cells^{21, 23}. Moreover, mechanisms of arbovirus host cell attachment and entry are becoming more completely understood and generally do not include specific receptor-ligand interactions that are required for successful infection of a given host.

Once within a host cell, arboviruses must suppress host innate antiviral immunity in order to replicate their RNA, assemble and be released from the cell. In general, virus-associated pathogen-associated molecular patterns (PAMPs) trigger host immunity that results in an antiviral state that interferes with these processes to varying degrees. Vertebrate and invertebrate antiviral pathways have notable similarities and differences. Many antiviral pathways converge around JAK/STAT, IMD and TOLL signaling pathways that are highly conserved across vertebrate and invertebrate taxa. Recently, *Aedes aegypti* mosquitoes were engineered to upregulate JAK/STAT signaling upon bloodfeeding, resulting in reduced DENV replication, but no reductions in chikungunya or Zika viruses²⁴. The more potent antiviral pathways, however, diverge between arthropods and vertebrates. In vertebrates, intracellular antiviral states are dependent on type I interferon signaling and production, while in arthropods (which lack interferon) RNA interference (RNAi) is the dominant antiviral pathway. In the flaviviruses, NS5 is a potent antagonist of interferon signaling through a variety of mechanisms²⁵, generally leading to degradation of STAT-2. In mosquitoes, NS5 likely functions in a similar manner due to the conservation of NS5-

targeted pathways, but the downstream effect of NS5 would not be conserved due to the lack of interferon in arthropod cells. Moreover, suppression of host antiviral mechanisms, in this case by the flavivirus NS5, appear to target upstream signaling molecules that have the capacity to suppress the formation of an antiviral state in a host-independent manner.

In addition, flaviviruses produce a small noncoding RNA derived from the 3' untranslated region (UTR) of their genome that is required for pathogenesis in vertebrates and cytopathology²⁶. Recently it has become clear that this subgenomic flavivirus RNA (sfRNA) functions in vertebrate cells to antagonize the interferon response by binding TRIM25²⁷. sfRNA also suppresses mosquito innate immunity due to stalling of the 5'-3' exonuclease XRN1²⁸, resulting in stoichiometric inhibition of Ago2 and Dcr, that bind to excess sfRNA and are thus unavailable to degrade full-length viral RNA²⁹. Indeed, sfRNA has now been shown to be a critical determinant of WNV infectivity to mosquitoes³⁰. Here again, a single virus-associated molecule, sfRNA, has the capacity to disrupt host antiviral responses in both hosts that are required for transmission in nature.

Virus-encoded secreted proteins also appear to have the capacity to mediate the two-host lifecycle that is integral to all arboviruses. This is clear in the case of the flaviviruses. The flavivirus nonstructural protein 1 (NS1) is another multifunctional viral nonstructural protein^{31, 32, 33, 34}. NS1 is secreted from flavivirus infected cells and reaches high levels in patient serum. Recent studies have demonstrated that NS1 is associated with vascular leakage in dengue hemorrhagic fever^{32, 35, 36}. NS1 alone from any of the four DENV serotypes led to vascular leak and secretion of proinflammatory cytokines. Further, anti-NS1 antibodies ablated these effects³⁵. However, severe pathology in human beings is not, in itself, strictly required for DENV perpetuation, although it may contribute under some circumstances. Importantly, NS1 of both dengue and Japanese encephalitis viruses appear to have a crucial function in mosquitoes, where it enhances flavivirus infection³⁷. Due to its significance to vertebrate pathogenesis and mosquito transmission, NS1 has thus been proposed as an attractive vaccine candidate because it could reduce the burden of disease directly in vaccinated individuals and decrease the intensity of transmission by interfering with infection of mosquitoes^{35, 37}.

Finally, the error-prone nature of the RNA-dependent RNA polymerases encoded by arboviruses confers adaptive plasticity that appears to significantly contribute to the ability of these viruses to infect two different host types. Studies conducted with West Nile virus demonstrated that mutational diversity within arbovirus populations contributes to their ability to escape from mosquito RNAi via a mechanism akin to negative, frequency-dependent selection^{38, 39}, a finding recently confirmed through studies of Powassan virus within host ticks⁴⁰. RNAi-mediated diversifying selection, combined with relaxed purifying selection within arthropods permits arboviruses to achieve relatively high levels of genetic diversity within these hosts. In contrast, purifying selection within birds leads to removal of deleterious mutations in a species-dependent manner, ultimately resulting in high-fitness virus populations⁴¹. Thus, the alternating host environments experienced by arboviruses may be beneficial because it allows for the exploration of a wide area of sequence space in arthropods while ensuring high fitness when the virus is transmitted back to vertebrates.

In sum, viruses that have adopted the phenotype of replicating in two distinct host types (arthropods and vertebrates) pose some of the most complex and difficult problems in public health. These viruses, commonly called arboviruses, appear to largely derive from a vast pool of arthropod-associated viruses that is not well understood and only partially characterized. These arthropod-associated viruses have, at various points during their evolutionary history, acquired (and lost) the ability to replicate not only in arthropods, but in the vertebrates that they feed upon. Several general characteristics of viruses, flaviviruses in particular, that possess this ability are the lack of requirement for a specific host receptor molecule for entry into host cells, giving them the ability to infect a broad array of organisms, and several different tissues within these organisms. Further, they have the ability to effectively suppress, circumvent or subvert both vertebrate and invertebrate immune pathways using virus-derived intracellular and secreted proteins and RNA structures. In many cases the same molecules function in vertebrates and invertebrates by targeting similar upstream signaling pathways or interfering with recognition of key viral PAMPS. Interestingly, in some cases it seems that a two-host phenotype may carry an adaptive benefit due to the different selective environments present in each host. While history has focused almost exclusively on arthropod-borne viruses that cause disease in humans and animals, it seems prudent to continue comparative studies of arbo- and insect-specific viruses to understand the underlying mechanisms that permit adoption of the “arbovirus” phenotype.

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Highlights

Adoption of a multi-host lifestyle, a phenotype that may be gained and lost, requires mechanisms for successful replication in hosts that are extremely taxonomically divergent.

Intense interest in metagenomics has led to the understanding that many medically important arboviruses derive from a vast pool of insect-associated viruses.

Arboviral structural and nonstructural proteins, and the viral RNA itself, meditates the multi-host lifestyle by subverting, suppressing and/or circumventing host immunity.