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West Nile virus and its vectors

Alexander T Ciota[1,2]

West Nile virus (WNV *Flaviviridae*; *Flavivrus*) is the most geographically widespread arbovirus in the world and the leading cause of arboviral encephalitis globally. Worldwide, WNV is maintained in an enzootic cycle between primarily *Culex* spp. mosquitoes and birds, with human infection and disease resulting from enzootic spillover. Dynamic and complex intrinsic and extrinsic factors contribute to the temporal and spatial variability in WNV transmission. The most current information on the relative contribution of each of these factors is reviewed and a case to incorporate detailed and localized environmental and genetic data into predictive models is presented.

Addresses
[1] The Arbovirus Laboratory, Wadsworth Center, New York State Department of Health, Slingerlands, NY, USA
[2] Department of Biomedical Sciences, State University of New York at Albany School of Public Health, Albany, NY, USA

Corresponding author: Ciota, Alexander T (alexander.ciota@health.ny.gov)

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History and epidemiology

West Nile virus (WNV) is a member of the *flavivirus* genus, in the family *Flaviviridae*, which is comprised of the most medically important mosquito-borne viruses in the world. In addition to WNV, notable flaviviruses include dengue virus (DENV), Zika virus (ZIKV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), St. Louis encephalitis virus (SLEV), and Murray Valley encephalitis virus (MVEV). Flaviviruses are enveloped RNA viruses with positive sense genomes encoding a single polyprotein which is pre- and post-translationally cleaved into three structural genes (capsid, pre-membrane/membrane, envelope), and seven non-structural genes (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) [1].

The first known isolation of WNV was from the blood of a febrile Ugandan woman in 1937 [2••]. Subsequent isolations were reported from the sera of Egyptian children in the 1940s [3] and Israeli adults as early as 1951 [4]. Early serological surveys indicated widespread dissemination of WNV in West African nations and a high prevalence of WNV in Egypt and India in the early 1950s [5–7]. A significant outbreak was reported in Israel in the summer of 1953, for which WNV was indicated as the causative agent of West Nile fever, a relatively mild, self-limiting febrile illness [8]. The first reports of an association between WNV and neuroinvasive disease were identified in elderly Israeli patients in 1957 [9], and subsequently in 1962 [10]. WNV arrived in Europe in 1958 [11] and sporadic and limited activity was reported through the early 1990s. Outbreaks of increasing intensity and severity were reported from 1996 to 2000 [12]. The first cases of WNV in the Western Hemisphere were reported in the New York City area in the summer of 1999 [13••], which also marked the first reports of significant virulence in avian hosts [14]. By 2005 WNV had reached across the U.S. and into Canada and Latin America [15]. Today, WNV is the most geographically widespread arbovirus in the world and the leading cause of arboviral encephalitis. In the U.S. alone there have been over 43 000 cases reported since 1999. Given that the large majority of infections are subclinical, this likely equates over 3 million infections [16,17].

Early experimental data confirmed the capacity of both *Aedes* and *Culex* spp. mosquitoes to transmit WNV [18•] and the first report of isolation of WNV from a mosquito was in 1952 in Egypt [19]. Extensive 3 year field and experimental studies in Egypt beginning in 1950 established the dominant transmission cycle of WNV as an enzootic cycle between predominantly *Culex* spp. mosquitoes and birds [20••]. Although there is significant variability in viremia levels and disease [21], over 300 species of birds have been identified as viable WNV hosts [22]. Given that competent hosts are readily available, the extent of WNV transmission is largely driven by the transmission potential of individual mosquito populations. This transmission potential, that is vectorial capacity, is governed by vector competence, feeding behavior and mosquito fitness. The factors contributing to each of these variables is reviewed.

Vector competence

Efficient transmission has been experimentally demonstrated in most mosquito species evaluated [23–26], with notable exceptions, including *Aedes aegypti* [27]. Despite the ubiquity of its competence, WNV is still predominantly vectored worldwide by members of the *Culex pipiens* L. complex, which includes *Cx. pipiens* and

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Culex quinquefasciatus in Africa, Asia and the Americas, and both *Cx. australicus* and *Cx. globcoxitus* in Australia [28]. *Cx. pipiens* is further separated into two bioforms, form *pipiens* and form *molestus*. *Cx. pipiens* form *molestus* are found in subterranean areas throughout the Americas and Europe [29]. Although hybridization between bioforms is relatively rare in most regions, *Cx. pipiens* form *pipiens* populations in the U.S. are known to possess some form *molestus* signature, while European populations tend to be 'pure', although recent studies demonstrate exceptions to this [30–34]. *Cx. quinquefasicatus* and *Cx. pipiens*, on the other hand, occupy similar niches and therefore readily hybridize in regions where they co-exist. In N. America., this hybridization zone stretches from 30°N to 40°N latitude, with *Cx. pipiens* to the north and *Cx. quinquefasciatus* to the south [35,36]. Although all members of the complex are efficient vectors, significant variability has been identified on both species and population levels [23,24,37–41]. Hybridization among species and bioforms within the complex has also been shown to significantly effect WNV competence [42,43]. Additional Culex species are known to dominate WNV transmission in some regions, including *Culex tarsalis* in the Western U.S [44], *modestusCulex* in parts of Europe [45], *Culex univittatus* in S. Africa [46] and *Culex annulirostris* in Australia [47]. The same mosquitoes that drive horizontal transmission during WNV outbreaks are also thought to be responsible for maintenance between these outbreaks. In milder climates this generally occurs through continued low level enzootic transmission, but in temperate regions in which mosquitoes enter diapause in winter months WNV is thought to overwinter in vertically infected adults [48•,49,50]. Vertical transmission, although relatively inefficient, has been demonstrated in Culex mosquitoes in the laboratory [51,52•].

While anatomical barriers regulating midgut infection, midgut escape, salivary gland infection, and transmission are well-documented and known to influence WNV competence [53–55], the molecular mechanisms that underlie susceptibility and transmission of WNV and other arboviruses in mosquitoes are still not well characterized [56]. RNA interference (RNAi) is thought to be the primary immune response to arboviruses in mosquitoes [57], and studies have shown that RNAi modulates WNV infection and replication in invertebrates [58•,59,60]. In addition, in a classic example of a host-pathogen 'arms race' evidence has recently emerged that flaviviruses have evolved strategies to evade both RNAi machinery and apoptosis in mosquitoes [61,62••,63•]. Although antimicrobial peptides (AMPs) and classical innate immune pathways of invertebrates have historically been associated with defense against bacteria and parasites [64,65], it is now clear that these pathways are also active against WNV and other viral pathogens. For instance, the blocking of specific AMPs has been shown to decrease WNV infectivity in mosquitoes [66], the Jak-STAT pathway can restrict WNV in mosquito cells [67•], the Toll pathway has been implicated in DENV control in *Ae. aegypti* [68,69], the Imd pathway contributes to defense against RNA viruses in Drosophila [70,71], and WNV infection in *Cx. quinquefasciatus* has been shown to lead to upregulation of markers of all these pathways [72,73]. Despite these robust immune responses, studies evaluating gene regulation during WNV infection have generally shown that genes involved in transporter and catalytic activity, rather than immune regulation, are most influenced by WNV infection [74].

It is now well established that interactions between microbial communities and mosquitoes can significantly impact competence for arboviruses [75]. Although the capacity of *Wolbachia pipientis* to modulate competence of DENV and other arboviruses, including WNV [76–78], has been most thoroughly studied, there are likely a range of direct and indirect effects of bacterial communities on mosquito immunity and WNV competence [73], and these communities likely vary substantially between and within populations [79]. An increased capacity for deep-sequencing using 'shotgun' approaches has begun to reveal the breadth of mosquito-only viruses that reside in vectors of WNV, including many flaviruses [80]. These include cell fusing agent virus [81], Kamiti River virus [82], *Culex* flavivirus [83], *Culex theileri* virus, Palm Creek virus, Quang Binh virus and Calbertado virus [84], among others. Evidence on the influence of these viruses on WNV competence is mixed [85], with some studies suggesting a suppressive effect on WNV with superinfection [86,87] and others suggesting an enhancement [88,89]. As with all of these interactions, the role of mosquito-only viruses in WNV competence is likely dependent on complex interactions between viral genotype, mosquito genotype, microbial communities and the environment.

The extrinsic factors that most directly influence vector competence of WNV and other arboviruses are dose and temperature. The probability of infection, dissemination and transmission are all highly dependent on WNV dose [90–92]. Dose is of course a product of host competence, specifically the magnitude and duration of avian viremia. Viremia levels are highly variable among avian species, and the role of host variability in WNV activity has been adequately reviewed elsewhere [93,94]. In general, increased temperatures increase viral replication rates in mosquitoes and therefore accelerates dissemination and abbreviates extrinsic incubation periods (EIPs; [95–97]). In addition to shorter EIPs, overall susceptibility and transmission rates are directly correlated to temperature, although the extent of which is dependent on both mosquito and viral genetics [98••]. Even small increases in transmission and, in particular small decreases to EIP, can have profound effects on overall vectorial capacity [99].

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