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Research Article

Diurnal Temperature Range and Chikungunya Virus Infection in Invasive Mosquito Vectors

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Abstract

Climate strongly influences the geographic distribution and timing of mosquito-borne disease outbreaks. Environmental temperature affects phenotypic traits of mosquitoes including vector competence for arboviruses mediated by changes in infection, extrinsic incubation period and in rates of transmission. Most experiments, however, are done at constant temperatures. In nature, mosquitoes are more likely to experience daily fluctuations in temperature. Here we compare disseminated infection (leg infection) and saliva infection of *Aedes aegypti* (L.) (Diptera: Culicidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) from Florida following oral exposure to an Asian genotype of chikungunya virus emergent in the Americas. We evaluated experimentally the effect of variable temperature regimens on disseminated infection and saliva infection of these *Aedes* species. Each of three temperature regimes had approximately the same average temperature (27–28°C), but differed in the magnitude of the diurnal temperature range (DTR). The large DTR was 8.0°C (range 23–31°C) and the small DTR was 4.0°C (range 26–30°C) which approximate ranges in different locations of Florida during July–October when risk of transmission is highest. The constant temperature was set at 27°C. Testing three geographic populations of each mosquito species, significant effects on disseminated infection were detected for an interaction between temperature regime and geographic population for both *Ae. aegypti* and *Ae. albopictus*. There were no significant treatment effects of temperature, geographic population, or temperature by geographic population interaction on saliva infection for either mosquito species. Constant temperature resulted in a higher viral load in the saliva of *Ae. albopictus*, but not *Ae. aegypti*, compared to conditions where the temperature fluctuated.

Key words: invasive mosquitoes, emergent arbovirus, environmental variation, disseminated infection, transmission

Intermittent outbreaks of chikungunya fever have occurred from the 1950s to 1970s in Southeast Asia and India (Kumar and Gopal 2010, Weaver 2014). However, chikungunya virus (CHIKV) has emerged as a major global health threat problem over the last decade. Local transmission of the Asian lineage of CHIKV was first detected in 2013 on St. Martin Island in the Caribbean (Leparc-Goffart et al. 2014, Lanciotti and Valadere 2014) and later spread throughout the Americas over the next 2 yr (Pan American Health Organization 2017). Human illness associated with CHIKV infection is usually self-limiting and results in headache, high fever, rash, joint swelling, and joint pain (Caglioti et al., 2013), although chronic musculoskeletal diseases may last for months to years and may lead to rheumatoid arthritis-like pathologies (Gasque et al. 2016). Domestic container mosquitoes *Aedes aegypti* (L.) (Diptera: Culicidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) are the primary transmitters of CHIKV to humans (Higgs and Vanlandingham 2015). Both these CHIKV vectors are present and abundant throughout much of the year in Florida which is considered a region of the United States at high risk for local transmission (Monaghan et al. 2016), which was observed in 2014 (CDC 2014).

Climate strongly influences the geographic distribution and timing of mosquito-borne disease outbreaks. In particular, environmental temperature affects arboviral infections in mosquitoes, through both the extrinsic incubation period (e.g., Dohm et al. 2002, Kilpatrick et al. 2008) and through the proportion of mosquitoes able to transmit (Dohm et al. 2002, Kilpatrick et al. 2008, Richards et al. 2012, Zouache et al. 2014). Most laboratory experiments, however, are done at constant temperatures. In nature, mosquitoes are more likely to experience daily fluctuations in temperature. A large diurnal temperature range (DTR) was shown to impede dengue-1 and -2 virus infection of the mosquito midgut and reduce transmission compared to a small diurnal range or constant temperature (Lambrechts et al. 2011). Analogous observations have been made in other mosquito-pathogen systems (e.g., malaria parasites;

Paaijmans et al. 2009, 2010), suggesting common effects of temperature on mosquito-parasite interactions.

The few studies to date on the influence of environmental temperature have demonstrated distinct differences in vector competence of *Aedes* vectors depending on CHIKV strain and geographic origin of the mosquitoes. Specifically, for CHIKV of the East/Central/South Africa lineage, transmission efficiency by *Aedes albopictus* (Skuse) (Diptera: Culicidae) depended on interactions between the geographic population of the mosquito, the virus, and environmental temperatures (Zouache et al. 2014). Despite high rates of viral dissemination among the treatments, these authors reported that transmission efficiency was lower and strongly depended on treatment interactions (e.g., genotype-by-genotype-by-environment), suggesting that salivary gland transmission barriers may influence vector competence among *Ae. albopictus*. That is, regardless of the rate of CHIKV dissemination, absence of virus in the salivary glands will prevent transmission by bite. Although the previous study focused only on *Ae. albopictus*, an assessment of 35 American *Aedes aegypti* and *Ae. albopictus* populations revealed that CHIKV dissemination was high for all, but transmission efficiency varied substantially among American populations (Vega-Rua et al. 2014). Taken together, these studies strongly suggest that salivary gland transmission barriers determine variation in vector competence among these *Aedes* species. The mechanism(s) responsible for these effects is not known, but environmental temperature strongly influences cellular and humoral immunity responses in mosquitoes which in turn impacts vector competence (Murdock et al. 2012, Adelman et al. 2013). Therefore, CHIKV emergence may vary among geographic populations of *Aedes* vectors and the direct or indirect influences of environmental temperature on virus replication or mosquito physiology. Also, *Ae. aegypti* and *Ae. albopictus* from Florida differ in their competence to transmit the three lineages of CHIKV (East/Central/South Africa, West Africa, Asian) (Sam et al. 2012, Vega-Rúa et al. 2014, Alto et al. 2017). Few studies have appraised the influences of environmental temperature on transmission efficiency of *Ae. aegypti* and *Ae. albopictus* for the CHIKV Asian lineage responsible for the current outbreak in the Americas (Vega-Rúa et al. 2015).

In this article, we examine the influence of constant versus fluctuating environmental temperature on CHIKV disseminated infection and saliva infection of *Ae. aegypti* and *Ae. albopictus* from Florida. We tested whether geographic populations of these species show regional differences in disseminated and saliva infections of CHIKV in different temperature environments.

Materials and Methods

Mosquito Collections and Rearing

Ae. aegypti and *Ae. albopictus* were collected as larvae from containers in Florida where these species are sympatric or allopatric (Lounibos et al. 2010, 2016; Murrell et al. 2011). Collections were made from sites in Manatee (Bradenton, sympatric), Okeechobee (Okeechobee, sympatric), Monroe (Key West, *Ae. aegypti*, allopatric), and Alachua (Gainesville, *Ae. albopictus*, allopatric) Counties. We chose these sites based on a previous study that showed small-scale variation in mosquito-CHIKV interactions among Florida *Ae. aegypti* and *Ae. albopictus* (Alto et al. 2017). Additionally, there is some evidence of genetic isolation of Florida Keys *Ae. aegypti* from mainland Florida (Damal et al. 2013). Larvae were reared at an approximate density of 150/liter water in plastic trays [25 × 30 × 5 cm (width by length by height)] with 900 ml of water and 0.4 g larval food (equal amounts of brewer's yeast and liver powder) at hatching and supplemented again with the same amount 3–4 d later. Mosquitoes were held at 26–28°C and a photoperiod of 13.5:10.5 light:dark. After pupation mosquitoes were transferred to 0.3 m^3 screened cages to house adults. Adults were provided with 10% sucrose solution from cotton wicks and bovine blood meals once per week using a hog casing membrane feeding system. On damp paper towels in cups with water females laid eggs, which were hatched and the resulting larvae used for experiments. One day before adults were fed CHIVK infected blood, females were placed in cages with mesh screening (height by diameter: 10 cm × 10 cm, 50 females/cage). The F_{1-3} generation progeny of field-collected *Aedes* mosquitoes were used for the CHIKV infection studies.

CHIKV Isolate and Propagation

An isolate of CHIKV from an infected human in the British Virgin Islands (Asian lineage, GenBank accession: KJ451624) in December 2013 was provided by the Centers for Disease Control and Prevention. The virus was propagated using two passages in cultured African green monkey (Vero) cells. Viral titer was determined by plaque assay using a modified procedure by Kaur et al. (2016).

Mosquito Infection

Adult females aged 11–14 d old were exposed for 1 h to CHIKV infected defibrinated bovine blood (Hemostat, Dixon, CA) through an artificial membrane feeding system (Hemotek, Lancashire, UK). Virus was prepared by propagating CHIKV in T-175 cm^2 tissue culture flasks with monolayers of Vero cells and media for 48 h using methods described by Alto et al. (2017). Infected blood was created by addition of media from infected cell cultures with defibrinated bovine blood and ATP (0.005 M). Mosquitoes were exposed to 6.3 to 6.8 $\log_{10}$ plaque forming unit equivalents (pfue)/ml of CHIKV for the experiments with *Ae. aegypti* and *Ae. albopictus*. Immediately following feeding trials, mosquitoes were transferred to one of three temperature treatments.

Temperature Regime

We evaluated experimentally the effect of temperature variations on transmission efficiency of these *Aedes* species using three temperature regimes. Mosquitoes were housed in environmental chambers (Percival, Models 130VL and 136VL, Perry, IA) with the coolest conditions being associated with the dark period of the diel (13.5:10.5, light:dark) with temperature changes possible on each hour (Fig. 1). Each temperature regime had approximately the same average temperature (27–28°C), but differed in the magnitude of the DTR (U.S. Climate data 2015). The large DTR was 8.0°C (range 23–31°C) which approximates West Palm Beach (adjacent to Okeechobee) during July-October with an average low of 23.6°C and high of 31.3°C. The small DTR was 4.0°C (range 26–30°C) which approximates Monroe Co. during July–October with an average low of 25.8°C and high of 31°C. On both DTRs, the day's peak temperatures occurred in late morning (Fig. 1). The DTR of Manatee Co. is like West Palm Beach and so a separate treatment was not included for this geographic location. The DTR of Alachua Co. is larger than the other treatments (DTR 10.7°C, low 20.1°C, high 30.8°C) and was not included in these studies. HOBO data loggers (Onset, Cape Cod, MA) were used to monitor temperatures on 30-min intervals during the experiments. We deliberately chose temperatures representative of a time of year of the highest expected numbers of imported cases of CHIKV, because this period coincides with the greatest risk for local transmission (FDOH 2014). The time of the year used also overlaps with the season when local transmission of this arbovirus in Florida was documented in 2014. Observed

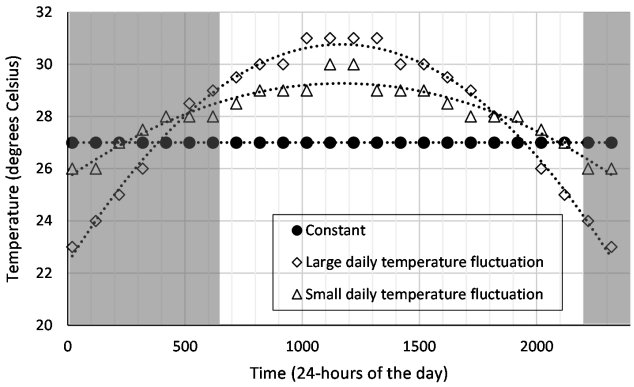

Fig. 1. Daily temperature regimes used in the chikungunya virus infection experiment in *Ae. aegypti* and *Ae. albopictus*. The constant temperature was set at 27°C, small daily fluctuating temperature was from 26 to 30°C (range of 4°C), and the large daily fluctuating temperature was from 23 to 31°C (range of 8°C). White and grey areas represent the light and dark periods of the photoperiod, respectively. The time during the diel on the x-axis is expressed in military time which ranges from 0000 to 2400. The observed temperatures deviated from the set temperatures as follows (range of SD): Constant (*Ae. aegypti*, 0.06–0.35; *Ae. albopictus*, 0.81–1.0), low fluctuating temperature temperatures (*Ae. aegypti*, 0.48–0.68; *Ae. albopictus*, 0.20–0.73), and high fluctuating temperatures (*Ae. aegypti*, 0.04–0.40; *Ae. albopictus*, 0.11–0.20).

temperatures (mean ± SD) measured at 30-min intervals during the experiments were as follows: *Ae. aegypti* (constant, 26.9 ± 0.25°C; small DTR, 28.9 ± 1.3°C; large DTR, 28.9 ± 2.9°C) and *Ae. albopictus* (constant, 27.1 ± 1.0°C; small DTR, 28.5°C ± 0.9; large DTR, 28.3 ± 2.8°C). The temperature regimes occur over a 24-h period and repeated daily (Fig. 1).

Temperature regimes may directly alter mosquito–virus interactions through factors such as altered immune function (e.g., RNAi pathways, Adelman et al. 2013), extrinsic incubation period (Davis 1932, Chamberlain and Sudia 1955), and infection of tissues and organs (Lambrechts et al. 2011). Conversely, fluctuating temperature regimes may indirectly alter mosquito–virus interactions through alterations in mosquito physiology during growth and development during the immature stages that have transstadial effects on the adult stage (Alto and Bettinardi 2013). Our study manipulates temperature regimes only during the adult stage to measure direct effects on mosquito–virus interactions.

Mosquito Disseminated Infection and Saliva Infection

Mosquitoes were tested for their potential to transmit CHIKV by means of collections of expectorated saliva 7 d after feeding on infected blood. This incubation period was chosen based on maximum transmission efficiency of Florida *Ae. aegypti* and *Ae. albopictus* for the Asian lineage of CHIKV in the Americas, after which a decline in saliva infection was observed later during infection (Alto et al. 2017). Also, our timing is based on anticipated maximum number of viral particles shed in the saliva during CHIKV infection for both species (Dubrulle et al. 2009). Mosquito saliva was collected in capillary tubes as described previously (Alto et al. 2014, 2017). For each mosquito, legs and wings were removed and the proboscis was inserted into a capillary tube containing immersion oil. After 1 h of salivation, immersion oil and saliva were expelled into 300 µl of media, which was then subjected to viral RNA isolation and real-time reverse transcription–polymerase chain reaction (qRT-PCR). Mosquito bodies and legs were stored at −80°C upon completion of each saliva infection assay and later tested separately for the presence of CHIKV RNA by qRT-PCR using methods of Reiskind et al. (2008). Legs were homogenized in 1000 µl of media in preparation for RNA isolation (Alto et al. 2017). Primers were designed to target a nonstructural polyprotein gene (accession ID of transcript, KU365292.1) with the following sequences: forward, 5′-GTACGGAAGGTAAACTGGTATGG-3′: reverse, 5′-TCCACCTCCCACTCCTTAAT-3′. The probe sequence was: 5′-/56-FAM/TGCAGAACCCACCGAAAGGAAACT/3BHQ_1/-3′ (Integrated DNA Technologies, Coralville, IA). Testing the legs of mosquitoes allowed us to determine disseminated infection, calculated as the percent of infected legs from the total number engorged with blood (Turell et al. 1984). Saliva infection was calculated as the percent of transmitting mosquitoes out of the total number of mosquitoes with infected legs.

A 140 µl sample of mosquito legs and saliva homogenate was used for RNA isolation using the QIAamp viral RNA mini kit (Qiagen, Valencia, CA) and eluted in 50 µl of buffer per the manufacturer's protocol. CHIKV RNA was detected using the Superscript III One-Step qRT-PCR with Platinum Taq kit by Invitrogen (Invitrogen, Carlsbad, CA) as described previously (Reiskind et al. 2008, Alto et al. 2017). Quantitative RT-PCR was performed with the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The program for qRT-PCR was as follows; 50°C for 30 min, 94°C for 2 min, 39 cycles at 94°C for 10 s and 60°C for 1 min, and 50°C for 30 s. The titer of CHIKV in mosquito samples was determined using a standard curve method by comparing cDNA synthesis for serial dilutions of CHIKV together with plaque assays on serial dilutions of CHIKV, expressed as plaque forming unit equivalents (pfue)/ml (Bustin 2000).

Statistical Analyses

Mosquito species, geographic population, temperature, and interaction effects on disseminated infection and transmission were analyzed using maximum likelihood categorical analyses of contingency tables based on the number of mosquitoes categorized for the presence or absence of CHIKV in mosquito legs and saliva. When significant treatment effects were found, follow-up analyses included pairwise comparisons of treatments, correcting for multiple comparisons using the sequential Bonferroni method (Rice 1989). These statistical tests enabled us to gauge barriers to infection and transmission (midgut escape and salivary gland barriers). Each infection experiment with *Ae. aegypti* and *Ae. albopictus* and CHIKV was conducted only once with four separate feeding trials on different days for each species. Similar number of mosquitoes from each geographic location and temperature treatment were used in each of the four feeding trials. Individual mosquitoes are the unit of replication, and we analyzed infection responses by means of frequency distributions (Sokal and Rohlf 1995). Analysis of variance was used to test for differences in mean virus titers in the legs and saliva of the individual mosquitoes. Significant effects were followed by Tukey-Kramer multiple comparisons among treatment least-squares means.

Results

Viral Titer in Infected Blood

Ae. aegypti and *Ae. albopictus* fed on a dose of 6.8 ± 0.2 $\log_{10}$ plaque forming unit equivalents (pfue)/ml and 6.3 ± 0.5 $\log_{10}$ pfue/ml of CHIKV, respectively. These titers are within the range of the viral load in patients with symptomatic CHIKV infection (Appassakij et al. 2013). Because viral titers of infected blood were significantly lower for *Ae. albopictus* than *Ae. aegypti* (t = 3.4; df = 22; P = 0.0026) we analyzed infection and saliva infection parameters of the two species separately.

Disseminated Infection and Saliva Infection by *Ae. aegypti*

There were no significant main effects of origin of *Ae. aegypti* population or temperature regimen on chikungunya disseminated infection. However, there was a significant interaction effect between geographic population of *Ae. aegypti* and temperature regimen on disseminated infections (Table 1). Because the interaction was significant, we compared each temperature regime within the geographic population treatment (e.g., constant versus low, constant versus high, and low versus high for Monroe Co., FL). High fluctuating temperatures resulted in a significantly greater number of mosquitoes with disseminated infections of CHIKV than constant (high fluctuating, 65%; constant, 41%; $\chi^2 = 12.3$; df = 1; $P = 0.0004$) and low fluctuating (high fluctuating 65%; low fluctuating, 37%; $\chi^2 = 16.8$; df = 1; $P < 0.0001$) temperature regimes for *Ae. aegypti* from Manatee Co., FL, but not other locations in Florida (Table 2). There were no significant treatment effects of temperature, geographic population, or temperature by geographic population interaction on CHIKV saliva infection (Table 1).

There were significant differences in the leg titer of tested populations of *Ae. aegypti* infected with CHIKV (Table 3). Viral titers were significantly higher among individuals originating from Monroe and Okeechobee than Manatee (All *P*-values <0.03, Fig. 2A). There were no significant treatment effects of temperature or temperature by geographic population (Table 3). There were no significant treatment effects of temperature, geographic population, and temperature by geographic population on saliva titer (Table 3, Fig. 2B).

Disseminated Infection and Saliva Infection by *Ae. albopictus*

There were no significant main effects of geographic population of *Ae. albopictus* and temperature regimen on chikungunya disseminated infection. There was a significant interaction effect between geographic population of *Ae. albopictus* and temperature regimen on disseminated infections (Table 1). Because the interaction was significant, we compared each temperature regime within the geographic population treatment (e.g., constant versus low, constant versus high, and low versus high for Alachua). However, after correcting *P*-values for multiple comparisons differences were only marginally significant. High fluctuating temperatures (19.3%) resulted in a lower number of mosquitoes with disseminated infection than constant (34.7%; $\chi^2 = 3.7$; df = 1; $P = 0.05$) and low fluctuating (37.6%; $\chi^2 = 5.4$; df = 1; $P = 0.02$) temperature regimes for Alachua, but not other locations in Florida (Table 2). There were no significant treatment effects of temperature, geographic population, or temperature by geographic population interaction on CHIKV leg titer (Table 3, Fig. 2C). There was a significant effect of temperature, but not geographic population or temperature by location interaction on CHIKV saliva titer (Table 3, Fig. 2D). Both small and large DTR led reduced viral load in saliva compared to constant temperature (large DTR, log10 pfue/ml 1.30 ± 0.23; small DTR, 1.19 ± 0.20; constant, 1.92 ± 0.20), although it was only significantly different for small DTR versus constant temperature ($P = 0.01$).

Table 1. Treatment effects on the chikungunya virus disseminated infection (leg infection) and transmission (saliva infection)

Ae. aegypti			
Disseminated infection			
Factor	χ^2	df	*P*
Location	4.65	2	0.0977
Temperature	4.52	2	0.1046
Location × Temperature	19.08	4	0.0008
Transmission			
Factor	χ^2	df	*P*
Location	1.38	2	0.5027
Temperature	0.66	2	0.7193
Location × Temperature	5.79	4	0.2157
Ae. albopictus			
Disseminated infection			
Factor	χ^2	df	*P*
Location	2.79	2	0.2483
Temperature	0.72	2	0.6963
Location × temperature	11.41	4	0.0223
Transmission			
Factor	χ^2	df	*P*
Location	1.09	2	0.5797
Temperature	2.22	2	0.3300
Location × temperature	4.18	4	0.3824

Discussion

We compared the number of mosquitoes with disseminated infection and saliva infection of an emergent Asian genotype of chikungunya virus from the Americas in *Ae. aegypti* and *Ae. albopictus* from multiple locations in Florida to examine the effects of constant and fluctuating daily temperatures. We found partial support that DTR alters vector competence for CHIKV in invasive *Aedes* mosquitoes. Specifically, a large DTR enhanced disseminated infection in *Ae. aegypti* and inhibited disseminated infection in *Ae. albopictus* relative to constant and low DTRs for select geographic populations. The idiosyncratic nature of the effects of the DTR among species and populations further complicate our ability to identify patterns in mosquito–virus interactions related to temperature. Model frameworks that account for heterogeneity in infection responses may improve links between environmental temperature and risk of transmission of mosquito-borne pathogens in Florida and other locations. In our study, temperature regime did not influence saliva infection and so, within ranges tested in this study, is not likely to lead to changes in CHIKV transmission potential among Florida mosquito vectors.

Comparison with existing studies shows that fluctuating temperature may enhance or impede the infection process in *Ae. aegypti*. A low mean temperature, but not temperature fluctuations at low and high mean temperatures, reduced susceptibility to dengue-1 virus infection of *Ae. aegypti* (Carrington et al. 2013b). In contrast, large daily fluctuating temperature reduced susceptibility to infection with dengue-1 and dengue-2 viruses of *Ae. aegypti* compared to constant and small daily fluctuating temperatures (Lambrechts et al. 2011, Carrington et al. 2013c). However, Carrington et al. (2013c) showed that daily fluctuating temperature did not influence dissemination of dengue-1 virus into head tissue. In a related study, Carrington et al. (2013b) showed that fluctuating temperature at low, but not high, mean temperatures shortened the extrinsic incubation period of dengue-1 of *Ae. aegypti*. These observations contrast those by Lambrechts et al. (2011) showing that large daily fluctuating temperature reduced the number of mosquitoes with midgut infections in *Ae. aegypti* but not the extrinsic incubation period for dengue-1 and dengue-2 viruses. These results, taken together with those of the current study, show that there is considerable variability of DTR on barriers to infection of mosquitoes and between mosquito–virus systems. The impact of fluctuating temperature on mosquito vector competence may depend on whether fluctuations occur around cool or warm mean temperatures (Carrington et al.

Table 2. Chikungunya virus disseminated infection and transmission for *Ae. aegypti* (*aeg*) and *Ae. albopictus* (albo) from different geographic regions of Florida

Geographic region[a]	Mosquito species	Daily temperature fluctuation	% disseminated infection (no. of mosquitoes)[b]	% transmission (no. of mosquitoes)[c]
Okeechobee Co.	*aeg*	Constant	53.3 (137)	23.5 (51)
		Low	57.9 (95)	29.0 (38)
		High	54.1 (85)	21.4 (28)
	albo	Constant	26.4 (106)	32.1 (28)
		Low	37.8 (68)	50.0 (18)
		High	41.3 (46)	11.1 (18)
Monroe Co.	*aeg*	Constant	64.0 (111)	31.5 (54)
		Low	51.8 (112)	21.2 (33)
		High	52.2 (113)	14.3 (42)
Manatee Co.	*aeg*	Constant	41.8 (153)	26.3 (57)
		Low	37.0 (135)	23.3 (30)
		High	65.9 (85)	35.6 (45)
	albo	Constant	23.1 (52)	40.0 (10)
		Low	20.7 (58)	33.3 (12)
		High	36.6 (41)	38.5 (13)
Alachua Co.	*albo*	Constant	34.7 (75)	32.0 (25)
		Low	37.6 (93)	28.1 (32)
		High	19.3 (57)	22.2 (9)

[a]Geographic location of mosquito populations collected in Florida.

[b]Disseminated infection corresponds to the percent of individual females with infected legs out of the total number engorged with blood. A total of 1,026 *Ae. aegypti* and 596 *Ae. albopictus* were tested.

[c]Transmission corresponds to the percent of individuals with infected saliva mosquitoes out of the total number of mosquitoes with infected legs. A total of 378 *Ae. aegypti* and 165 *Ae. albopictus* were tested.

Table 3. Treatment effects on titers of chikungunya virus in legs and saliva of *Ae. aegypti* and *Ae. albopictus*

Ae. aegypti			
Leg viral titer			
Factor	F	df	*P*
Location	3.87	2	0.0214
Temperature	0.96	2	0.3842
Location × temperature	1.13	4	0.3418
Error		522	
Saliva viral titer			
Factor	F	df	*P*
Location	1.25	2	0.2922
Temperature	0.30	2	0.7443
Location × temperature	1.28	4	0.2857
Error		89	
Ae. albopictus			
Leg viral titer			
Factor	F	df	*P*
Location	0.32	2	0.7263
Temperature	0.17	2	0.8460
Location × temperature	0.36	4	0.8378
Error		173	
Saliva viral titer			
Factor	F	df	*P*
Location	2.39	2	0.1038
Temperature	3.42	2	0.0420
Location × temperature	1.22	4	0.3151
Error		43	

2013c). Fluctuating temperature at low temperature (20°C), but not high temperature (30°C), increased the number of *Ae. aegypti* with disseminated infection of dengue-1 virus relative to constant temperature (Carrington et al. 2013b). Our results are consistent with these observations given that we used a warm mean temperature (27°C) and found that fluctuating temperature had a minimal impact on the number of mosquitoes with disseminated infection and saliva infection for *Ae. aegypti* and *Ae. albopictus* among select geographic populations. These results suggest that the midgut barriers may be more sensitive to fluctuations in temperature than salivary gland barriers. Ultimately, fluctuating diurnal temperatures may be more important for disease transmission through their effects on vector population dynamics and age structure (Beck-Johnson et al. 2017).

We found evidence that the number of mosquitoes with disseminated infection, but not saliva infection, in *Ae. aegypti* and *Ae. albopictus* was influenced by an interaction of geographic origin of mosquito and temperature regime, suggesting small scale geographic variation of CHIKV infection in potential *Aedes* vectors. High fluctuating temperature led to a significant (40%) increase in the number of *Ae. aegypti* with disseminated CHIKV infection from Manatee compared to constant and low fluctuating temperature regimes. In contrast, similar rates of disseminated infection were observed among the temperature regimes among *Ae. aegypti* originating from other locations in Florida. These results are consistent with observations of variation in vector competence among mosquito vectors in Florida for the Asian and Indian Ocean genotypes of CHIKV (Alto et al. 2017). Specifically, *Ae. aegypti* from Manatee had lower or similar rates of disseminated infection and saliva infection of the Indian Ocean genotype of CHIKV from one or more other locations in Florida. For *Ae. albopictus*, saliva infection of Indian Ocean CHIKV, but not disseminated infection, varied by Florida mosquito populations (Alto et al. 2017), with higher rates found among individuals originating from either Manatee or Indian River/St. Lucie compared to Alachua. In contrast, *Ae. albopictus* dissemination rates, but not saliva infection, varied among Florida mosquito populations, with

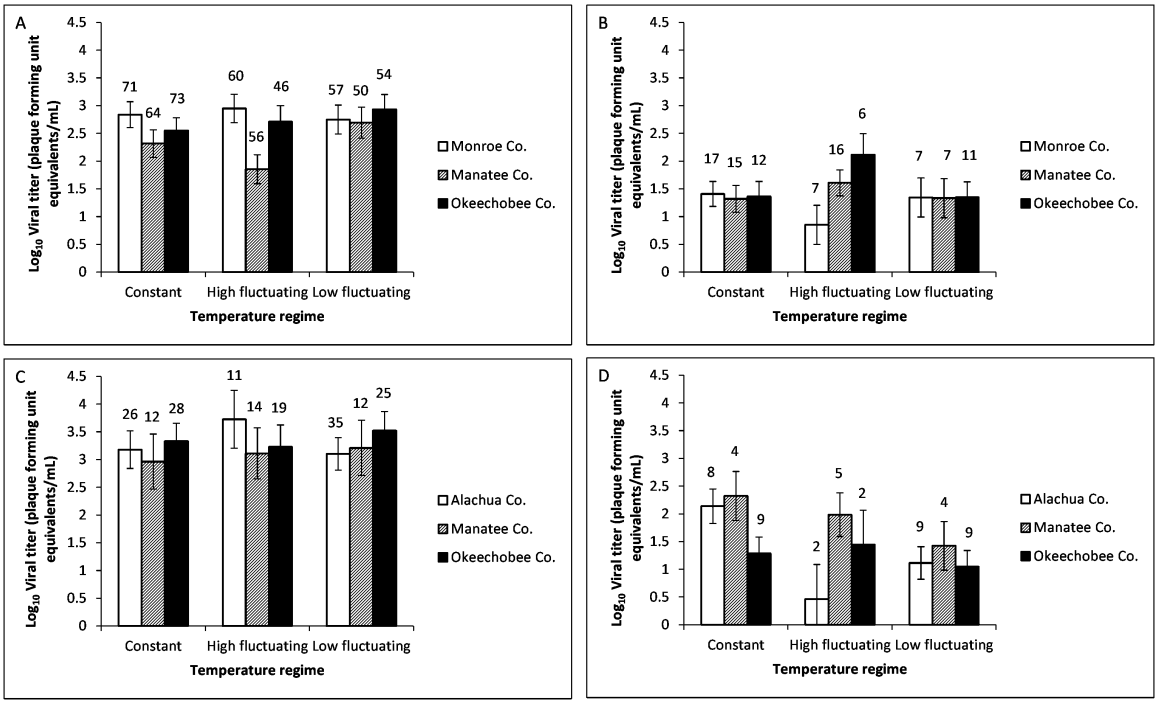

Fig. 2. Viral titer (LS mean ± SE) expressed in log10 plaque forming unit equivalents/ml of chikungunya virus in (A) *Ae. aegypti* legs, (B) *Ae. aegypti* saliva, (C) *Ae. albopictus* legs, and (D) *Ae. albopictus* saliva. Temperature regimes include constant (27°C), small daily fluctuating temperature from 26 to 30°C (range of 4°C), and a large daily fluctuating temperature from 23 to 31°C (range of 8°C). Numbers above columns show the number of mosquito samples tested.

higher rates found among individuals from Alachua than from Manatee or Indian River/St. Lucie. Taken together, these results suggest that midgut and salivary gland barriers may differ at small geographic scales, but the pattern is complex and depends on interactions between virus and mosquito genotypes and environmental temperature. Population genetic diversity of *Ae. albopictus* outside of Southeast Asia is high because of multiple introductions of different genotypes (Manni et al. 2017) which may also apply to select geographic populations of *Ae. aegypti*. The intra-populational variability of these invasive vector species, facilitated by 'chaotic dispersion' (Manni et al. 2017), may underlie the variable responses of local populations to infection and temperature parameters.

We tested a relatively narrow temperature range which may, in part, explain effects found on the number of mosquitoes with disseminated infection but not saliva infection. Fluctuating temperatures lowered viral titer in *Ae. albopictus* in some tissues given the lower viral load in the saliva in the two fluctuating temperature treatments compared to constant temperature, suggesting that temperature regime altered virus replication. Therefore, daily fluctuation in temperature may result in lower viral inoculum by bite and associated decreased infection compared to constant temperature conditions. These observations contrast measurements of viral loads in the saliva of *Ae. albopictus* infected with an Asian genotype of CHIKV where daily fluctuations of temperature with a mean of 20°C did not alter viral loads compared to a constant temperature of 20°C (Vega-Rúa et al. 2015). Although the reason for the discrepancy in results between this study and the current study is not known, it may be associated with different temperatures tested, a factor known to alter mosquito–virus interactions (Carrington et al. 2013b,c; Zouache et al. 2014) and immune function against CHIKV (Adelman et al. 2013).

In the current study, disseminated infection tended to be higher for *Ae. aegypti* than *Ae. albopictus*, whereas saliva infection was similar for both species. Species-specific differences in infection rates are consistent with other observations comparing *Ae. aegypti* and *Ae. albopictus* for the Asian genotype of CHIKV (Sam et al. 2012, Vega-Rúa et al. 2014, Alto et al. 2017), suggesting a more permissive midgut barrier to infection for *Ae. aegypti*. Additionally, higher disseminated infection rates may be, in part, attributable to the higher dose of virus in infected blood meals fed to *Ae. aegypti*. Disseminated infection and saliva infection rates were lower than those observed for an infection study with emergent CHIKVs from the Asian and Indian Ocean lineages (Alto et al. 2017) and *Ae. aegypti* and *Ae. albopictus*, most likely attributable to the lower infectious doses used here.

It should be emphasized that the current study did not consider whether fluctuating temperatures influenced other parameters of vectorial capacity (e.g., adult survival, biting rate, extrinsic incubation period; Lambrechts et al. 2011, Mordecai et al. 2017) and adult age structure (Beck-Johnson et al. 2017). Furthermore, fluctuating temperatures may alter life history traits attributable to temperature experienced during the immature stages which may indirectly alter CHIKV-mosquito interactions (e.g., dengue viruses, Alto and Bettinardi 2013, Carrington et al. 2013a). We only measured disseminated infection (not susceptibility to midgut infection) and saliva infection at a single time point after ingesting CHIKV infected blood, limiting our ability to detect temporal patterns in infection attributable to treatments. Future empirical studies and models are needed to address the cumulative effect of daily temperature variation on the entomological components of CHIKV epidemiology.

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