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Complexity of virus - vector interactions

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Introduction

Arboviruses are faced with the unique challenge of having two very disparate hosts in which they must replicate in order to perpetuate in the environment, the invertebrate vector and the vertebrate host. Together, these two hosts constitute the reservoir of the virus. The interrelationships among viruses, vectors and vertebrate hosts are complex and dynamic and shaped by biotic (e.g., viral strain, vector genetics, host susceptibility) and abiotic (e.g., temperature, rainfall, human land use) factors. Because arthropods are ectothermic, they are highly sensitive to increases in global temperatures [1], as are the viruses they transmit. It is anticipated that changes in climate, as predicted by the recent 5th Assessment Report of the Intergovernmental Panel on Climate Change [2], will result in landscape changes and consequent change in spatiotemporal patterns of arbovirus transmission. Understanding how arboviruses interact with mosquito vectors in such a dynamically changing environment is intrinsically important to estimate risk and design strategies to control arboviral pathogens. The intention of this review is to explore the interconnectedness of mosquito and virus biology, how that influences arbovirus transmission intensity through an impact on vector competence, survivorship, and feeding behavior; and how these aspects of mosquito biology affect vectorial capacity.

Vectorial capacity

The concept of vectorial capacity (VC), or R_0 of a pathogen, is the measure of a mosquito population's capacity to transmit an infectious agent to a new susceptible population. It integrates biotic and abiotic factors, enabling a clearer understanding of the impact of each on transmission of mosquito-borne pathogens. One basic formula for VC, a modification of [3] is $[ma^2 (I*T)p^n]/-\ln(p)$, where m is the vector density in relation to the host, a is the probability that a vector feeds on a host in 1 day. [A host preference index can be calculated as number of mosquitoes that blood-fed on the target host (e.g., human) minus the number that blood-fed on other vertebrates divided by the total number of mosquitoes that blood-fed on either host [4]].

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The probability that a vector survives 1 day is p; n is the duration of the extrinsic incubation period (EIP) in days; I (infection rate) * T (transmission rate) is equal to vector competence (b) or the proportion of vectors ingesting an infective meal that are later able to transmit the infection, and 1/−ln(p) is the duration of the vector's life in days after surviving the EIP. Vectorial capacity integrates viral factors with the biology of the mosquito vector. Further discussion of basic factors affecting vector competence, one aspect of VC, can be found in [5–7]. In addition, [8] among other publications, focuses on how VC of *Aedes aegypti* and *A. albopictus* impact the intensity of transmission of recent Aedes-transmitted viral threats.

Determination of VC is most informative when it takes into account subgroups of vectors that may contribute differentially to transmission risk. Therefore, refinements to the basic VC formula have been made by adding geographical, ecological, and epidemiological complexities to improve accuracy of the estimate, as VC varies spatially and temporally across a region. For example, impact of temperature on the EIP was incorporated into a dengue model [9]. A review of mathematical models of mosquito-borne pathogen transmission is presented by Reiner RC and colleagues [10]. In addition, there are a wide variety of behavioral, physiological, and morphological variations among local populations of mosquitoes that could potentially impact VC [11].

Vector competence

Vector competence is one aspect of VC. It defines the ability of the mosquito to become infected with and transmit virus following an infectious blood meal [12]. Physiologic barriers to infection and dissemination [5] and the immune response following infection [13,14] have been addressed thoroughly and thus won't be covered here. The major barrier is arguably the midgut infection barrier most likely due to mosquito and virus genetics, viral dose, receptor binding, uncoating, translation, or transcription. Research is actively addressing identification of cell receptors on the midgut, which was demonstrated by Ciota and Kramer to be a major bottleneck to West Nile virus (WNV) diversity [15]. This bottleneck and others the mosquito imposes on the virus, such as infection of the salivary glands, impact viral fitness and evolution [16]. With at least one alphavirus, Venezuelan equine encephalitis (VEE) virus at high doses, midgut escape also can present a bottleneck for the virus [16]. A still unresolved question is the means by which arboviruses disseminate from the midgut. Mechanisms behind these barriers have been reviewed thoroughly [17]. Surface structures of the virus particle itself may be responsible for efficient crossing of tissue barriers [18], and/or the barrier may be physically altered during virus replication [17].

Virus and vector genetics

Vector competence is affected by both virus and vector genetics. It is well known that mosquito species differ in their ability to become infected and transmit virus, but in addition, population differences are important within single species. For example, early studies demonstrated vector competence of *Culex tarsalis* for Western equine encephalitis (WEE) and St Louis encephalitis (SLE) viruses varied spatially in California. Differences in peroral susceptibility were observed among populations collected from different locations within a

contiguous geographical area as well as among cohorts of females collected as pupae from different breeding habitats sampled at the same location [19]. Similarly, vector competence of *Culex pipiens* for WNV was demonstrated [20] to vary spatially and temporally; as did Dengue virus (DENV) in different geographic populations of *A aegypti* [21].

Viruses also demonstrate inter- and intra- serotype-specific differences in infectiousness. For example, the ID50 for DENV-1 and DENV-2 were lower than for DENV-3 and DENV-4 in one study in Vietnam [22], and the American genotype of DENV-2 was more poorly transmitted by field populations of *A. aegypti* than the Asian genotype [23]. Even within the DENV-2 Asian-American genotype, one clade of virus had an early fitness advantage over another in *A. aegypti*, contributing to a clade replacement event [24] in Nicaragua.

Adaptive mutations in emerging virus strains may affect intensity of transmission by one species of mosquito and not another. For example, vector competence of East/Central/South African Chikungunya virus (CHIKV) by *A. albopictus* was facilitated by an amino acid change from alanine to valine at position 226 of CHIKV E1 glycoprotein (E1-A226 V), causing increased replication, midgut infection, dissemination, and transmission in this species, while no significant changes were observed in *A. aegypti*'s competence [25]. Further studies conducted on the importance of genotype x genotype x environment interactions with CHIKV examined *A. albopictus* transmission potential for CHIKV in six worldwide vector populations, with two virus strains and two ambient temperatures (20° and 28°C) [26]. The importance of the interaction between mosquito species, viral genetics, and temperature also was demonstrated with WNV where fixation of the E glycoprotein amino acid change A159V in WNV was facilitated by high temperatures and decreased extrinsic incubation period in *C. pipiens* and *C. tarsalis*, but not *C. quinquefasciatus* [27,28].

WNV and CHIKV adapted to the local mosquito species in naïve locations where they were introduced, as described above. Experimental studies have demonstrated that some arboviruses, e.g., WNV [29], have the capacity to adapt further in that they can evolve to replicate to higher titers and more efficient transmission in their arthropod hosts. Closely related viruses, such as SLEV, appear to exist at fitness peaks and do not demonstrate the same capacity to evolve [29]. Such adaptation may be associated with costs to the vector [30] and therefore experimentally adapted virus strains might not reflect what is found in nature. Resistance to infection also was found to be costly and was observed to have an equally negative impact on mosquito biology including survivorship and egg laying patterns following infection of *C. pipiens* with mosquito-adapted WNV [31]. This may result from cost of RNAi response in the mosquito, which plays a key role in immunity against infecting viruses [32]. Therefore, the mosquito is affected by the virus, and equally, the virus by the mosquito. Virus diversity is thought to be generated in the mosquito through relaxed purifying selection [33,34], although bottlenecks encountered as the virus replicates in the different tissues of the mosquito may restrict the presence of minority variants in the mutant swarm and thereby constrain evolution [15]. With DENV, more than 90 percent of the single nucleotide variants were lost with transmission from infected humans to *A. aegypti* and from mosquito abdomen to salivary glands, but new variants were generated at each stage of infection, thereby maintaining genetic diversity [35]. This heterogeneity of the virus population is likely important in allowing arboviruses to infect diverse hosts. The genetic

conservation generally observed in the consensus sequence of arboviruses may stem as much or more from intrahost pressures as interhost, but alternatively may be misleading in not reflecting heterogeneity of the intrahost viral swarm.

Survivorship

Two of the most important components of VC are survivorship and feeding frequency. Survivorship is important because the mosquito must become infected with virus in the initial blood meal and live long enough for virus to reach the salivary glands to be transmitted in a subsequent blood meal, thus EIP and survivorship are interconnected. Together they vary with the virus, dose, mosquito species, population genetics, and are affected by temperature. The interconnection of temperature and EIP was first demonstrated for yellow fever virus in *Aedes aegypti* by Davis [36] and has been demonstrated with many other arboviruses [27,37,38]. The importance of diurnal temperature range (DTR) as compared with mean temperature was demonstrated with *A. aegypti* infected with DENV [39], where mosquitoes were less susceptible to virus infection and died faster under larger DTR around the same mean temperature. But even these more realistic experimental designs have shortcomings, as mosquitoes may not be directly exposed to ambient temperatures, but rather choose optimal temperatures to rest, as in houses, in the grass, or in the shade.

Mosquito lifespan also has an impact on reproductive output of the mosquito, affecting population size and dispersal. It is difficult to measure survivorship in the field, and consequently good data are rare. Mark-recapture studies have been conducted to estimate lifespan [40], as well as analysis of cuticular hydrocarbons [41]. Most recently, investigators have been investigating age-related molecular and biochemical changes in mosquitoes including changing transcriptional and protein expression signatures. Age grading is based on changing gene transcription profiles measured using quantitative RT- PCR [42], and 2-D-differential in-gel electrophoresis to measure changes in *A aegypti* genome expression during aging [43].

Feeding frequency integrated with survivorship is critical because the mosquito must initially take an infective blood meal, become infectious, as described above, and feed again. Thus the oldest females are generally the most efficient vectors as virus is more likely to have become established in the salivary glands allowing successful transmission to occur when the female feeds. However, apoptosis has been observed in salivary glands infected with West Nile virus, increasing with time following infection [44], possibly inhibiting transmission. Smaller *A. aegypti* have been demonstrated to ingest blood more frequently than larger females [45], thereby allowing them to transmit virus more often, however another study found host-seeking was reduced with smaller *A. aegypti* [46]. Infected *A. triseriatus and A. albopictus* took smaller blood meals than uninfected siblings, potentially enhancing peroral transmission by causing the mosquito to feed more frequently. Mosquitoes infected with LACV and other viruses were demonstrated to probe more frequently during feeding attempts [47]. This behavior increases virus transmission, as virus is ejected each time the mosquito probes. Time required for feeding by DENV-infected mosquitoes also was shown to take significantly longer than the time required by uninfected mosquitoes. Similarly, the mean time spent probing was significantly longer in infected

mosquitoes than in uninfected mosquitoes [48], as was observed with LACV, but host seeking increased for LACV-infected *A. triseriatus*, but not *A. albopictus* [49]. Temperature also has an impact on feeding frequency. *A. aegypti* experiences reduced mobility and struggles to imbibe blood at temperatures below 14–16°C [50].

Impact of the larval environment

Not only is longevity of the adult mosquito affected by ambient temperature, but also the immature stages may be affected by temperature as well as other aspects of the environment. Nutrition availability and quality, and inter- and intra- specific competition for resources will affect survivorship, size and population density of the adult mosquitoes, nutritional storage and utilization, immunity, and reproduction [51]. Size and survivorship reflect energy reserves, and population density reflects fecundity and developmental success. Trade-offs exist between development time and both adult size and fitness, which can have significant downstream effects on feeding behavior and fecundity [52,53]. Alterations to these life history traits can lead to substantial variations in vectorial capacity of mosquitoes that harbor and transmit pathogens [54,55].

Temperature of the larval environment was demonstrated to significantly affect development time, immature and adult survival, mosquito size, blood feeding, and fecundity of both field and colonized populations of *Culex pipiens*, the northern house mosquito in the US and its sibling species, *C. quinquefasciatus*, the southern house mosquito, for WNV. However, in spite of their distinct geographic ranges, evidence of significant species-specific adaptation to temperature ranges was not seen [56]. Similarly, strong and consistent non-linear effects were measured in life history traits of distinct populations of *C. pipiens* across an altitudinal and latitudinal gradient in the eastern United States, with lack of support for local thermal adaptation [57]. Attempts to further adapt *C. pipiens* and *C. quinquefasciatus* to increasing temperatures as might occur during climate change, demonstrated adaptation of *C pipiens*, but not *C quinquefasciatus* using both field and colonized mosquitoes (Kramer and Ciota unpub). This result may reflect homogeneity of the *C. quinquefasciatus* population in the US [58] compared with the more heterogeneous *C. pipiens* [59]. Rearing temperature also had a significant effect on *C. tarsalis* developmental parameters, including shorter time to pupation and emergence and smaller female body size as temperature increased [60].

Other studies have shown that larval rearing temperature can affect mosquito competence for select arboviruses, including Murray Valley encephalitis (MVE) [61], Japanese encephalitis [62], and WEE [63], and DEN [64] viruses. However, infection, dissemination, and transmission rates for West Nile Virus (WNV) at 5, 7, and 14 days post infectious feeding were not consistently affected by rearing temperature [60]. Similarly, there was no change in vector competence of Culex spp for WEE, SLEV, MVE and Rift Valley fever (RVF) viruses, when larval rearing temperatures varied. Aedes spp on the other hand were affected following rearing at different temperatures when tested for vector comp to RVF, VEE, CHIK viruses.

Smaller nutrient-deprived mosquitoes have been noted to be more susceptible to infection with some arboviruses than larger mosquitoes, as has been observed with LaCrosse virus in

Ae triseriatus [6]. Dodson and colleagues found that nutritional stress increased *Culex tarsalis* larval development time and reduced adult size but as with rearing temperature, there was no significant effect on vector competence for WNV [65]. Nasci and colleagues found that smaller *Ae aegypti* acquired a higher concentration of virus per body weight than larger individuals [66]. Smaller-sized *A. aegypti*, females, regardless of rearing conditions, were significantly more likely to become infected and to disseminate DENV than larger individuals [67], A molecular basis for increased susceptibility of *A. aegypti* following nutritional stress was suggested to be upregulation of several cecropin transcripts in small mosquitoes.

Other components of mosquito biology that impact VC

Insect-specific viruses (ie, RNA viruses that replicate only in insects and not in vertebrate hosts) are now recognized as being pervasive and may alter vector competence. These viruses represent a broad range of families, including *Flaviviridae* and *Togaviridae* [68]. The microbiome of the mosquito also may affect virus infection of mosquitoes. Complex interactions have been reported between infecting microbes and virus infection [69–71]. Other factors such as competition, landscape, precipitation also influence VC and must be studied as well.

Conclusions

These studies point out the dynamic nature and complexity of virus-vector interactions and the inability to generalize from one mosquito or virus species to another, and equally one population to another. Temperature both in the larval and adult stages has an impact on nearly every aspect of vectorial capacity and must be considered in virus-vector interactions. Research is needed to elucidate how climate change and anthropogenic changes to the environment are affecting mosquito population biology and virus transmission.

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Highlights

- Virus-vector interactions are complex and dynamic, affected by biotic and abiotic factors.
- Vectorial capacity integrates viral factors with the biology of the mosquito.
- The two most important components of vectorial capacity are survivorship and feeding frequency on susceptible hosts
- Virus and vector genetics affect vectorial capacity and interact in a genotype x genotype manner.
- The larval environment has impact on vectorial capacity equal to the adult environment; both will be affected by climate change in variable manner