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Dynamics of West Nile virus evolution in mosquito vectors

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West Nile virus remains the most common cause of arboviral encephalitis in North America. Since it was introduced, it has undergone adaptive genetic change as it spread throughout the continent. The WNV transmission cycle is relatively tractable in the laboratory. Thus the virus serves as a convenient model system for studying the population biology of mosquito-borne flaviviruses as they undergo transmission to and from mosquitoes and vertebrates. This review summarizes the current knowledge regarding the population dynamics of this virus within mosquito vectors.

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Introduction

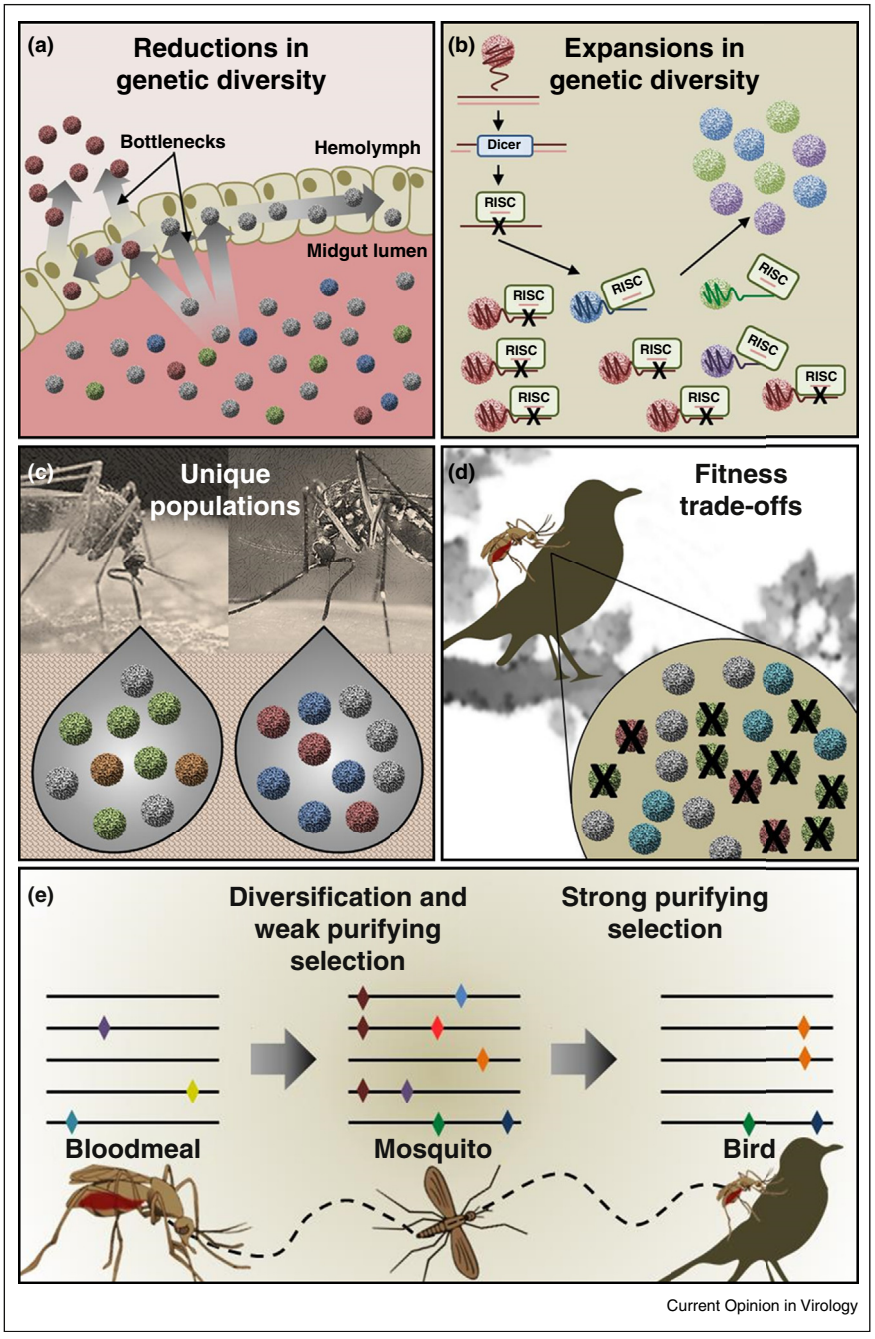
West Nile virus (WNV; *Flavivirus*; *Flaviviridae*) is a single-stranded positive sense RNA virus that exists in transmission cycles mainly involving *Culex* species mosquitoes and passerine birds. WNV was introduced to the Western Hemisphere in 1999 and was quickly spread throughout the US (reviewed by [1]). Understanding the mechanisms that contribute to rapid emergence and subsequent persistence of WNV almost 20 years later is critical for our understanding of other mosquito-borne outbreaks, such as the recent and ongoing epidemics of chikungunya virus (CHIKV) [2] and Zika virus [3] in the Americas. For example, molecular epidemiology demonstrated that WNV quickly adapted to local mosquito vectors during the invasion process [4–6], which likely enhanced transmission and facilitated its success [4,7]. CHIKV followed a similar pattern during the Indian Ocean epidemic when it adapted to be more efficiently transmitted by *Aedes albopictus* [8]. However, the current CHIKV epidemic in the Americas and some local emergences of WNV were not associated with previously observed vector-adaptive mutations [9,10]. What, then are the factors that favor the emergence of adaptive mutations within arbovirus populations? Although the answer is not entirely clear, experimental evolution studies of WNV are currently seeking to define these conditions.

WNV exists in nature as genetically diverse populations [11•]. Like other RNA viruses, genetic diversity is rapidly formed by error-prone polymerases ($\sim 10^{-4}$/site/round of replication [12•,13,14]), which seem to operate at optimal fidelity [15•,16,17]. Collectively, intrahost virus variants influence population fitness [18••,19], alter disease outcome [20••,21•], and provide opportunities for adaptation [22•,23]. However, the relationships between viral genetic diversity and phenotype become muddled once the temporal aspects of evolution are included: Viral populations are in constant flux. In general, WNV genetic diversity in mosquitoes is generated by strong diversifying selection [24••,25•], stochastically rearranged by bottlenecks [26••,27], and persist due to weak purifying selection [11•,28,29]. This produces greater diversity in mosquitoes than birds [30] and humans [31]. Here we outline the forces of selection and drift that alter WNV populations, microhabitat conditions that can direct the evolutionary pathway, and fitness costs during transmission (Figure 1).

Bottlenecks during systemic mosquito infection

Several physical barriers within mosquitoes impede systemic WNV infection and dramatically restructure viral populations. These mainly occur during entry and exit of the midgut and salivary glands (recently reviewed by [32,33]). Briefly, WNV must first infect the posterior portion of the midgut where contents of the bloodmeal are digested and absorbed. The virus must then pass through the basal lamina of the midgut and exit into the hemocoel to infect the hemocytes (invertebrate immune cells [34]), fat bodies, neurons, and muscle tissue [35]. Upon salivary gland infection, mature virions are transported and/or are directly released into an extracellular acinus (a holding place for saliva proteins). The contents of the acinus, including virus, are expectorated during mosquito probing and feeding. In general, *Culex* mosquitoes can expectorate 10^4–10^6 WNV plaque forming units during bloodfeeding [36]. Virus populations that pass

Figure 1



Dynamics of WNV evolution during mosquito transmission. **(a)** WNV population genetic diversity can be immediately reduced upon midgut infection through bottlenecks, introducing random genetic drift and founder's effects. These stochastic events occur during each major anatomical barrier to infection: midgut and salivary gland infection and escape. **(b)** WNV population genetic diversity can be rapidly restored through negative frequency-dependent selection introduced by RNAi. Essentially, common variants are more likely targeted by RNAi-mediated degradation while rare variants with mismatches between the template RNA loaded into RISC are allowed to replicate, increasing population complexity. **(c)** The influence of repeated random bottlenecks and RNAi-mediated diversification leads to the formation of unique subpopulations in different mosquito tissues and compartments, including what is expectorated in saliva. Furthermore, these processes influenced by the mosquito species, leading to very different WNV populations transmitted between different vectors. **(d)** The combined effects of bottlenecks, diversifying selection, and weak purifying selection lead to the accumulation many deleterious mutations into a population. In addition, mosquito-adapted variants are often not as fit in birds. Thus, there are fitness trade-offs in birds, which is predicted to remove many of the WNV produced within mosquitoes. **(e)** Together, the input WNV population taken up by mosquitoes during bloodfeeding drastically diverges and diversifies during mosquito infection, and weak purifying selection allows for many deleterious mutations to persist. During transmission to birds, strong purifying selection removes many of the variants, decreasing WNV population genetic diversity and maintaining fitness.