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Dissecting vectorial capacity for mosquito-borne viruses

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Abstract

The inter-relationship between mosquitoes and the viruses they transmit is complex. While previously understood barriers to infection and transmission remain valid, additional factors have been uncovered that suggest an “arms race” between mosquito and virus. These include the mosquito microbiota and interplay between mosquito and viral genetics. Following an infectious blood meal, the mosquito mounts an immune and transcriptional response, leading to altered expression of multiple genes. These complex interactions, specific to vector and virus genotypes, combine with external influences, particularly temperature, to determine vector competence. The mosquito’s response to the infecting agent may have consequences in terms of longevity, feeding behavior and/or fecundity. These factors, together with population density and the frequency of host contact determine vectorial capacity.

Introduction.

Arboviruses are dependent on intricate relationships with their arthropod vectors and vertebrate hosts, and these relationships are further shaped by environmental and other conditions, referred to as abiotic, or extrinsic, factors. Vectorial capacity is affected by both sets of factors. Abiotic factors affect replication of the virus, longevity of the mosquito, density and composition of the mosquito and vertebrate populations, and contact with appropriate vertebrate hosts. Intrinsic factors, on the other hand, may be inherited and influence vector competence, i.e., the ability of the mosquito to become infected with an arbovirus after an infective blood meal and subsequently transmit virus, as well as blood feeding patterns, and more. This review will focus on how the biology of the mosquito affects arboviral transmission.

Following ingestion of an infectious blood meal, initial replication of the virus takes place in midgut epithelial cells, then virus enters the haemocoel from where it disseminates to other tissues, most importantly the salivary glands and ducts. The transmission cycle is complete when arboviruses present in the arthropod's salivary glands are released into the saliva to infect susceptible vertebrate hosts as the mosquito takes subsequent blood meals. To understand the basic concepts of infection, dissemination, and transmission, the reader is directed to an excellent review by Hardy and colleagues [1]; new techniques in molecular biology have further elucidated the basic concepts put forth therein, and will be presented here.

Vector competence

Infection of the mesenteron.

The most important organ that determines vector competence for arboviruses is arguably the mosquito mesenteron (midgut). This organ consists of a single layer of epithelial cells

surrounded on the abluminal side by the basal lamina, a multilayered membrane[2]. Virus in the infective blood meal is primarily deposited in the posterior portion of the mesenteron[1, 11,12] although exceptions have been observed, for example[3]. A threshold level of virus is required to infect the mosquito successfully;this threshold is specific to the virus and mosquito species. For example,[4] demonstrated differences between St Louis encephalitis virus (SLEV;

Flaviviridae:Flavivirus) and Western equine encephalitis virus (WEEV;*Togaviridae:*

Alphavirus) infection of *Cxtarsalis*, and differences in both intraspecific and interspecific infection rates in *Cxtarsalis* populations. As another example, [5] demonstrated differences between infection and transmission rates of WNV with diverse mosquito species fed on a low and high dose of WNV. When a mosquito species is refractory to infection, it has a midgut infection barrier (MIB). Immunological and physical barriers including proteolytic enzyme upregulation, the RNA interference (RNAi) pathway, peritrophic matrix formation, the physical barrier of midgut epithelial cells, and the midgut flora contribute to the MIB [6].

The mechanism of viral entry into the midgut cell remains controversial. Direct fusion of Dengue virus(DENV; *Flaviviridae:Flavivirus*) with the plasma membrane of mammalian and mosquito cells has been proposed[7], as has entry via non-classical endocytic pathway independent from clathrin[8]. West Nile virus (WNV; *Flaviviridae:Flavivirus*) has been described to enter cells through clathrin-dependent endocytosis and co-localize with early and late endosomes[9]. The fusion of WNV with cellular membranes has also been demonstrated[10]. Using virus obtained directly from mosquito saliva, Vancini and colleagues demonstrated DENV and WNV may infect cells by a mechanism that involves direct penetration of the host cell plasma membrane as proposed for alphaviruses [11]. For example, EM studies of WEEV and *Culextarsalis* suggest

viral entry into mesenteron epithelial cells with some virus/mosquito pairs infecting via direct membrane fusion [12] rather than into vesicles as occurs in vertebrate cells.

Few cells in the posterior portion of the midgut are initially infected following an infectious blood meal. It was demonstrated that following feeding on Venezuelan equine encephalitis virus (VEEV; *Togaviridae: Alphavirus*) replicons, the average number of midgut cells infected was 28. WNV was demonstrated to undergo a bottleneck in *Cxpiens* during initial infection of the midgut following the infectious blood meal [13], as was also observed with *Cxquinquefasciatus* [14], although bottlenecks in peripheral tissues were not seen in the latter species while they were in *Cxpiens*. *Culex tritaeniorhynchus* and *Culex pipiens* infected with Japanese encephalitis virus (JEV; *Flaviviridae: Flavivirus*), and *Aedes albopictus* infected with DENV also demonstrate few cells infected in the posterior portion of the midgut following feeding on an infectious blood meal, suggesting a bottleneck will likely occur [15,16].

Dissemination.

Following replication in the midgut, virus must disseminate to the parenteral tissues in the mosquito. The majority of evidence supports passage of virus through the midgut basal lamina into the hemolymph, which then serves as the source of infection of hemocytes, fat body, reproductive tissue, and ultimately the salivary glands. But because the midgut basal lamina has pore sizes significantly smaller than arboviruses [16], a longstanding question has been how viruses bypass the noncellular basal lamina that coats the epithelia, including those of the midgut and salivary glands. A potential alternative route of dissemination of viruses from the midgut epithelium and into the body cavity is via the trachea [17]. Inability of virus to disseminate from the midgut is referred to as a midgut escape barrier [18]. This is largely genetic, but may also be related to the infecting dose [18,19]. Selected DENV strains have been demonstrated to have

equal infectiousness for the midgut, yet differ significantly in replication and dissemination throughout *Aedes aegypti* parenteral tissues, including the salivary glands, and therefore differ significantly in their potential to be transmitted to humans [20].

Transmission.

Although this review only will address peroral transmission from a vector to a vertebrate, other modes of transmission exist, e.g., vertical transmission, both transovarial (TOT; virus in egg; [21] and vertical (virus on surface of egg as for certain flaviviruses; [22]), co-feeding transmission from an infected arthropod to uninfected arthropods feeding in close proximity and without evidence of a substantial viremia in the host [23], first noted with tick-borne pathogens [24], and non-biologic transmission such as by mechanical transmission, as with Rift Valley Fever virus [25]. Peroral transmission, as with other aspects of vector competence, is virus- and vector species-specific (see references for infection differences). The time from ingestion of an infectious blood meal to transmission of virus is the extrinsic incubation period (EIP) and is dependent largely on viral dose, temperature of extrinsic incubation, and genetics. Rarely, a salivary gland escape barrier has been reported where virus is present in the salivary glands, but is inefficiently transmitted, as has been noted with Lacrosse virus (LACV, *Bunyaviridae: Orthobunyavirus*) infection in *Aedes hendersoni* [26].

During peroral transmission, substances injected with the salivary secretions during feeding have been demonstrated to contain antihemostatic substances, and have potent properties, such as to enhance viral replication or act as immunosuppressants [17]. Proteins in the saliva with antiplatelet, anticoagulation, and vasodilation activities have been characterized genetically and biochemically [27]. Replication and pathogenesis of numerous arthropod-borne viruses,

including DENV [28] and WNV [29], have been demonstrated to be enhanced by addition of mosquito salivary secretion to the inoculum, or following infected mosquito feeding [30].

Vectorial capacity.

Experimental studies have disproportionately focused on assessing vector competence exclusively to evaluate the potential for arbovirus emergence and spread among vector populations, yet vectorial capacity (VC) is influenced to a larger degree by population density, bloodfeeding behavior and vector longevity [31,32]. VC can be estimated by the basic formula:

$$VC = ma^2bp^n / -\log_e p \quad (1)$$

where m=number of female mosquitoes per host, a= daily blood feeding rate, b= transmission rate among exposed mosquitoes, p= the probability of daily survival, and n=extrinsic incubation period, based on [33]. Highly competent mosquitoes may be inadequate vectors if the frequency of contact of feeding on amplifying hosts is low or if vectors are short-lived relative to the EIP of the pathogen. Conversely, a poorly competent vector can sustain or expand an outbreak if EIP is short, feeding is frequent or population density is high. *Ae. aegypti* are unique in their frequency of bloodmeal acquisition from humans, and a study with Yellow Fever virus (YFV; *Flaviviridae:Flavivirus*) demonstrated that a poorly competent population of *Ae. aegypti* was responsible for facilitating an outbreak in Nigeria, likely due to both frequent blood feeding and high population density [34]. Although life history traits can be estimated in the laboratory, accurate quantification of these factors in the field is difficult and this is further complicated by the fact that it is now well-documented that pathogen infection can influence both bloodfeeding behavior and longevity [35-41].

Costs of infection.

The terms ‘vector’ and ‘extrinsicincubation’ imply a relatively benign, commensalvector-pathogen relationship, yet studies in recent years demonstrate that these interactions are instead highly complex and that infection of vectors can often be virulent. Decreases in longevity of mosquitoes has now been associated with infection of WNV[40,41], CHIKV [38], DENV[35],and Eastern equine encephalitis virus(EEEV; *Togaviridae: Alphavirus*)[36,37], among others. WNV infection of *Cx. quinquefasciatus* is associated with both salivary gland pathology [42]and apoptosis of midgut cells[43], and cytopathology has also been noted with alphavirus infection of mosquitoes [44,45]. It is not known whether it is pathology, metabolic costs or a combination of factors that ultimately result in decreased vector survival.

In addition to effects on longevity, infection has also been associated with decreased fecundity and increased blood feeding rates, which could ultimately increase transmissibility [39,46]. Our understanding of how costs of infection alter transmissibility are confounded by the fact that these relationships can be both vector population and virus strain-specific[35,38,40,41]. In addition to costs of infection, these studies demonstrate costs of resistance in vectors, with decreases in mosquito fitness associated with exposure to WNV and DENV in the absence of established infection[35,40,41]. This suggests that vector defenses, in addition to viral genotype and dose, contribute to infectivity and viral fitness in mosquitoes. From an evolutionary perspective, the maintenance of a resistance mechanismis consistent with infection being costly and tolerance not always being attainable. Such relationships could contribute to determining vector competence. Specifically, if the cost of resistance outweighs the cost of infection then competence is predicted to be high, and alternatively if infection is more costly then resistance competence should be low[40]. The potential flaw in the idea thatcompetence is a product of vector-virus co-evolution is that it requires that vector populations experience sufficient exposure

to pathogens to apply evolutionary pressure. Although prevalence of arboviruses is generally low, even during epidemic activity, the likelihood of exposure to potentially pathogenic RNA viruses during a mosquito's lifetime may be high. Consistent with this, recent studies of *Ae. aegypti* demonstrate that genes linked to the RNAi pathway, which is thought to be primarily responsible for RNA virus defense [47], are under relatively high levels of positive selection [48].

Genetic and microbial interactions.

Although there are multiple examples of viral genetic changes significantly altering competence in one or more species, including WNV in *Culex* spp. [49] and Chikungunya virus (CHIKV; *Togaviridae: Alphavirus*) in *Ae. albopictus* [50], it is somewhat surprising that more adaptive evolution is not reported given the pace at which these RNA pathogens accumulate mutation and explore sequence space. Although this is generally explained by the fact that adaptive trade-offs associated with host cycling suppress single host adaptation [51], this could partially be due to the fact that evolutionary pressures are population-specific and therefore generic adaptations are rare. Consistent with this idea are repeated data demonstrating strain and population-specific competence as well as genotype by genotype interactions [52-54]. Transcriptome analyses have shown that arbovirus infection leads to the upregulation of many genes in mosquitoes, including transcription factors, ion-binding proteins, and many metabolic proteins, as well as downregulation of protease and pupal cuticle protein genes, among others [55-57]. It has been shown that transcriptional response to DENV is largely dependent on vector genotype [58] and that DENV competence is partially dependent on interactions between viral genotype and genotype of *dicer-2*, the primary enzyme in the RNAi pathway [59]. Furthermore, the RNAi targeting of specific regions of WNV leads to genomic diversification of those regions [60]. In

addition to RNAi, the JAK-STAT and Toll signaling pathways have also been implicated in arboviral defense in mosquitoes [61-63] and up-regulation of specific genes broadly influencing innate immune expression is likely dependent on virus genotype and thereby a significant contributor to vector competence.

Variability in microbial communities can also have profound effects on vector competence [64,65]. The most established of microbial-arboviral relationships is the generally inhibitory effect of *Wolbachia* on arboviral fitness and competence [66-68], which is now being exploited as a DENV control measure [69]. Others have shown that there are likely complex interactions between microbial populations, mosquito immunity and arboviruses which may ultimately prove as important in determining population-specific competence as mosquito and virus genetics [70-73]. In addition, it is now well recognized that mosquito-specific viruses are likely ubiquitous and abundant [74-77] and direct or indirect interactions can result in heterologous and/or homologous interference and alterations in competence for human pathogens [78-81]. Together, these studies demonstrate that understanding and predicting vector competence requires a holistic approach.

Conclusions.

Vectorial capacity of mosquitoes for arboviruses is both vector population and virus strain-specific. It involves a complex interplay between biotic and abiotic factors, and between the mosquito and infecting virus. Pathogen exposure impacts the mosquito – survivorship, fecundity, blood feeding behavior - and the reverse is also clearly true where the virus is dependent on the competence of the mosquito to be transmitted to a susceptible host. Future studies should exploit molecular techniques to identify specific genetic signatures of virus and vector that contribute to fitness and transmissibility. The role of the microbiota of the mosquito is just beginning to be explored, as is the impact of mosquito-specific viruses on infection and transmission. To more

fully understand the ecology and emergence of arboviruses it is critical to focus on vectorial capacity and not vector competence alone. Furthermore, it is important to elucidate the factors in this complex interplay to find novel approaches to control.

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