4. Vector competence for arboviruses in relation to the larval environment of mosquitoes

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Abstract

Mosquito vector competence studies are reviewed to identify the species and environmental conditions that modify susceptibility to infection and transmission of pathogens. Most studies on vector competence have focused on environmental conditions experienced by adults, but the larval environment shapes the phenotypes of adult mosquitoes and may, thereby, alter vector competence for arthropod-borne (arbo)viruses. This review summarizes results of studies on the effects of nutrition, competition, temperature and insecticides during the larval stages on adult vector competence for arboviruses. A statistical analysis of previously published work supported the conclusion that larval environment may significantly alter susceptibility to infection, dissemination, and virus transmission. These effects show multiple and environmentally specific effects on barriers to virus infection. Involvement of multiple virus barriers in the adult mosquito suggests that several factors may be responsible for the alteration of vector competence by larval environment.

Keywords: arbovirus, competition, insecticide, nutrient limitation, temperature, vector competence

Introduction

Studies to the assess ability of mosquitoes to vector pathogens have played a central role in advancing our understanding of mosquito-borne disease transmission and led to improvements in control practices. Vector competence is the intrinsic permissiveness of an arthropod to infection, replication, and transmission of a pathogen (Hardy 1988, Woodring *et al.* 1996) and it serves as a measure of vectoring ability. Measurements of vector competence can be integrated with other ecological and behavioural characteristics of mosquitoes (abundance, longevity, feeding habit) to estimate vectorial capacity. Vectorial capacity, the average number of potentially infective bites received by a host in a single day, may be used to guide control efforts aimed at reducing contact rate between infected vectors and target hosts. A central goal of mosquito vector competence studies is to identify the conditions that modify competence in order to further our understanding of interactions between mosquitoes and pathogens and to aid efforts to minimize transmission risk. Both environmental and genetic factors influence arthropod-borne (arbo)virus infection processes in mosquitoes. Although numerous mosquito species may be capable of transmitting particular arboviruses, usually far fewer contribute substantially to transmission in nature because of genetic or environmental barriers related to vectoring ability, including vector competence. Most studies on vector competence have focused on environmental conditions experienced by adults (e.g. ambient temperature, viral dose) but environmental conditions of immature stages may have latent effects that continue to adulthood and alter arbovirus vector competence.

Growth and development of mosquitoes occurs during the aquatic stages, so the immature environment shapes traits of adults, some of which may relate to vector competence for arboviruses and disease transmission. The immature stages of mosquitoes occupy a variety of aquatic environments which vary in quality and quantity of nutrients, primarily from allochthonous detritus that forms the basal resources for microorganisms eaten by mosquito larvae (Fish and Carpenter 1982, Lounibos *et al.* 1993, Merritt *et al.* 1992, Walker *et al.* 1991, 1997). Different types and quantities of detritus influence individual development, growth, survival, as well as population dynamics and community structure (e.g. species assemblages), and these effects may be modified by ambient temperature. Equally as important are biotic interactions (e.g. predation, competition, parasitism), many of which primarily occur during the larval stages. Regulation of populations of mosquitoes in permanent ground water habitats is strongly influenced by predation, whereas competition appears to be a more important regulatory mechanism in ephemeral and container aquatic habitats (Juliano 2007, Service 1985).

Studies of mosquito vector competence, even when quantitative comparisons are made between species, rarely consider the possibility that biotic interactions may influence vector competence for arboviruses. For mosquitoes, biotic interactions shaping adult traits are largely experienced during the larval stages. Direct interactions include predation, interference, mutualism, and parasitism. Indirect interactions occur through intermediary species, and they can be most generally categorized as either feeding chains or other interactive modifications (Peacor and Werner 2000, 2001, Werner and Peacor 2003, Wooton 1994, 2002). Alterations in phenotypes from species interactions and other aspects of the larval environment are assumed to arise from phenotypic plasticity. Alternatively, alterations in phenotypes may also be due to selection among individuals for different phenotypes (Hetchel and Juliano 1997). Regardless of the mechanism, if alterations in phenotypes also translate to altered susceptibility to viral infection and transmission, then there is a possibility that the larval environment may influence disease transmission.

Typically, arbovirus transmission by mosquitoes involves acquisition in the midgut of an infectious blood meal, infection of midgut cells, dissemination of virus from the midgut to secondary tissues including the salivary glands, and inoculation of a vertebrate host during probing and feeding (Hardy *et al.* 1983, Woodring *et al.* 1996). Transmission from mosquito to host depends on whether the founding virus population, usually acquired by bite, successfully overcomes barriers to dissemination. The relative efficacy of barriers that impede the progression of virus infection in mosquitoes may depend on the specific virus, mosquito species, or even the geographic origin of the mosquito or virus (e.g. Bennett *et al.* 2002, Gubler *et al.* 1979, Tabachnick *et al.* 1985). Identified barriers to infection include the midgut infection and escape barriers and salivary gland infection and escape barriers (Hardy *et al.* 1983, Woodring *et al.* 1996). Both the midgut infection and escape barriers are dose-dependent so that higher doses of virus increase the probability of infection and its progression to other tissues. Differences in vector competence are reduced, sometimes to the point of eliminating resistance to viral infection in mosquitoes, when the midgut infection and escape barriers are bypassed via intrathoracic inoculation (Gubler and Rosen 1976, Hardy *et al.* 1978), underscoring the importance of natural infection processes to vector competence. The salivary gland infection and escape barriers ultimately determine whether a disseminated infection may be transmitted to a host through virus-infected saliva during probing and feeding.

Although our understanding of the molecular basis for barriers to virus infection is rudimentary, recent advances on the expression of immunity related genes have been possible by completion of annotated genome sequences for the mosquitoes *Aedes aegypti* (L.) and *Anopheles gambiae* Giles (reviewed in Fragkoudis *et al.* 2009). Ingestion of arboviruses is known to induce antimicrobial immune pathways including immune deficiency (Imd), Toll (Ramirez and Dimopoulos 2010, Sanders *et al.* 2005, Xi *et al.* 2008), Janus kinase-signal transducers and activator of transcription (JAK/STAT) (Fragkoudis *et al.* 2009, Sanders *et al.* 2005, Souza-Neto *et al.* 2009), and RNA interference (RNAi) (Cirimotich *et al.* 2009, Keene *et al.* 2004, Khoo *et al.* 2010). The relative role and sequence of immune signaling between linked pathways that lead to modification in viral infection and replication are only known in part for a few mosquito species and viruses *in vivo,* e.g. *Ae. aegypti* and Sindbis virus (Cirimotich *et al.* 2009, Khoo *et al.* 2010, Sanders *et al.* 2005); and *Ae. aegypti* and dengue virus (Ramirez and Dimopoulos 2010, Sánchez-Vargas *et al.* 2009, Xi *et al.* 2008). Moreover, immune pathways may be virus-specific, as is the case with *Drosophila melanogaster* Meigen, and modified by the environment (reviewed in Xi *et al.* 2008). For instance, exposure to temperature and other types of environmental stress may up-regulate the expression of heat shock proteins in mosquitoes (Zhao *et al.* 2010). In some cases these proteins may influence virus infection, as demonstrated by *An. gambiae* heat shock protein cognate 70B which reduces o'nyong-nyong virus replication (Sim *et al.* 2007). If the environmental conditions experienced by larvae lead to expression of immune-related genes in nature, then such molecular changes may affect vector competence for arboviruses (Muturi, unpublished results).

There is an intellectual gap to be traversed between the ecology of the immature stages and adult vectoring ability. The current chapter limits our investigation into this area of research to vector competence for arboviruses. Our aim is to connect the importance of different environmental influences on larvae to mosquito biology and vector competence (Table 1). Specifically, we will use this review as a basis to formalize tests of hypotheses generated from published literature regarding the nature and extent of larval environmental influences on vector competence, identify plausible mechanism(s) for altered vector competence, and discuss areas for future research to further our understanding of larval environmental influences on susceptibility to virus infection and transmission. For purposes of this review, assays of mosquitoes for virus infection are categorized into infection, dissemination and transmission. Our reasoning is that these categories enable us to better identify particular virus barriers that may be modified by the larval environment. Infection refers to the presence of virus in mosquito midguts or in mosquito bodies and absence of virus in other tissues (e.g. legs). Dissemination refers to the presence of virus in tissues other than the midgut. Evidence for transmission comes either from the presence of virus in mosquito saliva or in vertebrate hosts after infected mosquitoes are allowed to probe/feed.

Nutrition

Decomposing organic matter in the form of plant detritus and decomposing invertebrates serve as the basal resources for microorganisms that form the diet for mosquito larvae (Kaufmann *et al.* 2010, Merritt *et al.* 1992). Spatio-temporal variation in the availability of organic matter is likely to determine habitat quality for mosquitoes. The quantity and quality of nutrients available to mosquitoes largely determine growth and development during the larval stages. Upon eclosion, the size and nutrient reserves of adults are directly related to the nutrient conditions that the larvae experienced. It has been long suspected that mosquito size, and associated physiological status and fitness, may influence susceptibility to virus infection and transmission. Studies on the role of larval nutrition on vector competence are biased towards container-inhabiting mosquitoes due to their importance in the transmission of arboviruses as well as their ease of manipulation.

Large adult *Aedes triseriatus* Say from nutrient rich larval conditions were less likely to be infected and transmit LaCrosse encephalitis virus (LACV) than small adults from nutrient-deprived larvae (Grimstad and Haramis 1984, Grimstad and Walker 1991, Patrican and DeFoliart 1985). These nutrient-dependent effects on transmission were observed for both horizontal (by bite) and vertical (transovarial) routes of transmission (Patrican and DeFoliart 1985). Although mechanism(s) responsible for altered vector competence were not identified, differences in the midgut morphology were suggested to be responsible for higher rates of LACV dissemination from the midgut in small-sized adults due to a thinner basement membrane (fewer basal laminae) than detected in large-sized adults (Grimstad and Walker 1991). Field collections of pupae were

Table 1. Larval environments of mosquitoes and adult vector competence for arboviruses.

Mosquito species	Arbovirus	Larval environment	Altered performance	Infection[1]	Dissemination[1]	Transmission[1]	Reference
Ae. aegypti							
	CHIKV	elevated temperature	development, survivorship	.	+	.	Mourya *et al.* 2004
	DENV-2	elevated temperature	.	.	+	.	Yadav *et al.* 2005
		intra-, interspecific competition	size, survivorship, development, λ′	0	0	.	Alto *et al.* 2008a
			size	+	+	.	Alto *et al.* 2008b
		intraspecific competition	size	.	-	.	Sumanochitrapon *et al.* 1998
	RRV	nutrient deprivation	size	-	.	.	Nasci and Mitchell 1994
	SINV	elevated temperature	size, development, survivorship, λ′	+	+	.	Muturi, unpublished results
			size, development, survivorship	+	+	.	Muturi and Alto 2011
		insecticide malathion	size, development, survivorship, λ′	+	+	.	Muturi and Alto 2011; Muturi, unpublished results
			size, development, survivorship	0	+	.	Muturi *et al.* 2011
		intra-, interspecific competition	size, survivorship, development, λ′	0	0	.	Alto *et al.* 2005
		intraspecific competition	size, survivorship, development	0	+	.	Muturi *et al.* 2011
		nutrient deprivation	size, development, survivorship	+	+	.	Muturi, unpublished results
Ae. albopictus							
	CHIKV	elevated temperature	size, development, survivorship	-	-	.	Westbrook *et al.* 2010
	DENV-2	intra-, interspecific competition	size, survivorship, development, λ′	+	+	.	Alto *et al.* 2008a
			size	+	+	.	Alto *et al.* 2008b
		nutrient deprivation	.	.	+	.	Zhang *et al.* 1993
	SINV	insecticide malathion	size, development, survivorship	0	0	.	Muturi *et al.* 2011
		intra-, interspecific competition	size, survivorship, development, λ′	+	+	.	Alto *et al.* 2005
		intraspecific competition	size, development, survivorship	0	0	.	Muturi *et al.* 2011
Ae. taeniorhynchus							
	RVFV	elevated temperature	.	-	+	.	Turell 1993
	VEEV	elevated temperature	.	-	+	.	Turell 1993

Table 1. Continued.

Mosquito species	Arbovirus	Larval environment	Altered performance	Infection[1]	Dissemination[1]	Transmission[1]	Reference
Ae. triseriatus							
	LACV	field collected pupae	size	.	+	+	Paulson and Hawley 1991
		intra-, interspecific competition	size, survivorship	+	+	.	Bevins 2008
		nutrient deprivation	size	0	+	+	Grimstad and Haramis 1984
			size	+	+	+	Grimstad and Walker 1991
			size	.	+	+	Paulson and Hawley 1991
			size, development	.	+	+	Patrican and DeFoliart 1985
Ae. vigilax							
	RRV	elevated temperature	.	0	0	.	Kay and Jennings 2002
		nutrient deprivation	size	0	0	0	Jennings and Kay 1999
Cx. annulirostris							
	MVEV	elevated temperature	.	+	.	+	Kay *et al.* 1989b
		nutrient deprivation	size	0	.	0	Kay *et al.* 1989a
Cx. tarsalis							
	WEEV	elevated temperature	.	-	.	.	Hardy *et al.* 1990
Cx. tritaeniorhynchus							
	JEV	nutrient deprivation	.	.	.	+	Takahashi 1976
	WNV	elevated temperature	size, development, survivorship	0	.	.	Baqar *et al.* 1980
		intraspecific competition	size, development, survivorship	+[2]	.	.	Baqar *et al.* 1980
		nutrient deprivation	size, development, survivorship	0	.	.	Baqar *et al.* 1980

[1] Symbols +, -, and 0, show increases, decreases, and no change in infection parameters. Periods (.) indicate that these measurements were not recorded.

[2] Baqar *et al.* (1980) showed that *Culex tritaeniorhynchus* from intermediate larval rearing densities (2.0 larvae/ml) had significantly lowered rates of West Nile virus infection than mosquitoes from low (0.5 larvae/ml) and high (4.0 larvae/ml) densities.

used to further address whether the size of *Ae. triseriatus* in natural populations could be related to vector competence. Rates of disseminated infections and transmission after imbibing LACV infected blood were negatively related to mosquito size, as measured by both pupal weight and wing length for two strains of *Ae. triseriatus* (Paulson and Hawley 1991). These relationships appear to be attributable to environment and not genetic determinants (Anderson *et al.* 2005), since vector competence was indistinguishable among F1 progeny from well-fed larvae of small and large sized parental mosquitoes (Paulson and Hawley 1991). Similarly, small adult *Aedes albopictus* Skuse derived from nutrient-deprived larvae had significantly higher rates of dissemination of dengue-2 virus than large adults from nutrient rich larval conditions (Zhang *et al.* 1993). Electron microscopic observation of mesenteronal tissue showed that adults from nutrient-deprived larvae had thinner basement membranes (6-12 laminae) than adults from nutrient rich larval conditions (14-19 laminae). Moreover, adults from field collections of *Ae. albopictus* pupae had body sizes and rates of dengue-2 virus infection similar to those adults from nutrient-deprived larval conditions (Zhang *et al.* 1993). Takahashi (1976) showed a trend for reductions in the length of the extrinsic incubation period for Japanese encephalitits virus in *Culex tritaeniorhynchus* Giles from nutritionally-deprived larvae. However, the relationship between nutrition and vector competence for other mosquito species and viruses has yielded inconsistent outcomes ranging from no effects, e.g. *Aedes vigilax* Skuse and Ross River virus (Jennings and Kay 1999); *Culex annulirostris* Skuse and Murray Valley encephalitis virus (Kay *et al.* 1989a), to decreasing competence with nutrient deprivation, e.g. *Ae. aegypti* and Ross River virus (Nasci and Mitchell 1994). Thus, nutrient-dependent changes in competency for arboviruses may, at least in part, depend on the species of mosquitoes and viruses.

Intra- and interspecific competition

Density-dependent interactions play a critical role in regulating populations and may have implications for understanding vector biology, control, and improved predictability for risk of disease transmission. For mosquitoes these regulatory forces act strongly on the immature aquatic stages and subsequently impact the size and fitness of the adult population. Regulation of populations of mosquitoes using container and ephemeral habitats appears to be strongly influenced by density-dependent competition, a source of mortality (e.g. Arrivillaga and Barrera 2004, Barrera and Medialdea 1996, Barrera *et al.* 2006, Juliano 2007, Service 1985, Southwood *et al.* 1972). Along the same lines, management of mosquito-borne diseases largely depends on the application of larvicides that induce mortality and subsequently reduce the density of adult mosquito vectors. Surprisingly, we know very little in terms of how these multiple sources of mortality influence arboviral vector competence of surviving adults.

One of the earliest studies to investigate the relationship between competition and vector competence focused on *Cx. tritaeniorhynchus* and infection with West Nile virus (Baqar *et al.* 1980). Moderate competition (2 larvae/ml) significantly reduced susceptibility of *Cx. tritaeniorhynchus* to West Nile virus infection relative to low and high competition (0.5 larvae/ml and 4 larvae/ml, respectively). Although a clear pattern of larval density effects on infection with West Nile virus was not identified, the study showed that the larval environment has effects that continue to influence interactions between the adult mosquito and arboviruses. Nearly two decades later a competition experiment using F1 *Ae. aegypti* from field collections in three geographic locations in Thailand demonstrated that large mosquitoes from low intraspecific competition had higher dissemination of dengue-2 virus than small mosquitoes from high competition (Sumanochitrapon *et al.* 1998). These effects also appeared to be stronger in mosquitoes from some geographic locations over others, suggesting a possible interaction between genotype and competition in dengue vector competence.

Only recently have researchers begun to investigate the role of interspecific competition in modifying arbovirus vector competence in mosquitoes. Research in this area was prompted by widespread invasions by the Asian tiger mosquito *Ae. albopictus* (reviewed in Benedict *et al.* 2007). *Aedes albopictus* immature stages occupy water-filled containers along with other mosquito species. In the USA, the treehole mosquito *Ae. triseriatus* and yellow fever mosquito *Ae. aegypti* are common occupants of containers and vectors of LaCrosse and dengue viruses, respectively. Competitive interactions between these species are well documented and influence individual life history traits, population dynamics and community structure. The establishment of *Ae. albopictus*, a competent laboratory and natural vector of several arboviruses, in new regions poses a risk to human health because it may become involved in existing disease transmission cycles of established pathogens or newly introduced pathogens. Additionally, interspecific interactions between *Ae. albopictus* and other mosquito species may alter the relative abundance of the adult populations of vector species or affect the phenotypes of mosquitoes that relate to vectoring ability such as vector competence.

Ae. albopictus is competitively superior in its larval stages to native *Ae. triseriatus* (Aliabadi and Juliano 2002, Livdahl and Willey 1991, Novak *et al.* 1993, Teng and Apperson 2000). Therefore, interspecific interactions between the larvae of these species are predicted to impose asymmetric nutritional stress on *Ae. triseriatus* via resource depletion, perhaps with similar consequences for susceptibility to LACV infection as was the case among nutritionally-deprived *Ae. triseriatus* (see above). Using a replacement series design, Bevins (2008) investigated competitive interactions between larvae of *Ae. triseriatus* and *Ae. albopictus* and their consequences for *Ae. triseriatus* infection with LACV. The presence of *Ae. albopictus* increased *Ae. triseriatus* mortality but the surviving individuals were larger than in treatments containing only *Ae. triseriatus*, perhaps attributable to release from competition among survivors. Reciprocal effects on *Ae. albopictus* performance measurements were not observed, consistent with other studies that demonstrated the competitive superiority of *Ae. albopictus*. Large *Ae. triseriatus* from interspecific treatments (containing *Ae. albopictus*) had higher infection and dissemination rates of LACV compared to intraspecific treatments of *Ae. triseriatus* (Bevins 2008). In contrast to studies on nutrition and LACV infection in *Ae. triseriatus* (see above), larger, not smaller, mosquitoes had enhanced vector competence, suggesting that size alone is not an accurate predictor of susceptibility to infection and transmission of LACV. Despite the apparent release from competition for *Ae. triseriatus* in interspecific treatments, the viral competency of these large sized individuals was still enhanced due to interactions with *Ae. albopictus*, a superior competitor. Studies on competitive interactions in mosquitoes commonly measure multiple population growth correlates as well as an estimate of the per capita rate of change (e.g. r', Livdahl and Sugihara 1984) because in many instances the effects of competition are complex, and interpretation of single or a few performance measurements may not accurately reflect outcomes at the population level. It is therefore plausible that the large *Ae. triseriatus* with enhanced competence for LACV from interspecific treatments experienced stress in other ways that were not fully captured by measuring size-related effects of competition.

The invasion of *Ae. albopictus* in the USA has been well-documented, including its influence on established mosquito species (Moore 1999, O'Meara *et al.* 1995, 1993). The spread of *Ae. albopictus* in southeastern USA in the 1980-1990s was associated with a decline in the abundance and geographic distribution of the yellow fever mosquito *Ae. aegypti* (Hobbs *et al.* 1991, Mekuria and

Hyatt 1995, O'Meara *et al.* 1995). Interspecific interactions between these two species appear to have contributed to declines in *Ae. aegypti* (Juliano *et al.* 2004). Several mechanisms have been proposed to explain displacements of *Ae. aegypti* populations (reviewed by Lounibos 2002, 2007), but superiority of *Ae. albopictus* in larval resource competition has received the most attention (e.g. Juliano 1998). Using a small response surface design, Alto *et al.* (2008a) showed intraspecific and interspecific larval competition altered individual life history traits, population performance, and vector competence for dengue-2 virus (DENV-2). Specifically, for laboratory strains of *Ae. albopictus,* but not *Ae. aegypti,* high levels of competition enhanced susceptibility to infection and dissemination with DENV-2, and these effects were presumed to be attributable to reductions in midgut infection and escape barriers (Figure 1) (Alto *et al.* 2008a). In a companion study, smaller *Ae. aegypti* and *Ae. albopictus* were more likely to become infected and to disseminate DENV-2 than larger individuals, independent of rearing conditions (Alto *et al.* 2008b). In a similarly designed experiment completed earlier using the same mosquito strains, competition-enhanced infection in *Ae. albopictus* with Sindbis virus (SINV) was observed (Alto *et al.* 2005). A separate study using more recently colonized Florida strains of mosquitoes showed that intraspecific competition enhanced dissemination of SINV in *Ae. aegypti* but not *Ae. albopictus* (Muturi *et al.* 2011). Although reasons for differences in the results between species and strains remain unclear, these studies showed a general relationship between competitive stress (lengthened development, reduced growth and survival) and weakening of midgut barriers. Also, it appears that competitive stress may alter other aspects of viral infection because high competition significantly increased SINV, but not DENV-2, viral titer in *Ae. albopictus* with disseminated infections, suggesting a reduction in the innate immune response to limit virus replication (Alto *et al.* 2005). Although the mechanism(s) responsible for these observed effects were unclear, it appears that multiple immune processes may be compromised because midgut barriers and viral titer were modified by the effects of

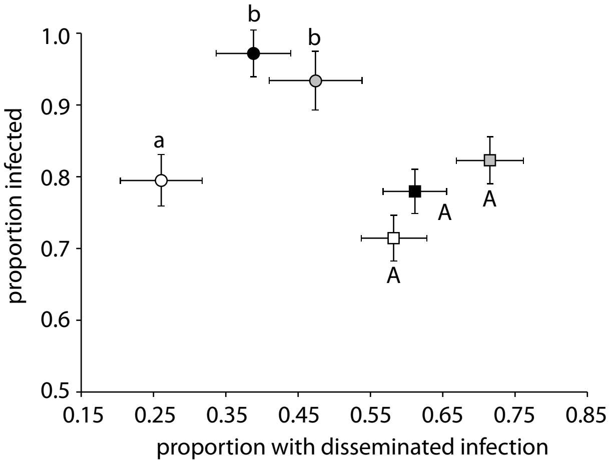

Figure 1. Proportion of Aedes aegypti *and* Aedes albopictus *infected and with disseminated infections after fed blood containing dengue-2 virus. Mosquitoes were exposed to intra- and inter-specific competition as larvae (Alto* et al. *2008a). Competition treatments consisted of initial number of larvae of* Ae. albopictus *:* Ae. aegypti *per container – 160:0 (open circle), 320:0 (filled circle), 160:160 (grey circle and grey square), 0:320 (filled square) and 0:160 (open square). Bivariate means followed by different lower- and uppercase letters show significant differences for* Ae. albopictus *and* Ae. aegypti*, respectively. Circles show* Ae. albopictus *and squares show* Ae. aegypti.

competition. Differences in competitive effects on virus replication may be related to the type of arbovirus being considered (e.g. SINV = *Alphavirus*, DENV = *Flavivirus*) as well as the species and strain of mosquito.

Temperature

Temperature is one of the most commonly studied of environmental factors affecting biological processes, including seasonal and geographic differences in temperature as well as anticipated climate change effects. Understanding temperature impacts on interactions between arthropods and pathogens will assist in predicting disease transmission patterns which are likely to be altered by anticipated climate change (Lafferty 2009, Rogers and Randolph 2006, Tabachnick 2010). The body temperature of mosquitoes is directly proportional to ambient temperature, thereby influencing growth, development, population dynamics (Alto and Juliano 2001a,b, Lounibos *et al.* 2002) and arbovirus infection including the extrinsic incubation period (EIP) which is the time from initial acquisition of virus infection to capacity to transmit (Chamberlain and Sudia 1955, Hardy *et al.* 1983). Consistent observations have documented that increases in adult maintenance temperatures are associated with enhanced vector competence as measured by higher net rates of infection, transmission and reductions in EIP for several viruses and mosquito species (e.g. Anderson *et al.* 2010, Chamberlain and Sudia 1955, Davis 1932, Hardy *et al.* 1983, Kay and Jennings 2002, Kay *et al.* 1989a,b, Richards *et al.* 2009, 2007, Turell 1993).

Although most investigations of temperature effects on vector competence have focused on adult mosquitoes, fewer studies have determined whether ambient temperatures acting on the immature stages of mosquitoes affect the vector competence of adults for arboviruses. However, temperatures experienced during the immature stages shape the adult phenotype e.g. nutritional reserves (Briegel and Timmermann 2001, Briegel *et al.* 2001a,b), so it seems plausible that such temperature effects also modify interactions between arboviruses and mosquito adults. *Aedes taeniorhynchus* Wiedemann reared at 19 °C had significantly higher infection, but not dissemination, rates of Rift Valley fever and Venezuelan equine encephalitis viruses than mosquitoes from 26 °C (Turell 1993). Larval rearing temperatures of 18, 24, and 32 °C altered development and growth rates of *Ae. albopictus* and resulted in adults with different competence for chikungunya virus. Infection rates did not differ among mosquitoes, but *Ae. albopictus* from 18 °C were significantly larger and had dissemination rates six times higher than mosquitoes from the warmest larval rearing conditions (32 °C) (Westbrook *et al.* 2010, Figure 2). These results suggest that the global spread of CHIKV vector *Ae. albopictus* in recent decades (Juliano and Lounibos 2005, Lounibos 2002) coupled with emergence of chikungunya virus in Italy (Powers and Logue 2007, Rezza *et al.* 2007), La Réunion (Paquet *et al.* 2006), Indonesia, Sri Lanka, and Singapore (Seneviratne *et al.* 2007) may pose greater epidemiological risks. In particular, high altitudes such as highlands of La Réunion where *Ae. albopictus* develops in cooler temperatures, may enhance risks of disease transmission. However, we caution against firm conclusions at this point since temperature is known to alter other parameters of vectorial capacity, and the net effect on risk of disease transmission is unclear. Results from this study are consistent with other field studies where cooler temperatures correlate with enhanced vector competence of Western equine encephalitis virus in a population of *Culex tarsalis* Coquillett (Hardy *et al.* 1990) and Murray Valley encephalitits virus transmission by *Cx. annulirostris* (Kay *et al.* 1989b). Taken together, these studies suggest that temperature effects experienced during the immature stages have opposite effects on arboviral competence compared to incubation temperature of adults (i.e. positive associations between temperature and vector competence). Low rearing temperature of immature stages typically results in larger sized adult mosquitoes (e.g. Lyimo *et al.* 1992; Westbrook *et al.* 2010) that imbibe larger volumes of blood and

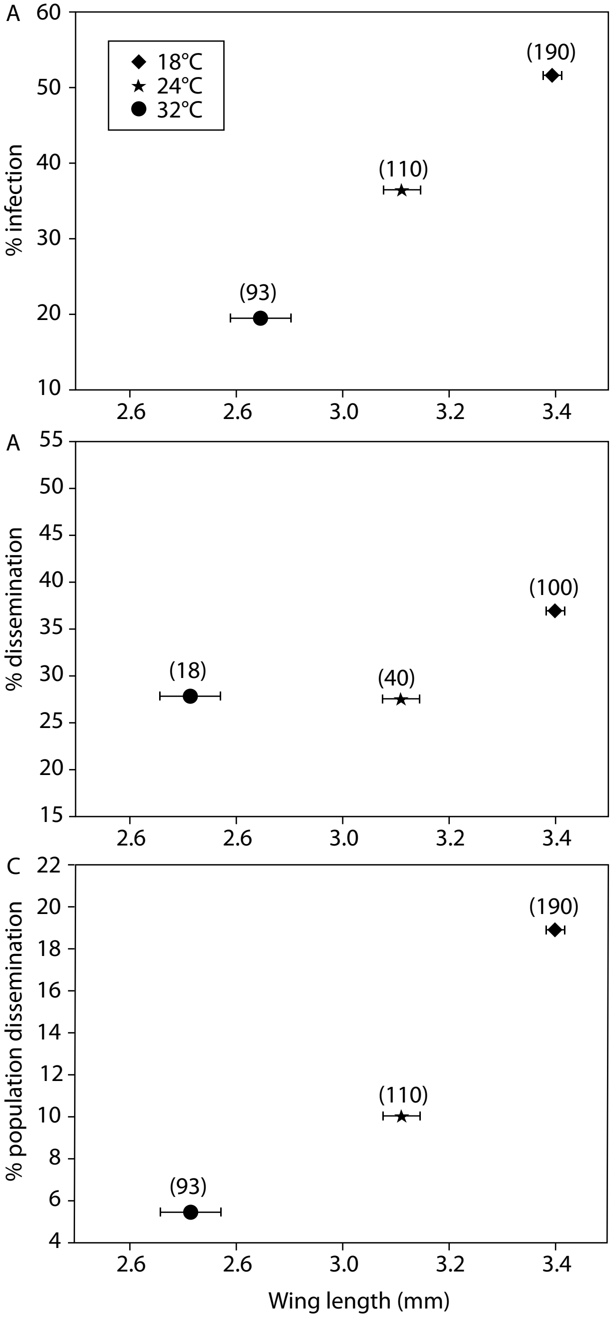

Figure 2. Percent of Aedes albopictus *infected and with disseminated infections after fed blood containing chikungunya virus. Mosquitoes were exposed to different temperatures as larvae. Numbers in the parentheses show the number of mosquitoes assayed for viral infection (Westbrook* et al. *2010).*

presumably more virus. It is therefore plausible that higher initial doses of viruses imbibed by large mosquitoes reared at cool temperatures may play a role in altered vector competence.

Other studies have found little or no identifiable relationships (Baqar *et al.* 1980, Kay and Jennings 2002) or opposite effects between larval rearing temperature and viral competence in mosquitoes. Elevated rearing temperature (30 vs. 20 °C or 32 vs. 25 °C) during larval and pupal development led to enhanced rates of infection and dissemination with adult *Ae. aegypti* and Sindbis virus (Muturi and Alto 2011, Muturi, unpublished results). Similarly, short-term exposure of larvae to heat shock (range from 36-44.5 °C) enhanced vector competence of *Ae. aegypti* for chikungunya virus (Mourya *et al.* 2004) and DENV-2 (Yadav *et al.* 2005). The molecular basis for observed temperature-dependent enhancement or depression of infection or dissemination is not well studied, but some researchers have hypothesized that temperature-induced up-regulation and down-regulation of stress (e.g. heat shock proteins) and immunoresponsive genes may affect susceptibility of mosquitoes to viral infection (Mourya *et al.* 2004, Muturi, unpublished results, Yadav *et al.* 2005).

Insecticides

Integrated mosquito management heavily relies on the use of insecticides directed at adult and immature mosquitoes. Control efforts assume that an externally applied source of mortality (e.g. insecticide) will act additively with other sources of mortality in nature to reduce overall numbers of adult mosquitoes. However, there may be environmental conditions that promote alternative outcomes, a possibility when two or more sources of mortality act in non-additive ways to reduce or increase the numbers of adult mosquitoes (Juliano 2007, Service 1985). For example, simulated mortality through removal of larvae in water-filled containers with suboptimal nutrients increased the number and size of *Ae. aegypti* that emerged to adulthood (Agudelo-Silva and Spielman 1984). If these results hold true under natural conditions then mosquito control by larviciding in nutrient-limited populations may increase the number of adults and perhaps life span of adults due to evidence for positive relationships between size and life expectancy in *Aedes* mosquitoes (Briegel *et al.* 2001, Haramis 1985, Reiskind and Lounibos 2009, Steinwascher 1982). Furthermore, insecticide-induced alterations in surviving mosquitoes may be associated with phenotypic variations in arboviral vector competence, either through sublethal exposure of insecticides or indirectly by modifications in the environment (e.g. release from competition). For example, insecticide resistant genes may in some instances have pleiotropic effects and result in changes in vector ability such as longevity, behaviour, and arboviral vector competence (reviewed in Rivero *et al.* 2010). Phenotypic variation related to mosquito vectoring ability could be due to a plasticity which would occur in the life span of the mosquito or selection where an alteration in the genetic structure of the population has occurred across generations (e.g. insecticide resistance).

To examine the interaction between competition and insecticide treatment, Muturi *et al.* (2011a) exposed Florida strains of *Ae. aegypti* and *Ae. albopictus* to intraspecific larval competition and a low concentration of the organophosphate insecticide malathion. They tested the hypothesis that the presence of malathion would alleviate larval competition and alter vector competence for Sindbis virus. This investigation, as well as follow-up studies, deliberately focused on container-inhabiting *Aedes* because the effects of competition are known to affect their vector competence and regulation of populations. As predicted, competition and malathion treatments reduced survival to adulthood. The presence of malathion appeared to have reduced competition as demonstrated by faster development and larger mosquitoes among survivors. However, it is also possible, but perhaps less likely, that malathion selectively favoured larger and faster developing mosquitoes. Exposure to malathion and competition independently led to a doubling of the rate of virus dissemination in *Ae. aegypti* but not *Ae. albopictus*. Enhanced viral competence was observed among small, competitively stressed mosquitoes and large individuals exposed to malathion, an indication that size alone was not responsible for altered competence. These effects of malathion on vector competence suggest a degree of complexity that is not simply attributable to an alleviation of larval competition. If this were the case, then we would expect that *Ae. aegypti* exposed to malathion should have similar competence as *Ae. aegypti* in low competition intraspecific treatments in the absence of malathion. On the contrary, malathion-exposed *Ae. aegypti* had elevated rates of dissemination, similar in magnitude to those observed for *Ae. aegypti* from high larval competition treatments in the absence of malathion. Therefore it seems that direct exposure to malathion, and not indirect effects of malathion-mediated release from competition, alters arboviral vector competence, perhaps attributable to differential expression of immunity related genes induced by the presence of malathion. Additionally, the effects of malathion on viral competence may be modified by other environmental factors. For example, exposure to malathion during development of the immature stages at high temperature (30 °C) enhanced *Ae. aegypti* viral infection and dissemination with Sindbis virus, but these effects were not observed at low temperature (20 °C) (Muturi and Alto 2011). This latter study reaffirmed the notion that size *per se* does not dictate vector competence since the sizes of mosquitoes with enhanced competence in the presence and absence of malathion at 30 °C were similar (Muturi and Alto 2011).

Muturi (unpublished results) exposed Florida strains of *Ae. aegypti* to one of several environmental stressors during the aquatic stages (starvation, nutrient limitation, insecticide malathion or elevated temperature) to identify differential expression of stress and immunity-related genes and to relate these to vector competence for Sindbis virus. Differential expression of stress and immunity-related genes were measured in larvae and adult female *Ae. aegypti*. In larvae, elevated temperature was associated with up regulation in gene expression of cecropin, defensin and CYP6Z6, whereas suboptimal nutrients and exposure to a low dose of malathion increased expression of cecropin and transferrin, respectively, relative to the controls. In contrast, starvation led to down-regulation of defensin, cecropin, transferrin, HSP70, HSP83, and CYP6Z6. A subset of these genes was investigated in adult females. Transferrin was up-regulated in all treatments except starvation, and defensin was up-regulated in starvation and the elevated temperature treatments. Variation in mosquito performance (survival, development, size) was specific to the particular environmental stressor, but all stressors enhanced susceptibility to viral infection and dissemination among infected mosquitoes. Although the specific details of immune pathways still require resolution, these observations suggest that environmental conditions experienced by the immature stages may modify the expression of genes related to viral infection in mosquitoes.

Synthesis of environmental influences on vector competence

Our review of the literature suggested that environmental influences on larvae have, in some instances, consequences for vector competence for arboviruses. Further, the relationship between larval environment and vector competence may differ depending on the particular environment. In order to provide additional resolution on the issue, we extracted information on sample sizes and susceptibility to infection, dissemination, and transmission from all the studies presented in Table 1 to construct tests for the following null hypotheses:

Null hypotheses:

H_0: Nutrient deprivation does not alter vector competence
H_0: Competition does not alter vector competence
H_0: Elevated temperature does not alter vector competence
H_0: Exposure to insecticide does not alter vector competence

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Hypotheses were tested by maximum likelihood categorical analyses of contingency tables (PROC CATMOD, SAS 2002) based on counts of individual mosquitoes being categorized as + or – for the presence or absence of virus, respectively. That is, responses from individual mosquitoes were used as data points. Separate maximum likelihood (ML) ANOVA tests were used for each larval environment and measure of vector competence (infection, dissemination, transmission) in order to more clearly identify viral barriers that may be modified by the environment. In instances where more than two levels were available within larval environmental treatments we used only the extremes (e.g. low, high) and excluded intermediate levels. The goal of these tests was to identify overall patterns of larval environment and vector competence for arboviruses, so no attempt was made to separate analyses by virus or mosquito species.

A total of 6,377 mosquitoes was used for the analysis for susceptibility to infection, 7,073 mosquitoes for the analysis of disseminated infection and 1,665 for the analysis of virus transmission. The ML ANOVA showed no significant nutrition effect for susceptibility to infection with arboviruses. However, mosquitoes from nutrient rich conditions were less likely to disseminate and transmit virus than individuals from nutrient-deprived conditions (Table 2). Thus, larval nutrition appears to have negligible effects on midgut infection barrier and primarily alters vector competence through changes in the midgut escape and transmission barriers. The ML ANOVA demonstrated a significant competition effect for susceptibility to infection and virus dissemination with enhanced susceptibility to infection and dissemination in adults from high competition larval environments (Table 2). These results suggest that both midgut infection and escape barriers are compromised by larval competition. An assessment for competitive effects on virus transmission was not feasible since no transmission studies have been performed. The ML ANOVA showed that warm rearing temperature significantly decreased susceptibility to infection and virus transmission but did not significantly influence dissemination in infected mosquitoes (Table 2). Rearing temperatures appear to influence both midgut and virus transmission barriers. However, we caution the interpretation of the generality of temperature effects on virus transmission because it was based on a single study. The ML ANOVA demonstrated that exposure to low concentrations of insecticide enhanced rates of infection and dissemination (Table 2). Insecticidal exposure effects on virus transmission have not been performed to date.

An overall assessment of these results suggest that larval environment (1) does alter vector competence for arboviruses, (2) influences multiple virus barriers in the adult mosquito, and (3) appears to be directionally similar in its influence on virus barriers for a given larval environment (e.g. midgut infection and escape barriers for competition).

Table 2. Maximum likelihood analyses of variance for the effects of larval nutrition, competition, temperature and insecticide on susceptibility to infection, dissemination and transmission of arboviruses.

Source Vector competence measure	Direction of test[1]	Estimate	Std. Error	df	χ^2	*P*-value	References
Nutrition							
Infection	High, +	-0.0286	0.0314	1	0.83	0.36	Baqar *et al.* 1980, Grimstad and Haramis 1984, Grimstad and Walker 1991, Jennings and Kay 1999, Kay *et al.* 1989a, Muturi unplished results, Nasci and Mitchell 1994
Dissemination	High, +	-0.1767	0.0246	1	51.74	<0.0001	Grimstad and Haramis 1984, Grimstad and Walker 1991, Jennings and Kay 1999, Muturi unpublished results, Patrican and DeFoliart 1985, Paulson and Hawley 1991, Zhang *et al.* 1993
Transmission	High, +	-0.1736	0.0261	1	44.31	<0.0001	Grimstad and Haramis 1984, Grimstad and Walker 1991, Jennings and Kay 1999, Kay *et al.* 1989a, Patrican and DeFoliart 1985, Paulson and Hawley 1991, Takahashi 1976
Competition							
Infection	High, +	0.1140	0.0257	1	19.66	<0.0001	Alto *et al.* 2005, 2008a, Baqar *et al.* 1980, Bevins 2008, Muturi *et al.* 2011
Dissemination	High, +	0.1934	0.0234	1	68.08	<0.0001	Alto *et al.* 2005, 2008a, Bevins 2008, Muturi *et al.* 2011, Sumanochitrapon *et al.* 1998
Transmission[2]	.	.	.	.	.	.	.
Temperature							
Infection	High, +	-0.1505	0.0219	1	47.05	<0.0001	Baqar *et al.* 1980, Hardy *et al.* 1990, Kay and Jennings 2002, Kay *et al.* 1989b, Muturi and Alto 2011, Muturi unpublished results, Westbrook *et al.* 2010
Dissemination	High, +	0.0267	0.0238	1	1.26	0.2624	Kay and Jennings 2002, Mourya *et al.* 2004, Muturi and Alto 2011, Muturi unpublished results, Turell 1993, Westbrook *et al.* 2010, Yadav *et al.* 2005
Transmission	High, +	-0.2652	0.1265	1	4.39	0.0361	Kay *et al.* 1989b
Insecticide							
Infection	Control, +	-0.0713	0.0283	1	6.34	0.0118	Muturi and Alto 2011, Muturi *et al.* 2011, Muturi unpublished results
Dissemination	Control, +	-0.0966	0.0316	1	9.33	0.0023	Muturi and Alto 2011, Muturi *et al.* 2011, Muturi unpublished results
Transmission[2]	.	.	.	.	.	.	.

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[1] Symbols + and – denote the presence or absence of virus. 'High' refers to the level of nutrients, competition and temperature. The 'direction of the test' denotes the reference point from which the test generates maximum likelihood (ML) estimates. Take for example nutrition effects on virus dissemination; there is a significant negative relationship (ML estimate of -0.1767) between high nutrition and disseminated virus infection.

[2] Periods (.) indicate that data are lacking to construct a statistical test.

Plausible mechanisms

The mechanisms responsible for changes in adult vector competence for arboviruses attributable to the larval environment are not entirely clear. In most instances published studies aimed to identify the nature of larval environmental effects on adult competence for viruses but not the mechanism(s) responsible. It seems reasonable to postulate that the mechanism(s) responsible for changes in adult competence attributable to nutrient limitation and competition may be similar, given that both these factors deprive larvae of nutrients and induce similar alterations in life histories (delayed development, reduced growth and survival). However, determinants of adult competence attributable to ambient temperature and exposure to insecticides during larval growth and development are entirely different stressors and so, their mechanism(s) may differ.

Studies investigating effects of larval nutrition attempted to identify plausible mechanisms by pointing out relationships between adult traits (body size, midgut basement membrane) and competence. Grimstad and Walker (1991) suggested that the strength of the midgut escape barrier in *Ae. triseriatus* was related to size of adult females. These authors hypothesized that the weakening of the midgut escape barrier was caused by nutrient-induced reductions in the basal lamina of the midgut epithelium. These results were consistent with some but not all other investigations of larval nutrient effects on adult vector competence for arboviruses (Table 1). Studies that have identified positive relationships between size and vector competence have suggested that large mosquitoes ingest higher numbers of viruses in blood meals than small mosquitoes (Nasci and Mitchell 1994, Westbrook *et al.* 2010). The expectation in these instances is that higher viral doses associated with larger blood meals increases the probability of midgut infection.

It is likely that size alone is not causally related to altered vector competence, at least when not taking into account the particular larval conditions that produced different sized mosquitoes (e.g. temperature, nutrients, insecticide). The most convincing evidence to support this conclusion comes from studies that produce a range of mosquito sizes with enhanced vector competence. *Ae. aegypti* larvae exposed to insecticide malathion and competition/nutrient limitation resulted in the production of large and small sized adults, respectively, with enhanced viral infection and dissemination relative to control treatments (Muturi *et al.* 2011, unpublished results). These studies demonstrated that in a majority of cases larval environment does indeed alter adult competence for arboviruses, usually with competence enhanced by stress, but the mechanism(s) are probably not simply related directly to mosquito size. Different larval conditions may alter adult vector competence by different mechanisms and these effects may be specific to the virus and mosquito species. Also, for any given larval environment, there may be multiple mechanisms influencing adult competence during the viral infection process, from initial infection in the midgut to transmission. Further advances in identifying plausible mechanisms responsible for altered adult competence have emerged from studies relating differential expression of immune and stress-specific genes to vector competence, suggesting multiple and complex immune responses which need further clarification to identify the details of the immune pathways (Muturi unpublished results).

Conclusions and future directions

This review demonstrates that the larval environment alters adult competence for viruses under laboratory conditions as well as in collections of mosquitoes from the field (Paulson and Hawley 1991). The latter area of research is understudied and we need to address whether larval environment factors in nature alter adult competence. For instance, much of the environmental variation in the field cannot adequately be represented under laboratory conditions. Additionally, laboratory colonies of mosquitoes, even after only a few generations breeding in a laboratory setting, may not accurately reflect field populations. Laboratory colonization may therefore have consequences for environmental effects on vector competence for arboviruses (e.g. environment x genotype interaction).

Although some studies have attempted to associate adult traits (body size, midgut basement membrane) to competence there appears to be inconsistencies in these relationships. What is the mechanism(s) responsible for the observed changes in adult competence? It is likely that several factors are responsible, especially since it appears that multiple barriers to virus infection and transmission may be influenced by different larval environments (Table 2). Recent advances in molecular biology and our understanding of the expression of immunity related genes have improved the capacity to identify plausible mechanisms responsible for altered adult competence. A further understanding of larval environmental influences on adult competence for viruses will come from the application of these newly developed methods. Molecular studies are valuable as correlates to alterations in vector competence but evidence for causation of actual mechanism(s) probably will require additional experimental manipulations (e.g. gene silencing).

Currently, we have only a rudimentary knowledge of the relationships between larval conditions, altered expression of immunity related genes and viral competence (Muturi unpublished results). Ideally, studies should identify the differential expression of immunity related genes but also the immune pathways including the sequence of immunity events in the mosquito that relate to the viral infection process from initial midgut infection to transmission. These studies will also elucidate whether antiviral molecular responses to infection are similar between different mosquito species and viruses, the latter which differ in genomic structure and replication. Lastly, vector competence is only one parameter that goes into determining disease transmission and studies will need to incorporate other factors as well such as adult life span, biting behaviour, and adult density. Models that incorporate these factors will assist in identifying the net effect of larval environment on disease transmission.

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