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# Promiscuous viruses—how do viruses survive multiple unrelated hosts?

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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.

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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 2013–2016 [1]. 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 emergence of several agricultural arboviruses such as bluetongue virus [2], 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 Americas [5]. Chikungunya virus has recently spread throughout much of the worlds' tropics, causing massive epidemics of debilitating arthritis [6]. Zika virus, of course, has now similarly spread in the tropical Americas, with devastating consequences [7]. 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 [8–12]. 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 invertebrates [13•]. 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 lost [16]. 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 and Ebel [17]). Vectorial capacity is the entomological restatement of the basic reproductive rate of a pathogen (R0), 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 though interactions between viral envelope glycoproteins and various host cell molecules including C-type lectins in vertebrates [18,19] and invertebrates [20], 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 viruses [24]. 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 mechanisms [25•], 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.