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Dynamics of West Nile virus evolution in mosquito vectors

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Abstract

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.

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? While 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 (~10^{-4}/site/round of replication [12–14]), which seem to operate at optimal fidelity [15–17]. Collectively,

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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 through these physiological barriers are subjected to genetic bottlenecks (small effective population sizes) [26,27,37,38]. This process can dramatically alter population demographics through random (i.e. nonselective) selection of only a few viruses that establish infection in the next tissue (genetic drift, founder's effects [39]). The number of infectious viruses that pass through a bottleneck is associated with the strength of the anatomical barrier; a weak midgut infection barrier tends to impose a weaker bottleneck than a strong barrier [26]. Most bottlenecks have a net negative effect on the virus population because they randomly fix low fitness mutations in a population [40,41]. Furthermore, systematic introductions of deleterious mutations can drive the population towards extinction unless mutation and/or recombination [42] can restore fitness (see Muller's ratchet [43]). In some cases, however, bottlenecks may be beneficial. For example, high fitness variants may be suppressed and remain at low population frequencies when the effective population size remains large [18]. However, if the variant can survive a bottleneck, it can reach dominance because it encounters fewer competitors.

RNAi-mediated diversification

Following genetic homogenization caused by bottlenecks during systemic spread, WNV populations must rapidly diversify in each tissue to evade the mosquito's primary antiviral response, RNA interference (RNAi) [44–46]. Viral RNA is targeted for degradation by sequence complementarity to a small template RNA loaded into the RNA-induced silencing complex (RISC). Mutant viruses are poorer matches to common RISC-loaded guide strands than are un-mutated viruses and therefore evade silencing. Thus RNAi creates an intracellular milieu that promotes diversification by allowing rare viral haplotypes to

replicate quickly until they are no longer rare [24,25]. This gives more genetically diverse populations a competitive advantage in mosquitoes [47].

Weak purifying selection

d_N/d_S ratios from intra-mosquito WNV populations are consistently greater than 1, suggesting that purifying selection is weak [11,26,29]. This could be directly related to the RNAi response, where selection happens at the nucleotide level and neither synonymous nor nonsynonymous mutations are favored. In addition, coinfection of multiple viral genomes within cells may also decrease purifying selection as they permit the persistence of deleterious mutations through complementation [26,48,49]. Together, we would expect that WNV genetic diversity would increase overtime with continued exposure to RNAi-mediated diversification and weak purifying selection. However, WNV diversity does not increase with longer extrinsic incubation periods during *Cx. quinquefasciautus* infection [49]. Diversifying selection and weak purifying selection therefore appear to be balanced by other forces that shape WNV populations within mosquitoes.

Variables altering the course of evolution

WNV is composed of as many as eight genetically distinct lineages and sublineages (reviewed by [1]), is an ecological generalist that can infect many mosquito species, and persists in several environments (reviewed by [50]). Therefore, the trajectory of WNV evolution is probably influenced by many different virus-, host-, and environment-dependent factors at a given time. Several of these are known to alter WNV demographics during mosquito infection. First, high frequency variants are more likely to survive bottlenecks [27] and the midgut bottleneck severity is inversely proportional to amount of virus in the bloodmeal [37]. Taken together, a WNV strain that can cause higher viremia in birds (e.g. [51,52]) may be more likely to maintain its diversity during initial mosquito infection. Second, we recently described how the species of mosquito involved in transmission can also strongly influence virus divergence, which may be associated with host susceptibility (virus replication and purifying selection) and the strengths of anatomical barriers (vector competence and bottleneck severity) [26]. These factors can be influenced by both the virus and the vector. For example, an enzootic pairing of Venezuelan equine encephalitis virus (VEEV) and *Cx. taeniopus* mosquitoes are more likely to maintain viral genetic diversity in than an epizootic pairing because more midgut cells become infected allowing for a larger effective population size (i.e. a weaker bottleneck) [53]. In addition, factors influencing the mosquito immune response, such as mosquito genetics [54] and its microbiome [55,56], can significantly alter vector competence. For example, pre-treating *Ae. aegypti* with antibiotics prior to dengue virus exposure increases the midgut viral titers due to lower immune activation in the absence of an intact microbiota [57]. In other cases, the presence of certain microbes, such as the endosymbiotic bacterium Wolbachia, can increase host resistance to WNV [58] and other mosquito-borne infections [59,60]. Third, environmental conditions impact virus-vector interactions in several ways. One of the most important of these is temperature. Higher temperatures can increase the ability of *Culex* mosquitoes to transmit WNV [61–63]. Given global climate change and that viruses are constantly emerging into new ecological niches, determining how temperature can drive mosquito-borne virus

evolution is of upmost importance. It may be that the positive correlation between temperature and vector competence also helps maintain viral genetic diversity and thus aids avoidance of the negative consequences of bottlenecks. Additionally, the frequency of spontaneous vesiculovirus mutation doubles when the temperature is raised from just 39 to 39.8 °C [64], suggesting that the WNV mutation rates could profoundly change during the 5–10 °C variations in mean temperature during the transmission season [65]. Ultimately, the numerous virus-vector-environment interactions that determine vector competence (e.g. [66–68]) may all slightly redirect WNV evolution and impact virus population structure.

Positive selection

Perhaps due to the requirement to cycle in two different hosts and the strong influences of genetic drift in mosquitoes, there is very little evidence for adaptive mosquito-borne virus evolution [69–73]. In fact, there are only a few known examples of positive selection the enhance virus replication or transmission within mosquitoes. The alphaviruses VEEV and CHIKV both utilized single amino acid substitutions in the envelope glycoprotein to increase vector competence of *Ae. taeniorhyncus* [74,75] and *Ae. albopictus* [8] mosquitoes, respectively. During the early years (2001–2003) of the North American WNV invasion, a locally derived variant (WN02) rapidly displaced the original (NY99) [4–6]. Again, the WN02 variant was demonstrated to contain a single amino acid substitution in the envelope protein (A159V) that conferred a fitness advantage by requiring a shorter extrinsic incubation period in *Culex* mosquitoes [4,7]. While the CHIKV E1 glycoprotein mutation (A226V) promoting enhanced infectivity in *Ae. albopictus* was experimentally reproduced in the laboratory [76], the same has not been demonstrated for the WN02 mutation. Specifically, the key mutation to WNV did not arise after NY99 infection of four species of birds [30] and mosquitoes [26]. One possible explanation for this is the homogeneity of the clone-derived, NY99 input virus population used in these studies. As demonstrated for CHIKV, epistatic interactions of mutations on the same haplotype have resulted in several multistep adaptive pathways [76–78]. The inclusion of genetic diversity in the founding WNV population may then allow the virus to follow more natural adaptive pathways, which may include the A159V mutation. Alternatively, adaptation and fitness are context specific. Possibly the conditions used in our experimental evolution studies, such as temperature [62], did not resemble the conditions that led to the NY99 displacement. What seems to be required is that a variant must have a very high fitness value to overcome its competitors within a mosquito. Alternatively, it needs be lucky enough to survive a random bottleneck and arrive in a more favorable environment with less competition. To summarize, we are critically lacking knowledge of the conditions that favor adaptive virus mutations to arise in mosquitoes and how they survive repeated bottlenecks.

Fitness trade-offs during transmission

Fundamental theories of evolution predict that genetic diversity provides viruses with opportunities for rapid selection, and therefore adaptation, during host shifts [79,80]. Mosquitoes can transmit unique and diverse virus subpopulations in their saliva allowing the virus to explore a tremendous amount of sequence space [26,27,49,76]. While the genetic

diversity provided to WNV during mosquito-borne transmission may be beneficial in some circumstances [22], in general, there is a fitness trade-off from mosquitoes to birds [81].

Our data suggests that surviving the mosquito environment, including (a) repeated bottlenecks imposing genetic drift, (b) RNAi-mediated diversifying selection, and (c) weak purifying selection, collectively imposes detrimental effects on WNV [26]. We therefore would predict that the WNV population will quickly revert towards the master sequence in birds contributing to the observed slow evolutionary rates of mosquito-borne viruses [82,83].

Conclusions

During mosquito infection, WNV populations change rapidly due to genetic drift and diversifying selection. The WNV genetic diversity thus produced likely provides opportunities for rapid adaptation and the emergence of new virus genotypes, but at the cost of lower relative fitness during transmission. Therefore WNV cycles between periods of genetic expansion in mosquitoes and selective constraint in birds. We hypothesize that periods of rapid transmission, such as during an explosive outbreak, are more likely to produce local adaptation because there are more opportunities for selection. Moreover, we also hypothesize that certain transmission cycles can increase these odds. For example, transmission involving *Cx. quinquefasciautus* vectors [26] and American robins [30] is more likely to allow the virus to explore new sequence space and select for highly fit variants. While a tremendous amount of progress has been made towards understanding the dynamics of WNV evolution, many questions remain regarding the factors that alter its path.

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Highlights

- WNV populations encounter several bottlenecks during systemic mosquito infection.
- Genetic diversity can be rapidly recovered by RNAi-mediated diversifying selection.
- Weak purifying selection acts to maintain deleterious WNV mutations.
- Many virus-, vector-, and environment-dependent factors can alter WNV evolution.
- WNV populations transmitted to vertebrates encounter a fitness trade-off.

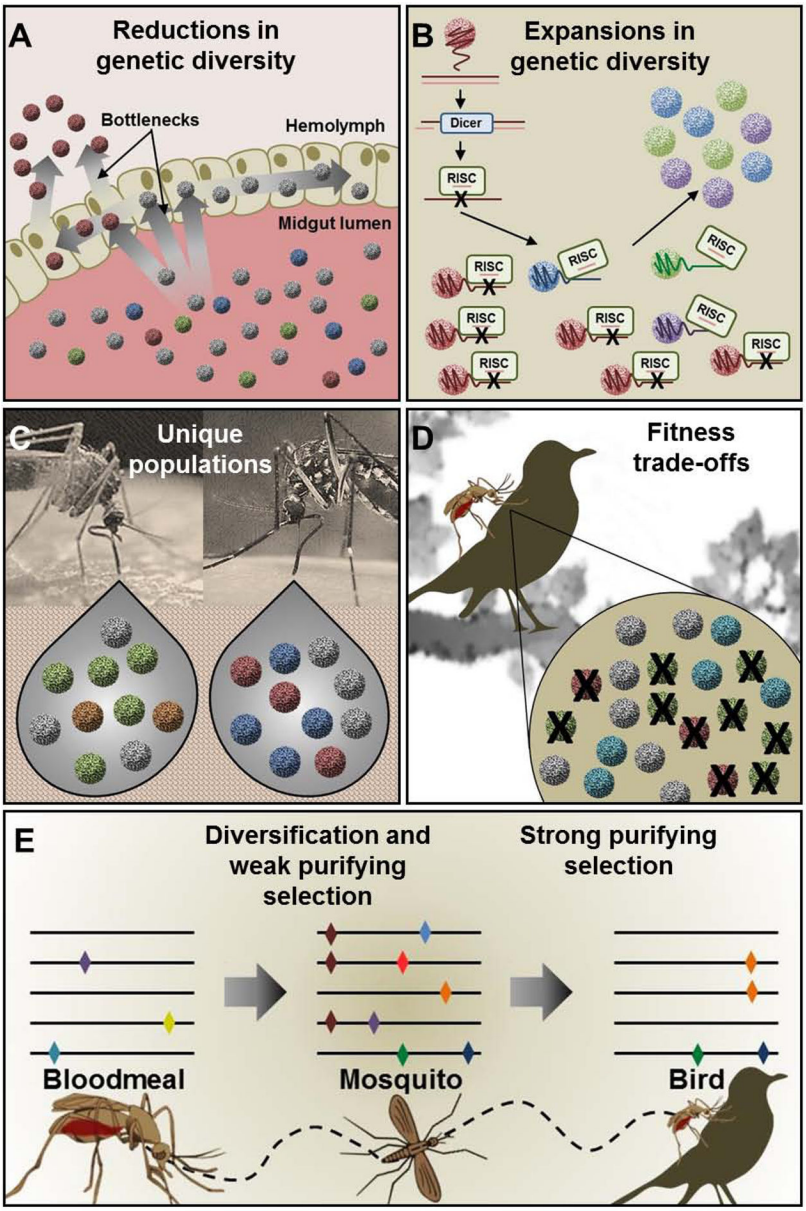

Figure 1.

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.