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Vector competence of Florida mosquitoes for chikungunya virus

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Chikungunya virus (CHIKV, family *Togaviridae*, genus *Alphavirus*) has re-emerged and caused human epidemics in Europe, Africa, Asia, and India in recent years (Bessaud et al. 2006, Powers and Logue 2007, Peyrefitte et al. 2008, Leo et al. 2009). The debilitating disease caused by CHIKV results in fever, skin rash, and arthritis-like pain in small peripheral joints that lasts for weeks or months (Beltrame et al. 2007, Chhabra et al. 2008). There is no licensed vaccine or treatment for CHIKV infection (Couderc et al. 2009).

International travel can transport CHIKV to other locales within an infected human or mosquito. In 2007, an outbreak in Italy was traced back to an infected traveler who had recently returned from an Indian region experiencing a CHIKV epidemic (Rezza et al. 2007). This outbreak was attributed to travel, presence of *Aedes albopictus* (Skuse), insufficient mosquito control, and environmental factors (Chretien and Linthicum 2007, Seyler et al. 2008). Some recent examples of human CHIKV cases have been reported in Singapore (Leo et al. 2009) and the United States (Beltrame et al. 2007). Cases of traveler-imported CHIKV were reported in the Florida counties of Volusia in 2007 and Miami-Dade in 2010 (E. Radke, personal communication).

Aedes aegypti (Linnaeus) and *Ae. albopictus* are implicated in the CHIKV transmission cycle due to vector competence (Mangiafico 1971, Dubrulle et al. 2009) and infected field isolates (Bonilauri et al. 2008, Sang et al. 2008, Leo et al. 2009, Paupy et al. 2009). These mosquitoes are opportunistic feeders but prefer to feed on humans (Ponlawat and Harrington 2005, Valerio et al. 2010). Florida *Ae. albopictus* and *Ae. aegypti* can become infected with CHIKV (Reiskind et al. 2008, Pesko et al. 2009). However, no studies have evaluated the transmission ability of Florida mosquitoes for CHIKV.

Culex pipiens quinquefasciatus Say are also opportunistic feeders known to feed primarily on humans and birds (Molaei et al. 2007). An African field study reported CHIKV detected in ornithophilic *Cx. ethiopicus* Edwards (Diallo et al. 1999, Chevillon et al. 2008). In Tanzania and Reunion Island, *Cx. fatigans* Wiedemann (Ross 1956) and *Cx. p. quinquefasciatus* (Bessaud et al. 2006) have respectively been found infected with CHIKV. Jupp et al. (1981) provides the only known laboratory evaluation of vector competence of *Culex p. quinquefasciatus* for CHIKV, showing that feeding on a viremic animal resulted in no infections in 19 mosquitoes.

Extrinsic incubation temperature (EIT) affects vector competence in several insect-virus systems and a higher EIT shortens the period required for virus transmission (Mellor 2000). No studies have examined the influence of EIT on the vector competence of *Ae. aegypti*, *Ae. albopictus*, and/or *Cx. p. quinquefasciatus* for CHIKV. An understanding of how environmental factors such as EIT affect vector competence is essential for evaluating the importance of potential vectors in the transmission cycle.

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Consequently, we evaluate the effects of EIT on vector competence, here measured as infection, dissemination, and transmission, for an emergent strain of CHIKV in potential Florida vectors. Information from this study provides necessary baseline data on the vector competence of Florida mosquitoes and informs risk prediction models as mosquito control and public health agencies prepare for the possibility of a CHIKV outbreak in the United States.

Three Florida mosquito colonies were used: *Ae. aegypti* from Alachua County (generation > F_{50}), *Ae. albopictus* from Pinellas County (generation > F_{50}), and *Cx. p. quinquefasciatus* from Indian River County (generation = F_{10}). Mosquitoes were held in cages at 28° C and 80% humidity at a 14:10 L:D cycle and given 20% sugar and water *ad libitum*. The CHIKV strain we used (LR2006-OPY1) was propagated according to standard procedures described elsewhere (Reiskind et al. 2008) and mixed with citrated bovine blood (Hemostat, Dixon, CA) prior to mosquito feeding.

Two samples (0.1 ml) of the blood meal were each mixed with 1.0 mL BA-1 diluent and stored at −80° C until further processing to determine blood meal virus titer. Five-day-old mosquitoes were fed at either 25° or 28° C for 30 min on pledgets soaked with CHIKV-infected blood warmed (35° C) for 10 min. These temperatures were chosen based on average daily temperatures observed in central Florida during spring/early summer (25° C) and late summer/early fall (28° C) (National Oceanic Administration Association: http://cdo.ncdc.noaa.gov/ulcd/ULCD). Fully engorged specimens were transferred to one liter cardboard cages with mesh screening and maintained in incubators for seven days at either 25° or 28° C. Partially engorged or unfed mosquitoes were discarded. We used an incubation period of seven days as others have reported that the maximum amount of virus is transmitted by *Ae. aegypti* and *Ae. albopictus* six to seven days post-infection (Dubrulle et al. 2009). After seven days, 30–50 mosquitoes of each species were removed from cages and legs and wings were detached and transferred to sample tubes containing 1.0 ml BA-1 diluent. Saliva was collected in capillary tubes with established methods (Anderson et al. 2010). Mosquito bodies were transferred to separate tubes containing 1.0 ml BA-1 diluent. All samples were frozen at −80° C until later processing.

Homogenization and viral RNA extraction used previously established methods (Richards et al. 2009). Quantitative real-time Taqman reverse transcriptase polymerase chain reaction was used to quantify the amount of CHIKV in each sample using established methods (Reiskind et al. 2008).

The infection rate was the percentage of all mosquitoes tested having infected bodies. The dissemination rate was the percentage of mosquitoes with infected bodies that also had infected legs. The transmission rate was the percentage of mosquitoes with infected bodies that also had infected saliva. Statistical analyses were conducted in SAS (SAS Institute, Cary, NC). χ^2 tests were used to analyze significant differences ($P < 0.05$) in infection, dissemination, and transmission rates for each EIT. Titers of bodies, legs, and saliva were log-transformed [log (x +1)] prior to analysis to normalize data. Analysis of variance (ANOVA, PROC GLM) was carried out separately for each body part to show differences in titers between EITs and species. If significant differences were observed, then Duncan's multiple-range test was used to determine which means were significantly different. Few *Cx. p. quinquefasciatus* became infected and none showed dissemination or transmission, so this species was excluded from χ^2 and ANOVA tests.

Mosquitoes were fed blood with 5.5 ± 0.1 (mean ± standard deviation) logs PFU CHIKV/ml. The highest infection, dissemination, and transmission rates were observed in *Ae. albopictus*, followed by *Ae. aegypti*, and *Cx. p. quinquefasciatus* (Figure 1). Virus titers of

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bodies, legs, and saliva at the end of the IP are in Table 1. Infection rates at both 25° and 28° C were significantly affected by the mosquito species; however, dissemination and transmission rates were not significantly different between species, regardless of EIT (Figure 1) (infection rate: 25° C: $\chi^2 = 55.85$, df = 1, $P < 0.0001$, 28° C: $\chi^2 = 49.44$, df = 1, $P < 0.0001$; dissemination rate: 25° C: $\chi^2 = 2.05$, df = 1, $P = 0.153$, 28° C: $\chi^2 = 1.24$, df = 1, $P = 0.265$; transmission rate: 25° C: $\chi^2 = 0.91$, df = 1, $P = 0.340$, 28° C: $\chi^2 = 0.25$, df = 1, $P = 0.253$). The EIT did not affect infection, dissemination, or transmission rates in tested species (all $P > 0.05$).

Titers of bodies, legs, and saliva are listed in Table 1. The highest ($P < 0.05$) body titers were observed in *Ae. albopictus*, followed by *Ae. aegypti* and *Cx. p. quinquefasciatus*, respectively (Table 1). Titers of bodies were significantly affected by the mosquito species, but not by EIT or species*EIT (Table 2). Titers of legs and saliva were not affected by species, EIT, or the species*EIT interaction (Table 2).

Under the conditions of this test, not all *Ae. albopictus* and *Ae. aegypti* with body infections and infections disseminated to the legs are infectious, i.e., capable of transmitting CHIKV in saliva. However, the current study only provides estimates of *in vitro* transmission, which does not necessarily represent the amount of virus inoculated in a host during natural blood feeding, and there is a limitation with regard to the sensitivity of the assay that must be considered. We found no differences in leg or saliva titers between *Ae. albopictus* and *Ae. aegypti*, regardless of EIT, showing that virus titers in both species level off at a similar rate under the conditions used here. *Aedes albopictus* showed the highest infection, dissemination, and transmission rates. It is possible that the increased vector competence of *Ae. albopictus* was due to less efficient infection barriers caused by a mutation in the emergent CHIKV strain (Tsetsarkin et al. 2007).

Our results are similar to another investigation using Florida *Ae. aegypti* and *Ae. albopictus* populations (Pesko et al. 2009), but the previous study did not test transmission rates as in the current study. We conclude that Florida *Ae. aegypti* and *Ae. albopictus* are competent vectors of CHIKV. These results support the findings of others that *Ae. albopictus* is a better vector of CHIKV than *Ae. aegypti* (e.g., Turell et al. 1992, Tsetsarkin et al. 2007). Additional studies are warranted to evaluate differences in vector competence of these mosquito species for different CHIKV strains, as well as differences in behavioral traits between species influencing mosquito-human interactions contributing to transmission. While *Cx. p. quinquefasciatus* did become infected with CHIKV, this species is a poor vector under the conditions of our test and shows that infected mosquitoes may not always be infectious. Vector-virus interactions contributing to vector competence are complex (Richards et al. 2009, 2010), hence additional populations, virus doses, environmental conditions, and factors would need to be tested in order to begin to evaluate the relative importance of these mosquitoes in the natural CHIKV transmission cycle. These issues must be addressed and we are in the early stages of understanding such complexities.

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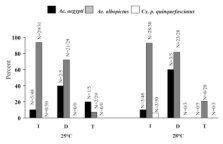

Figure 1.
Infection (I), dissemination (D), and transmission (T) rates for *Ae. aegypti*, *Ae. albopictus*, and *Cx. p. quinquefasciatus* orally infected with CHIKV and incubated for seven days at 25° C or 28° C.

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Table 1

The mean titers (logs plaque-forming units CHIKV/mL) ± standard deviation and rates of infection, dissemination, and transmission for *Ae. aegypti*, *Ae. albopictus*, and *Cx. p. quinquefasciatus* fed a CHIKV-infected blood meal and incubated for seven days at 25° C or 28° C.

No. tested	Body titer[1]	Leg titer[1]	Saliva titer[1]
	25° C		
	Ae. aegypti		
48	3.0 ± 2.4[b]	4.8 ± 0.3[a]	2.9[a]
	Ae. albopictus		
31	5.3 ± 0.6[a]	4.2 ± 1.2[a]	1.9 ± 1.2[a]
	Cx. p. quinquefasciatus		
50	-	-	-
	28° C		
	Ae. aegypti		
48	3.7 ± 2.5[b]	3.3 ± 2.2[a]	-
	Ae. albopictus		
30	5.4 ± 0.8[a]	4.5 ± 1.1[a]	1.6 ± 0.7[a]
	Cx. p. quinquefasciatus		
50	1.0 ± 0.3[c]	-	-

[1] Treatment groups with the same letter in the same column are not significantly different by means comparisons.

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Table 2

Results of analysis of variance (PROC ANOVA) of effects of species and EIT on CHIKV body, leg, and saliva titers of *Ae. aegypti* and *Ae. albopictus*.

Effect	F	df numerator, denominator	P
Body titer			
Species	28.20	1, 63	< 0.0001
EIT	0.75	1, 63	0.268
Species*EIT	0.50	1, 63	0.480
Leg titer			
Species	0.24	1, 45	0.627
EIT	1.03	1, 45	0.317
Species*EIT	2.51	1, 45	0.120
Saliva titer			
Species	1.24	1, 6	0.308
EIT	0.22	1, 6	0.653
Species*EIT	-	-	-