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THE INFLUENCE OF CLIMATE CHANGE AND EVOLUTION ON MOSQUITO LIFE HISTORY TRAITS AND PATHOGEN TRANSMISSION

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Jordan Ruybal

September 2016

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ABSTRACT

Jordan Ruybal

THE INFLUENCE OF CLIMATE CHANGE AND EVOLUTION ON MOSQUITO LIFE HISTORY TRAITS AND PATHOGEN TRANSMISSION

Many aspects of mosquito biology are highly sensitive to variation in temperature, which has led to predictions that climate change will alter the transmission of many vector-borne pathogens. However, it is unknown how mosquitoes will evolve in response to changing climates. We utilized common garden experiments and novel time-compressed climate change scenarios to examine standing geographic variation, species variation, and evolutionary change in the temperature dependence of four life history traits of mosquitoes (larval and adult survival, development rate, and biting rate).

First, we quantified spatial variation in life history traits for four populations of *Culex pipiens* mosquitoes, a primary vector of West Nile Virus in North America, to examine the extent to which mosquitoes might be adapted to local thermal environments. We found substantial variation in life history traits among mosquito populations that was uncorrelated with local thermal conditions. This variation will shape the response of mosquito species to changing climates and will make the impact of climate change on vector-borne disease more variable and less predictable than previously thought.

Second, we quantified variation in life history traits and vectorial capacity for the two dominant mosquito vectors of Zika, dengue, chikungunya, and yellow fever viruses, *Aedes aegypti* and *Aedes albopictus*. Differences in life history traits were mixed with *Ae. albopictus* having faster development, higher larval survival, and more frequent feeding, whereas *Ae. aegypti* had higher adult survival. *Ae. aegypti* was a slightly more efficient vector of all four viruses, and vectorial capacity was highest for yellow fever virus followed by Zika, Chikungunya and dengue.

Lastly, we reared *Ae. aegypti* under three rates of temperature increase (+2°C, +4°C, and +5°C) and three control conditions (a 2°C decrease, and two temperature profiles with no net change) for one year to examine the potential evolutionary response to climate change. Although there was significant among-treatment variation in four life history traits between the starting population and the six climate treatments, these differences were uncorrelated with the temperature regime the mosquitoes experienced, suggesting that the mosquitoes were adapting more to the increased variance in temperatures in the experiment than the differences in mean temperature.

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INTRODUCTION

Many aspects of mosquito biology are highly sensitive to variation in temperature, which has led to predictions that climate change will alter the transmission of many vector-borne pathogens (Rogers & Randolph 2000; Patz *et al.* 2002; Hay *et al.* 2004; Chaves *et al.* 2012; Kilpatrick & Randolph 2012; Siraj *et al.* 2014). However, it is unknown how mosquitoes will evolve in response to changing climates and how this might affect predictions that are often based on assumptions that mosquitoes will not evolve fast enough (Egizi *et al.* 2015). We utilized common garden experiments and a novel time-compressed climate change experiments to examine standing geographic variation and evolutionary change in the temperature dependence of four life history traits of mosquitoes (larval and adult survival, development rate, and biting rate).

Life history traits of different populations often differ significantly (Armbruster & Conn 2006; Sternberg & Thomas 2014). Specifically, performance across a range of temperatures is likely to vary due to local adaptation to temperature and other factors. This variation can cause spatial variation in pathogen transmission and will influence the impact of climate change on the transmission of vector-borne pathogens.

In chapter 1 **Geographic Variation in the Response of *Culex pipiens* Life History Traits to Temperature,** we quantified spatial variation in life history traits for four populations of *Culex pipiens* (Linnaeus) mosquitoes (a primary vector of West Nile Virus and other arboviruses in urban and residential areas of North

America) to examine the extent to which mosquitoes might be adapted to local thermal environments. The populations were distributed along altitudinal and latitudinal gradients in the eastern United States that spanned ~3°C in mean summer temperature, which is similar to the magnitude of global warming expected in the next 3-5 decades. We measured larval and adult survival, development rate, and biting rate at six temperatures between 16-35°C, in a common garden experiment, and integrated these results into a model of population dynamics and vectorial capacity.

We found substantial variation in life history traits among populations of *Cx. pipiens* that was uncorrelated with local thermal conditions, but will nonetheless shape the response of mosquito species to changing climates. This suggests that the impact of climate change on vector-borne disease will be more variable and less predictable than previous studies have suggested, but our study provides an estimate of this uncertainty.

Quantifying variation in vectorial capacity for multiple pathogens and multiple species of mosquito provides insight into spatial and temporal variation in potential pathogen transmission cycles and targets for control. Both *Aedes aegypti* and *Aedes albopictus* (Linneaus) mosquitoes are invasive and expanding their geographic distribution in the United States. These two species are also the dominant vectors for four of the most important mosquito-borne viruses of humans, Zika, dengue, chikungunya, and yellow fever virus. Determining their vectorial capacity for these viruses can be used to assess the likelihood of an outbreak of one of these viruses and is thus critical for developing efficient vector control strategies.

Thus, in chapter 2 **Variation in vectorial capacity for Zika, dengue, chikungunya, and yellow fever virus for North American *Aedes aegypti* and *Aedes albopictus* mosquitoes**, we collected *Ae. aegypti* and *Ae. albopictus* from New Orleans and measured four life history traits, larval and adult survival, development rate, and biting rate, across six fluctuating temperature regimes, with mean daily temperatures of 13.5-35.5°C, which spanned the conditions that these mosquitoes experience in much of their distribution. We then used these life history data and published data on vector competence to estimate the vectorial capacity of these two species for Zika, dengue, chikungunya, and yellow fever virus.

Differences in life history traits between *Ae. aegypti* and *Ae. albopictus* were mixed with *Ae. albopictus* having faster development, higher larval survival, and feeding earlier, but *Ae. aegypti* had higher adult survival. Integrating these differences into models of pathogen transmission suggested that *Ae. aegypti* was a slightly more efficient vector of all four viruses. Vectorial capacity of these two species was highest for yellow fever virus followed by Zika, Chikungunya and dengue.

Most studies that predict the impact of climate change on vector-borne diseases assume that traits of the pathogen, hosts and vectors are static and project the impacts of warming based on temperature-trait relationships measured in the lab, or previous patterns of incidence. However, evolutionary adaptation could be an important way for populations to respond to climate change. Here we tested the assumption that mosquito temperature-traits will remain static under predicted

climate change. In doing so, we are testing a key assumption of the current paradigm for projecting the impacts of global warming on vector borne disease.

Thus, in chapter 3 **Adaptation of *Aedes aegypti* to simulated climate change reveals sensitivity to temperature variability**, we collected *Aedes aegypti* (Linnaeus) mosquitoes from New Orleans, Louisiana and reared them under three rates of temperature increase (+2°C, +4°C, and +5°C) and three control conditions (a 2°C decrease, and two temperature profiles with no net change) for one year. All temperature trajectories incorporated stochastic daily (mean= 8°C, max= 17°C, min= 1°C), seasonal (mean= 17°C, max= 24°C, min= 10°C) and yearly fluctuations in temperature (mean= 23°C, max= 28°C, min= 20°C), as well as seasonal variation in photo-period. At the start and end of the experiment we measured four life history traits, larval and adult survival, development rate, and biting rate. We performed measurements across six fluctuating temperature regimes, with mean daily temperatures of 13.5-35.5°C, which spanned the conditions that these mosquitoes experience in much of their distribution.

There was significant among-treatment variation in the thermal response curves for the four life history traits between the starting population and the six climate treatments. Larval development rate increased at higher temps and decreased at lower temperatures, larval survival decreased across all temperatures, while adult survival increased across all temperatures, and biting rate decreased. However, the magnitude of these changes was uncorrelated with the temperature regime the mosquitoes experienced, suggesting that the mosquitoes were adapting more to the

increased variance in temperatures in the experiment than the differences in mean temperature.

CHAPTER ONE

Geographic Variation in the Response of *Culex pipiens* Life History Traits to Temperature

ABSTRACT

Background: Climate change is predicted to alter the transmission of many vector-borne pathogens. The quantitative impact of climate change is usually estimated by measuring the temperature-performance relationships for a single population of vectors, and then mapping this relationship across a range of temperatures or locations. However, life history traits of different populations often differ significantly. Specifically, performance across a range of temperatures is likely to vary due to local adaptation to temperature and other factors. This variation can cause spatial variation in pathogen transmission and will influence the impact of climate change on the transmission of vector-borne pathogens.

Methods: We quantified variation in life history traits for four populations of *Culex pipiens* (Linnaeus) mosquitoes. The populations were distributed along altitudinal and latitudinal gradients in the eastern United States that spanned ~3°C in mean summer temperature, which is similar to the magnitude of global warming expected in the next 3-5 decades. We measured larval and adult survival, development rate, and biting rate at six temperatures between 16-35°C, in a common garden experiment.

Results: Temperature had strong and consistent non-linear effects on all four life history traits for all four populations. Adult female development time decreased

monotonically with increasing temperature, with the largest decrease at cold temperatures. Daily juvenile and adult female survival also decreased with increasing temperature, but the largest decrease occurred at higher temperatures. There was significant among-population variation in the thermal response curves for the four life history traits across the four populations, with larval survival, adult survival, and development rate varying up to 45%, 79%, and 84% among populations, respectively. However, variation was not correlated with local temperatures and thus did not support the local thermal adaptation hypothesis.

Conclusion: These results suggest that the impact of climate change on vector-borne disease will be more variable than previous predictions, and our data provide an estimate of this uncertainty. In addition, the variation among populations that we observed will shape the response of vectors to changing climates.

INTRODUCTION

The impact of climate change on the transmission of vector-borne diseases is a hotly debated topic (Rogers & Randolph 2000; Patz *et al.* 2002; Hay *et al.* 2004; Chaves *et al.* 2012; Kilpatrick & Randolph 2012; Siraj *et al.* 2014). Early predictions suggested that climate change would increase the global burden of tropical diseases, such as malaria, as temperate regions warmed (Githeko *et al.* 2000). However, other researchers have argued that warming will also cause a decrease in transmission in some tropical regions which will become too hot, and this will result in a geographic shift in distribution but little change in overall disease burden (Rogers & Randolph 2006; Lafferty 2009). Further, many have argued that changes in other factors such

as socioeconomic development, land use, drug treatment and bed-net use will be more important than climate change in determining disease incidence, and that transmission will be limited in temperate regions by public health systems and highly developed living conditions (e.g. screened windows and air conditioning) (Gething *et al.* 2010).

An issue that has received far less attention, despite its potential impact, is variability in the response of vectors to temperature (Lafferty 2009; Sternberg & Thomas 2014). Determining the extent of variation in temperature responses, is necessary to predict current and future spatial variation in transmission of tropical vector-borne diseases such as dengue virus and malaria, and to determine the extent of uncertainty in model predictions (Sternberg & Thomas 2014). If variation among vector populations exists, and the variation is strongly correlated with local thermal regimes (as would be expected if adaptation to local temperatures were the strongest driver), then this variation could be incorporated into model predictions. However, organisms are simultaneously under a diverse set of selective pressures, and selection on life history traits from other factors, as well as drift, could result in unpredictable variation (Berven *et al.* 1979; Conover *et al.* 2009). Several studies have found either inverse or counter-gradient variation (a phenomenon in which variation in genotypes counteracts environmental influences across a gradient such that phenotypic variation is diminished) along temperature gradients (Conover & Schultz 1995), or significant variation, but little evidence of local thermal adaptation (Levins 1969; Armbruster & Conn 2006). If variation among populations in the response to temperature is substantial, but idiosyncratic, then predictions of the impact of climate change will be

far less accurate, and models should incorporate this additional source of uncertainty into the predictions.

Only a handful of studies have been conducted on local adaptation to temperature in mosquito vectors. In *Anopheles gambiae,* an important vector of malaria in Africa, populations along aridity and latitudinal clines in Cameroon and Nigeria had increased frequencies of a genetic trait (the 2La chromosomal inversion) that confers increased heat and desiccation tolerance (Coluzzi *et al.* 1979; Gray *et al.* 2009; Rocca *et al.* 2009; Cheng *et al.* 2012). In contrast, a study of two populations of *Culex tarsalis* in California did not find variation in life history traits that correlated with local temperatures (Reisen 1995). Clearly, additional studies are needed of variation in vector traits that influence transmission from multiple populations along temperature gradients (Sternberg & Thomas 2014).

We examined spatial variation in life history traits of *Culex pipiens* mosquitoes along altitudinal and latitudinal gradients to determine the extent of local thermal adaptation. *Cx. pipiens* is the primary enzootic (bird-to-bird) and bridge (bird-to-human) mosquito vector of West Nile Virus (WNV) and other arboviruses in urban and residential areas of North America north of approximately 36° latitude (Turell *et al.* 2002; Kilpatrick *et al.* 2005; Hamer *et al.* 2008; Farajollahi *et al.* 2011; Kilpatrick & Pape 2013), and a vector of WNV and Usutu virus in Europe (Vinogradova 2000; Joy & Clay 2002; Farajollahi *et al.* 2011). WNV is a significant public health issue in North America, with ~2.8 million human infections, >20,000 cases of encephalitis and 1,902 deaths since it was introduced in 1999 (Kilpatrick

2011; “Centers for Disease Control and Prevention” 2014). Additionally, WNV has also killed millions of birds and caused regional declines in some species of up to 50% (Kilpatrick *et al.* 2007b, 2013; LaDeau *et al.* 2007). The wide geographic distribution of *Cx. pipiens* and its importance in transmitting several arboviruses makes it a useful model species to examine the extent of adaptation to local thermal regimes.

We conducted a common garden study of *Cx. pipiens* mosquitoes from four populations along altitudinal and latitudinal gradients with average summer temperatures differing by 2-3°C (Fig. S1). This variation in temperature is similar to that predicted to occur over the next few decades due to anthropogenic climate change (Parry *et al.* 2007). We combined altitudinal and latitudinal gradients to generate temperature gradients with different confounding variables (e.g. day length, atmospheric pressure, etc.). We measured four life history traits: larval and adult survival, development rate, and biting rate, across a range of temperatures that spanned the seasonal climate of these four populations to characterize their performance along a thermal gradient. We hypothesized that high temperature populations (lower latitude and elevation) would experience selection for faster development rate, and increased survival at hotter temperatures,whereas colder populations would exhibit the opposite tradeoff(Brown *et al.* 1998; Byars *et al.* 2007).

METHODS

Study Sites

We collected an average of 24.5 (6, 15, 32, and 45 rafts, respectively from the coolest to the warmest site) *Cx. pipiens* egg rafts at each of four sites between July 25 and July 28, 2011 (Fig. 1B). Site names describe the latitude and/or elevation for each population relative to the low elevation/latitude population (Fig. 1B). *Culex pipiens* hybridize with *Culex quinquefasciatus* across a wide latitudinal band of North America (Smith & Fonseca 2004; Kothera *et al.* 2009), and although previous genetic analyses in the study area found little evidence of *Culex quinquefasciatus* ancestry in these populations (Kilpatrick *et al.* 2007a), introgression of selected alleles from this tropical species could influence the response of populations to temperature.

Rearing and Handling

All field collected eggs were hatched at 25°C (± 2°C) under a photoperiod of 14:10 hrs (L:D), and larvae were morphologically identified to the *Cx. pipiens* complex using published keys (Darsie & Ward 2005). Larvae were reared at 25°C in groups of ~200 in Sterilite® plastic trays (27.9cm L x 16.8cm W x 7.0cm H), filled with 1 liter of deionized water. Larvae were fed a finely ground 1:1:1 mixture of MP® Liver Powder Bovine, Kaytee Koi's Choice® Premium Fish Food, & Small World® alfalfa rabbit feed. Adults were transferred to a 30.5cm^3 aluminum mesh collapsible cage (BioQuip) and held at 25°C. Five day-old mosquitoes were deprived of sucrose, but not water, overnight (~12-15 hrs) and then fed a mixture of defibrinated chicken blood (Rockland Immunochemicals) plus a final concentration of 2.5% sucrose and 1% ATP, warmed at 37°C for 5 minutes in a water bath. Females oviposited en masse and we collected an average of 61 egg rafts (20-85 rafts

per population). Larvae from each population were pooled together, and 600 larvae from each population were placed into 3 trays (200 larvae each), except for the high elevation population which only had one tray with 200 larvae. This first laboratory-raised generation of offspring was used for measuring the response of each population to variation in temperature.

We used six fixed temperatures (one per incubator) that spanned the minimum and maximum summer temperatures experienced across study populations: 16, 20, 24, 27, 31, and 35°C. Relative humidity (70±10%) and photoperiod (16:8 hrs. (L:D)) were held constant in all five incubators and larvae (and adults) were maintained as described above. Each day dead larvae and pupae were counted and removed. However, counts revealed that larvae had also disappeared due to cannibalism. Pupae from replicate trays were combined and transferred to a single emergence jar (BioQuip). The number of emerged males and females was counted daily and adults were immediately transferred to one-gallon cardboard containers with mesh tops. At 35°C no larvae survived to become adults. As a result, we used the remaining larvae from the initial rearing temperature (25°C) to measure adult daily survival and biting rate at 35°C.

Adult mosquitoes were fed ad libitum Domino® sugar cubes and water. We monitored adult mortality by inspecting each cage daily and counted and removed dead adults. Females had constant access to an oviposition site—a small cup filled with deionized water. Blood meals were offered every 2 days for populations in the 35 & 20°C incubators, every 3 days for populations in the 24, 27 & 31°C incubators,

and every 4 days for populations in the 16°C incubator. We offered blood meals at different intervals in the different temperature treatments due to limited personnel and logistical difficulties, but this reduced our power to detect differences among populations, and should be avoided in future studies, if possible.

Statistical Analysis

All statistical analyses were done in R v3.1.1 (R Development Core Team 2012). We used generalized linear models to quantify the effects of population and temperature on female development time, larval emergence, larval cannibalism, and larval survival, and included two-way interactions between population and temperature to allow for the effect of temperature to vary among populations. We included linear and quadratic terms for temperature because residuals from linear models showed obvious evidence of nonlinearity. We calculated the Q_{10} temperature coefficient for larval development rate as $(R2/R1)^{10/(T2/T1)}$ where R is the developmental rate and T is the temperature (Bennett 1985). We used the fraction of larvae emerging as adults to quantify larval survival rather than survival analyses, because larval death included both individuals that were found dead on a known day, and cannibalism, in which larvae disappeared and the date of larval death from cannibalism could not be determined. We used Cox proportional hazard models with Weibull distributions and right-censored data to analyze differences in female adult survival with temperature among populations (Cox *et al.* 2007). We illustrate population response to temperature using the fraction of adult mosquitoes alive 9 days after emergence, which coincides with average lifespan of *Cx. pipiens* in the field for

the lowest elevation population (Jones *et al.* 2012). We used generalized linear mixed models with a binomial distribution and a logit link to analyze factors influencing the probability of mosquitoes taking their second blood meal including age, source population, and temperature, as fixed effects, and emergence group, and individual as random effects. For each life history trait, we compared the full fitted model to models that were each missing one fixed effect, by AIC. Finally, we combined the best fitting models for each trait, and models from a previous study (Kilpatrick *et al.* 2008a) to simulate population dynamics. For each population we estimated the number of infectious biting adults (i.e. those taking their second bloodmeal and therefore infectious for WNV) at temperatures 20-35°C.

RESULTS

In total, we measured juvenile survival for 9,659 individuals (2,415 ± 976 (mean ± SD) per population), development time for 4,099 females (1,025 ± 79), adult mortality for 922 females (230 ± 56), and biting rate for 39 females (10 ± 5). The best fitting models by AIC for larval and adult survival, and development rate were the full models which included population, temperature, temperature^2, population*temperature, and population*temperature^2 (Tables S1-S4). For biting rate, the best fitting model did not include population or population-temperature interactions, but did include temperature, age when taking the first blood meal, and the number of days between bloodmeals (Table S5).

Adult female development time decreased monotonically and nonlinearly with increasing temperature, and the greatest decrease occurred at cold temperatures (Fig. 2). A 4°C increase in temperature from 16 to 20°C decreased female development time by 57% (7.8 days) across the four populations, whereas the same 4°C increase from 27 to 31°C only decreased development time by 10% (0.85 days) (Fig. 2). Patterns were similar for adult female development rate – the inverse of development time – which increased at a decelerating rate with increasing temperature (Fig. S3). The Q_{10} temperature coefficient decreased with temperature, from 2.1, between 16 and 27°C, to 1.5 between 20 and 31°C.

Variation among populations in developmental time and rate were greatest at extreme temperatures, and smaller than the effects of temperature. The low latitude/altitude population had the fastest development rate across all temperatures. Specifically, at 16°C the low elevation/latitude population developed 1.9 days (9%) faster than the high elevation population, and at 31°C the low elevation/latitude population developed 1.2 days (15%) faster than the high elevation population (Figs. 2 and S3).

Average daily larval survival over the developmental period decreased almost linearly with increasing temperature until 31°C above which it declined more sharply (Fig. 3A). At 35°C all larvae died before reaching the fourth instar. Overall, a 15°C increase in temperature from 16°C to 31°C tripled daily mortality from 1 to 3% per day (Fig. 3A). Variation in daily larval survival among populations was substantial but variable across different temperatures. The low elevation/latitude population had

the highest larval daily survival (mortality was 6-fold lower than the coolest high-elevation population) at low temperatures (16°C) but the 2nd lowest survival at 31°C (mortality was 67% higher than the mid-latitude population). Additionally, the high elevation population had the highest larval mortality (2.2 times greater than the mid-elevation population).

Larval stage survival (the product of average daily survival and larval development time) increased with increasing temperature at temperatures below 27°C because development rate increased faster than mortality rate. However, between 31°C and 35°C stage survival decreased sharply because the increase in daily mortality overwhelmed the smaller decrease in developmental rate (Fig. 3B). Across all four populations, a 4°C rise from 16°C to 20°C increased stage survival by 12%, whereas stage survival decreased by 16% from 27°C to 31°C (Fig. 3B). Approximately 67% of larval mortality was due to larval cannibalism, which showed essentially the same trends as total larval mortality (Fig. S2).

Differences among populations in larval stage mortality were substantial. For example, mortality of the mid-latitude and high elevation populations were twice as high as the low elevation population at 16°C, and high elevation populations had markedly lower stage survival at most temperatures (Fig. 3). As with patterns across temperatures, these differences among populations in larval stage survival were mostly explained by differences in cannibalism. High elevation larvae were three times more likely to be cannibalized than those from the mid-elevation population,

which had the lowest cannibalism and highest overall stage survival across most temperatures (Figs. 3, S2).

Adult female survival decreased nonlinearly and monotonically with increasing temperature, and, as with larval survival, the largest decrease occurred at higher temperatures (Figs. 4, S4). For all populations, a 4°C rise, from 16 to 20°C, resulted in a nearly negligible 0.6% decrease in female survival, whereas, a similar increase in temperature, from 27 to 31°C decreased survival 25% (Fig. 4). Variation among populations was again substantial, with the coldest (high-elevation) population having 2.2 fold lower mortality than the warmest low elevation population at 16°C (0.19% vs. 0.42% daily mortality resulting in average lifespans of 5.3 and 2.4 days, for the high elevation and low elevation populations, respectively). At 27°C the high elevation population had 88% higher mortality than the mid-elevation population, but at 35°C this difference was reversed with the mid-elevation population having a 40% higher mortality than both the high and low elevation populations which had almost identical survival.

The cumulative fraction of females taking a second blood meal increased linearly with temperature and the number of days between blood meals, but did not differ significantly among populations, possibly due to small sample sizes (Fig. 5A-D). The effect of age on biting rate was also substantial (Table S5). For example, at 27°C, the probability that females would take a second blood meal 7 days later increased from 0.15 to 0.88 as the age when they took their first blood meal increased from two to 14 days (Fig. 5E).

We integrated the best fitting models described above for each trait, and the extrinsic incubation period for West Nile virus (Kilpatrick *et al.* 2008a), to simulate the number of larvae, adults, and infectious biting adults taking their second blood meal over time for each population and temperature (Fig. 6). For all populations, 24°C produced the highest fraction of infectious biting adults (a peak of 11.3% of the starting larval population occurring 52 days after hatching; Fig. 6A). At warmer temperatures there were fewer infectious biting mosquitoes, but they were produced earlier (at 24°C and 31°C, infectious mosquitoes peaked at 11.3%, and 8.0% of the starting larval population occurred on days 52 and 28 post-hatching, respectively; Fig. 6A). Differences among populations were very large, and the rank order varied with temperature (Fig. 6B). The mid-elevation population had the highest number of infectious biting adults at 24°C, which was more than twice as many as the high elevation population at this temperature (Fig. 6B). In contrast, at 31°C the mid-latitude population had the highest number of infectious biting adults.

DISCUSSION

Many studies have quantified the effect of temperature on mosquito life-history traits for single populations of a species (Rueda *et al.* 1990; Maharaj 2003; Delatte *et al.* 2009; Ciota *et al.* 2014). As in other studies, we found that temperature had strong and relatively consistent effects among populations on development time, larval survival and adult survival, the three most well-measured life history traits, and the patterns were strongly nonlinear. Development time, larval survival, and adult

survival decreased with increasing temperature, and biting rate increased. Temperature effects were strongest at higher temperatures for survival and at lower temperatures for development and biting rate.

Although the direction of the relationships we observed between temperature and life history traits are mostly consistent with previously observed patterns, the shape of the temperature performance relationships sometimes differed between our results and previous studies. We found that adult survival decreased monotonically with temperature and female development rate increased monotonically with temperature, whereas some previous studies presented unimodal relationships with temperature (Mordecai *et al.* 2013). It is worth noting that decreases in life history traits at very high temperatures can be due to rapid death of larval or adult mosquitoes rather than a lack of development or gonotrophic cycling, as we also observed for larval development.

We found significant differences among populations in how life history traits varied with temperature. However, these differences were rarely consistent with a local thermal adaptation hypothesis, which was similar to results from a previous study of two populations of *Culex tarsalis* in California (Reisen 1995). Some populations of *Cx. pipiens* had uniformly higher or lower performance for some traits across all temperatures, such as the uniformly faster development rate of the warmest population (Fig. 2) or the uniformly lower larval survival of the coldest population (Fig. 3A). The lower larval survival of the highest elevation population may have been influenced by a low number of egg rafts collected from this site. However, the

high survival of adults from this population at high and low temperatures demonstrates that not all traits were lower than other populations. In addition, some populations had the worst performance at temperatures where local adaptation would have resulted in them performing the best and vice-versa (e.g. the warmest population in Fig 3A). Although studies with a larger number of populations or from across a larger spatial temperature gradient might find some evidence for local thermal adaptation, our results suggest that variation that is uncorrelated with local temperatures is substantial and must be incorporated into uncertainty estimates in efforts to predict spatial and temporal variation in disease under climate change scenarios. Our results, and specifically, the magnitude of the site and site-temperature coefficients, provide an estimate of the magnitude of this variation.

CONCLUSION

Our results show that the impact of climate change on mosquitoes will be more variable than previous predictions due to the substantial variation that exists in the response of populations to temperature. At the same time, these differences among populations are likely to contribute to spatial variation in transmission, and will be an important source of variation for selection to act on as climate warms (Rohr *et al.* 2011; Egizi *et al.* 2015). The combination of standing variation and mosquitoes' evolutionary response will determine the impact of changing climates on vector borne disease.

Competing Interests: The authors have declared that no competing interests exist.

Authors' contributions JR, LDK, and AMK designed the study. JR collected the data. JR and AMK analyzed the data and wrote the first draft. All authors edited, read, and approved the final version of the manuscript.

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FIGURE LEGENDS:

Fig. 1. Map of study sites. Site names describe the latitude and/or elevation for each population of *Cx. pipiens* relative to the low elevation/latitude population and the elevation height is in parentheses.

Fig. 2. Adult female development time for *Cx. pipiens* (egg hatch to female emergence). All larvae died at 35°C. Error bars show 95% confidence intervals for replicate flats (200 mosquitoes). For the high population there was only one replicate flat with 200 mosquitoes. Points are jittered along the x-axis to facilitate presentation.

Fig. 3. *Cx. pipiens* larval survival. All larvae died at 35°C. Error bars show standard error for replicate flats (200 mosquitoes). For the high population there was only one replicate flat with 200 mosquitoes. Points are jittered along the x-axis to facilitate presentation. **A.** Daily juvenile survival. **B.** Fraction of larvae that survived to emerge as adults.

Fig. 4. Estimated survival of adult female *Cx. pipiens* to 9 days post-emergence based on Cox proportional-hazard models (see Fig. S1 for raw survival plot). Error bars show binomial errors for individual female mosquitoes. Points are jittered along the x-axis to facilitate presentation.

Fig. 5. *Cx. pipiens* biting rate. **A-D.** Cumulative fraction of females taking two blood meals. Each line represents a group of females that took their first blood meal on the same day. Each group was then followed over time until they took a second blood meal. A y-value of 100% means that of the initial females who took their first blood meal on the same day all females within that group went on to take a second blood meal before the study was terminated. Error bars are binomial errors based on the number of individuals within a group. **E.** Predicted Generalized Linear Mixed-effects Model values for probability of taking a second blood meal, given that age at first blood meal was 5 days old.

Fig. 6. Simulated population dynamics and vectoral capacity for 100 larval *Cx. pipiens* mosquitoes. **A**. The effect of temperature on the number of larvae emerging as adults and later becoming infectious biting adults, using the average model coefficients across all populations (Tables S1-5), and the relationship between temperature and time and the fraction of mosquitoes transmitting West Nile virus (Kilpatrick *et al.* 2008a). Each line color shows a cohort of 100 mosquitoes over time (hatching on day 0) at a specific temperature and line style indicates the mosquito life stage. Comparison of similar line types indicates the effect of temperature. At 35°C all larval mosquitoes died before pupation. **B**. Variation among populations and

temperature on the number of infectious biting adults over time starting from 100 larvae on day 0, as in panel A. Line color indicates temperature and line style indicates population. Note the difference in y-axis scales in panels A and B.

FIGURES

Fig. 1.

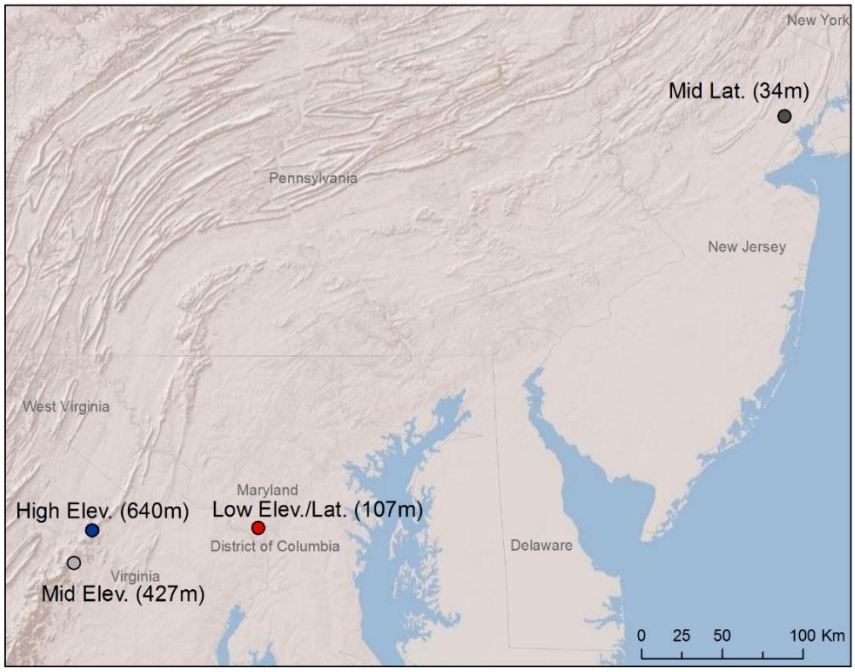


Fig. 2.

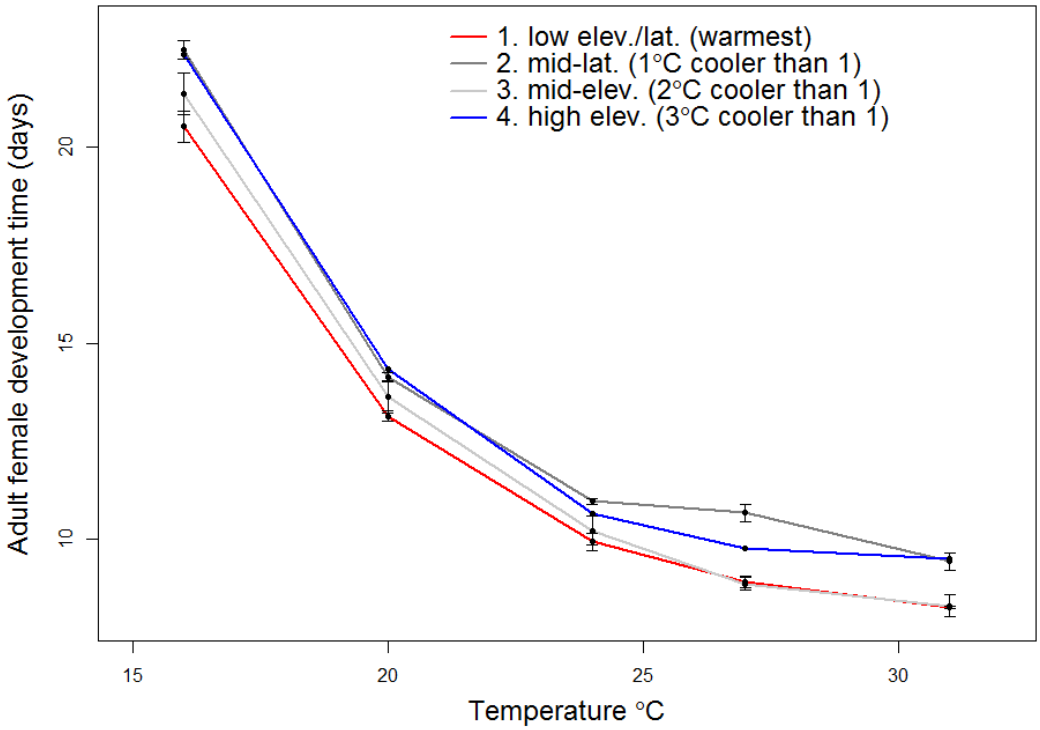

1. low elev./lat. (warmest)
2. mid-lat. (1°C cooler than 1)
3. mid-elev. (2°C cooler than 1)
4. high elev. (3°C cooler than 1)
Adult female development time (days)
10
15
20
15
20
25
30
Temperature °C

Fig. 3.

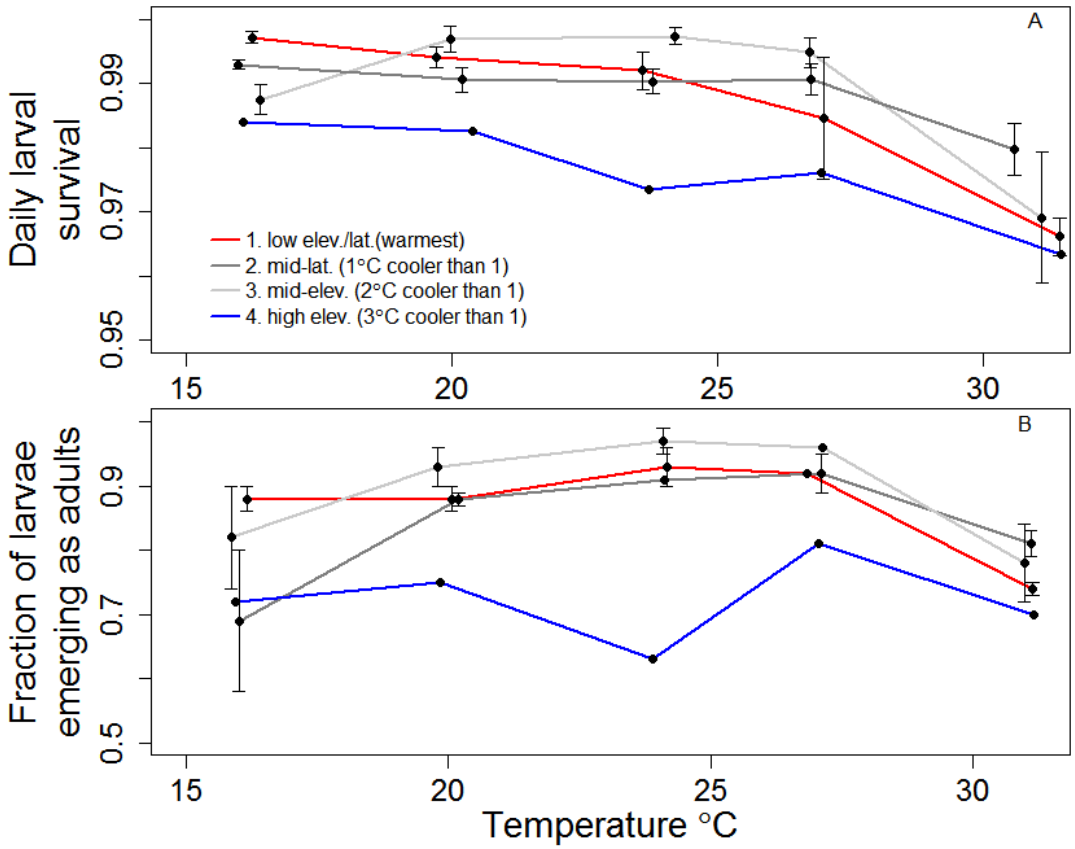

A
B
Daily larval survival
Fraction of larvae emerging as adults
0.95
0.97
0.99
0.5
0.7
0.9
15
20
25
30
Temperature °C
1. low elev./lat.(warmest)
2. mid-lat. (1°C cooler than 1)
3. mid-elev. (2°C cooler than 1)
4. high elev. (3°C cooler than 1)

Fig. 4.

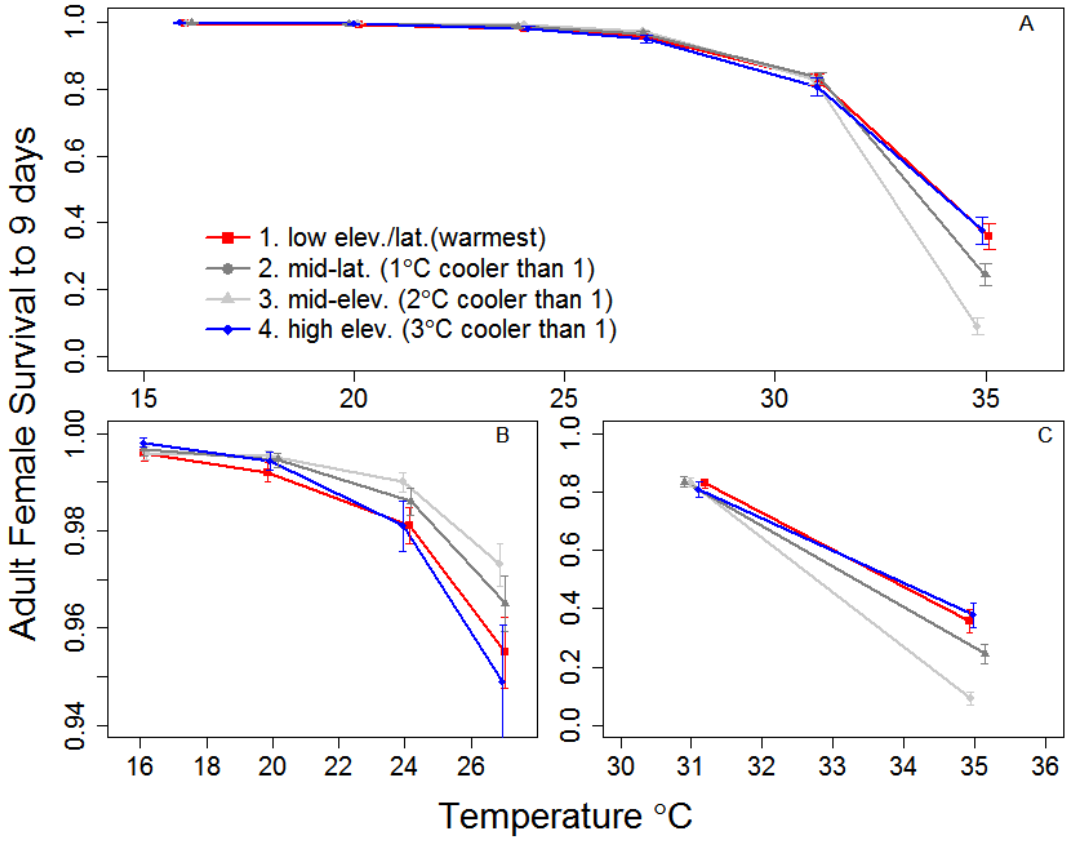

A
B
C
1. low elev./lat.(warmest)
2. mid-lat. (1°C cooler than 1)
3. mid-elev. (2°C cooler than 1)
4. high elev. (3°C cooler than 1)
Adult Female Survival to 9 days
Temperature °C

Fig. 5.

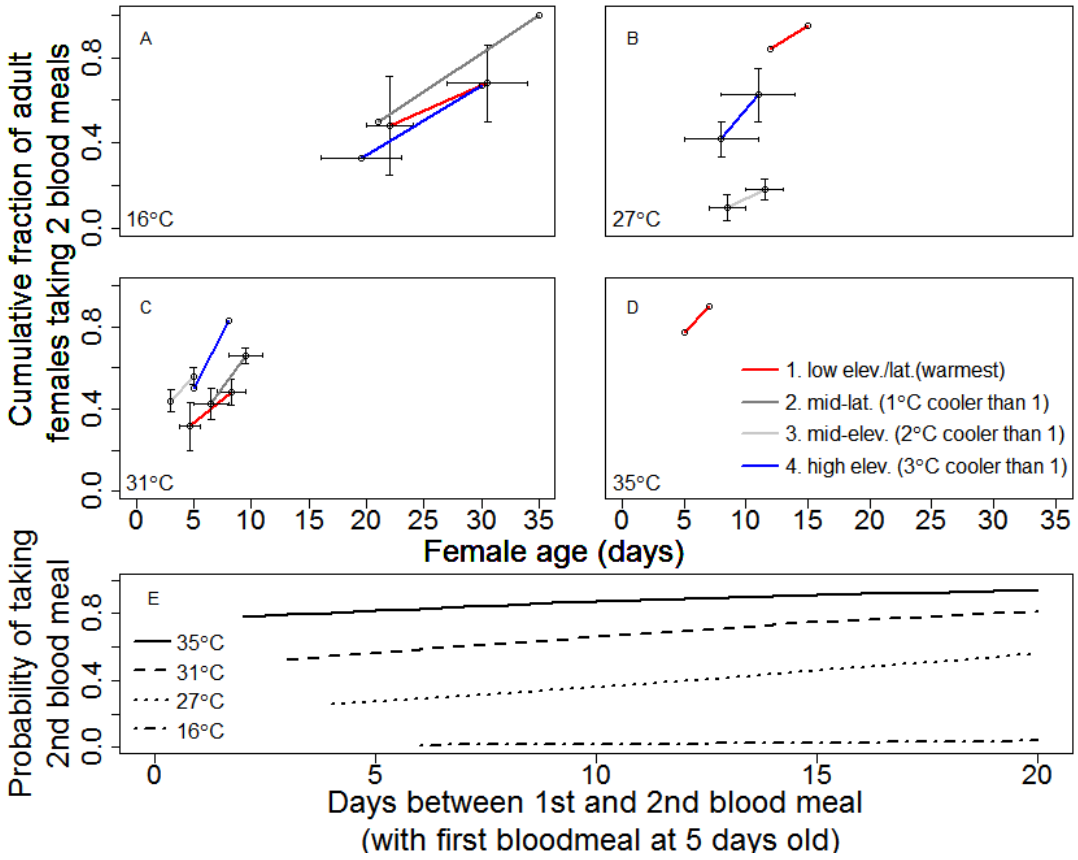
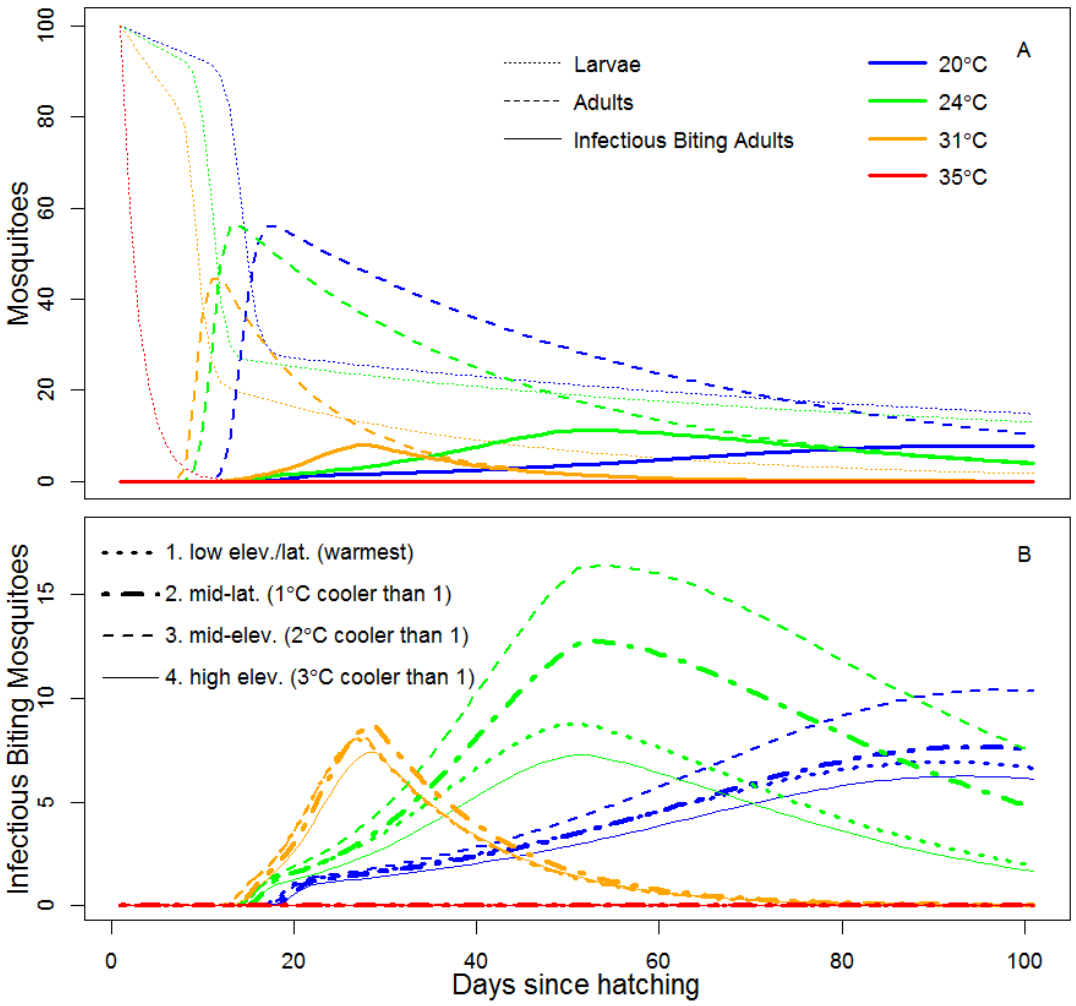

A
16°C
B
27°C
C
31°C
D
35°C
Cumulative fraction of adult females taking 2 blood meals
0.0
0.4
0.8
0
5
10
15
20
25
30
35
Female age (days)
1. low elev./lat.(warmest)
2. mid-lat. (1°C cooler than 1)
3. mid-elev. (2°C cooler than 1)
4. high elev. (3°C cooler than 1)
E
35°C
31°C
27°C
16°C
Probability of taking 2nd blood meal
Days between 1st and 2nd blood meal
(with first bloodmeal at 5 days old)

Fig. 6.


Mosquitoes
Larvae
Adults
Infectious Biting Adults
20°C
24°C
31°C
35°C
A
Infectious Biting Mosquitoes
1. low elev./lat. (warmest)
2. mid-lat. (1°C cooler than 1)
3. mid-elev. (2°C cooler than 1)
4. high elev. (3°C cooler than 1)
B
Days since hatching

CHAPTER TWO

Variation in vectorial capacity for Zika, dengue, chikungunya, and yellow fever virus for North American *Aedes aegypti* and *Aedes albopictus* mosquitoes

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Background: Quantifying variation in vectorial capacity (the vector component of the pathogen reproductive ratio, R_0) for multiple pathogens and multiple species of mosquito provides insight into spatial and temporal variation in potential pathogen transmission cycles and targets for mosquito control. Both *Aedes aegypti* and *Aedes albopictus* (Linneaus) mosquitoes are invasive and expanding their geographic distribution in the United States. These two species are also the dominant vectors for four of the most important mosquito-borne viruses of humans, Zika, dengue, chikungunya, and yellow fever virus. Determining their vectorial capacity for these viruses can be used to assess the likelihood of an outbreak of one of these viruses, guide control efforts, and is thus critical for developing efficient vector control strategies.

Methods: We collected *Ae. aegypti* and *Ae. albopictus* from New Orleans and measured four life history traits, larval and adult survival, development rate, and biting rate, across six fluctuating temperature regimes (mean= 8.2°C; SD= 1.3), with only mean daily temperatures varying between 13.5-35.5°C, which spanned the

conditions experienced by mosquitoes in much of their distribution. We then used these life history data and published data on vector competence to estimate the vectorial capacity of these two species at 27°C for Zika, dengue, chikungunya, and yellow fever virus.

Results: Differences in life history traits between *Ae. aegypti* and *Ae. albopictus* were mixed with *Ae. albopictus* having faster development, higher larval survival, and feeding earlier, but *Ae. aegypti* had higher adult survival. Integrating these data into models of vectorial capacity suggested that *Ae. aegypti* was a slightly more efficient vector of all four viruses in areas where abundance of the two species was similar. Vectorial capacity of these two species was highest for yellow fever virus followed by Zika, Chikungunya and dengue viruses.

Conclusion: These results suggest that the transmission potential for Zika virus in the southeastern USA may be higher than it is for dengue or Chikungunya viruses which have so far had only limited local transmission. Disease control efforts may need to be increased to prevent an outbreak of Zika virus, and efforts should be made to prevent the introduction of yellow fever virus that is currently circulating in Africa.

Keywords: Species comparison, thermal response, vector-borne disease, New Orleans, Louisiana, Zika, Chikungunya, dengue, yellow fever virus

INTRODUCTION

In the past five years two previously obscure viruses, Zika and Chikungunya, have emerged to cause serious public health impacts (Chouin-carneiro *et al.* 2016; Richard *et al.* 2016). At the same time, dengue virus transmission has increased, and yellow fever has recently seen a resurgence (Kilpatrick & Randolph 2012; Green 2016). *Ae. aegypti* and *Ae. albopictus* are the most important mosquito vectors for these four viruses (Johnson *et al.* 2002; Alto *et al.* 2014; Vega-Rúa *et al.* 2014). This is due to the fact that these species thrive in human environments (Jansen & Beebe 2010). Both species are present on all continents except Antarctica, with *Ae. aegypti* having a more tropical distribution ranging up to 40° latitude, while *Ae. albopictus* even further poleward ranging up to 50° latitude in some regions (Kraemer *et al.* 2015). Generally, *Ae. aegypti* prefers to feed on humans (Sivan *et al.* 2015) whereas *Ae. albopictus* is more variable and sometimes feeds on birds and non-human mammals (Niebylski *et al.* 1994; Ponlawat & Harrington 2005; Richards *et al.* 2006; Faraji *et al.* 2014). When *Ae. aegypti* and *Ae. albopictus* overlap in space, larvae compete for resources and *Ae. albopictus* usually wins, causing *Ae. aegypti* abundance to decrease at some sites (Juliano *et al.* 2004). Previous work has compared the vectorial capacities (the abiotic and biotic factors that influence a vectors ability to transmit pathogents) for *Ae. aegypti* and *Ae. albopictus* for dengue and Chikungunya viruses (Manore *et al.* 2014) but no comparisons have been made for Zika or yellow fever virus and none using life history data from North American mosquitoes.

We collected *Ae. aegypti* from the field and measured life history traits (larval and adult survival, development rate, and biting rate) across six fluctuating temperature regimes, with mean daily temperatures of 13.5-35.5°C. We then used our life history data and published data on vector competence to estimate the vectorial capacity of these two species for Zika, dengue, Chikungunya, and yellow fever virus. We hypothesized that *Ae. aegypti* would have a higher vectorial competence than *Ae. albopictus* at warmer temperatures due to its more tropical distribution.

METHODS

Study populations

Each night, for two weeks during September 2013, we set three oviposition traps at each of 40 sites in New Orleans, LA that spanned 32 square-miles. Oviposition traps consisted of 1 quart plastic containers filled with water and lined with garden seed paper. In total, we collected 1,287 *Ae. aegypti* eggs and 1,058 *Ae. albopictus* eggs.

Rearing and Handling

All field collected eggs were hatched at 25°C under a photoperiod of 14:10 hrs (L:D), and pupae were morphologically identified as *Ae. aegypti* and *Ae. albopictus.* For each species, all the pupae were pooled together in a 16oz (Solo®) cup filled to approximately ¼ volume with DI water. Adults emerged directly from the pupal cup into a 30.5cm^3 white plastic cage with mesh lining (BioQuip). Adults had constant access to cotton soaked in a 0.3M sugar solution. Every other day adults were offered an anaesthetized mouse (IACUC #Kilpm1207) and had constant access

to an oviposition cup (the bottom half of a 16oz Solo® cup) filled with water and lined with absorbent lab paper. Every few days the egg paper was removed from the oviposition cup, air dried, and then stored in a Ziploc® bag. This first laboratory-raised generation of offspring was used for our experiment.

Four life history traits—larval and adult survival, development rate, and biting rate—were measured inside Percival Biological Incubators (GSI-36VL). We used six temperatures, with the following average temperature profiles: 13.5°C (min 9.8; max 17.3), 18°C (14.2; 21.8), 21.6°C (16.4; 26.3), 26.9°C (22; 31.4), 30.2°C (27.1; 33.6), and 35.5°C (31.2; 39.6). These performance assays had diurnal variation (mean 8.2°C; SD ± 1.3) around the maximum temperature but there was no seasonal or between-day variance in temperature (Fig. S1). We raised juveniles in plastic Ziploc® trays (17.8cm x 11.4cm x 5.1cm) filled with 0.3L of DI water. Each of the six temperature treatments was initiated with two trays of 100 larvae per species. Every day larvae were fed finely ground TetraFin® Goldfish Flakes ad libitum, and dead larvae and pupae were counted and removed. Surviving pupae were transferred to a single emergence cup (as described above). The number of emerged males and females was counted daily and adults were immediately transferred to plastic 1-quart containers with mesh tops. We inspected each cage daily, and counted and removed dead adults. Once approximately 10 females had emerged, we offered an anesthetized mouse (blood meal) every day for the duration of the experiment. Females had constant access to a 2oz (Solo®) oviposition cup filled with DI water and lined with lab absorbent paper.

Quantifying vectorial capacity

We used published literature values to determine vector competence (fraction of mosquitoes that feed on an infected host and transmit the pathogen later) and extrinsic incubation period (the time between a mosquito feeding on an infectious blood meal and transmitting the pathogen) for *Ae. aegypti* and *Ae. albopictus* at 27°C for Zika, dengue, yellow fever, and chikungunya viruses (Table S7). We chose 27°C because there was a lack of studies measuring vector competence and extrinsic incubation period for these pathogens over a range of temperatures. For dengue and Zika, only data on the fraction of mosquitoes with disseminated infections (virus present in legs) were given, we estimated the fraction of mosquitoes with disseminated infections that would actually transmit the pathogen based on a study that presented data on both of these measures of vector competence (conversion factor =0.2) (Chouin-carneiro *et al.* 2016). We estimated the extrinsic incubation period for each species and pathogen by fitting generalized linear models to each species and virus, setting a threshold vector competence value and reporting the predicted incubation period. We then used the vector competence, extrinsic incubation period, and our measured life history traits, to estimate relative vectorial capacity using the following equation (Dye 1992):

$$\text{Vectorial Capacity} = (M\beta^2 C_V e^{-\mu EIP})/\mu$$

Where M is relative abundance, β is biting rate, C_V is vector competence, μ is mosquito mortality, and EIP is extrinsic incubation period.

Statistical analysis

All statistical analyses were done in R v3.2.2 (R Development Core Team 2012). We used a generalized linear model to quantify the effects of species and temperature on juvenile daily survival, and included two-way interactions between population and temperature to allow for the effect of temperature to vary between species. We included linear and quadratic terms for temperature because residuals from linear models showed obvious evidence of nonlinearity. We used Cox proportional hazard models with Weibull distributions and right-censored data to analyze how temperature and species influenced female adult survival (Cox *et al.* 2007). We display patterns of adult survival across temperatures using the fraction of adult mosquitoes alive 9 days after emergence, which coincides with average lifespan of *Ae. aegypti* in the field (Hugo *et al.* 2014). We used generalized linear mixed models to quantify the effects of population and temperature on adult female development time, fraction of larvae emerging as adults, and the probability of a female taking a second blood meal. We used the fraction of larvae emerging as adults to quantify larval survival rather than survival analyses, because larval death included both individuals that were found dead on a known day, and cannibalism, in which larvae disappeared and the date of larval death from cannibalism could not be determined. For female development time and the fraction of larvae emerging as adults, population and temperature were included as fixed effects while replicates were included as random effects. For the probability of a female taking a second blood meal (biting rate) days between blood meals (time) and temperature were

included as fixed effects and individual mosquitoes were random effects. For each life history trait, we compared the full fitted model to models that were each missing one fixed effect predictor, by likelihood ratio tests.

RESULTS

In total, we measured adult mortality for 603 females (302 ± 33), and biting rate for 91 females (45.5 ± 23). The best fitting model by AIC for adult survival was the full model which included species, temperature, temperature^2, population*temperature, and population*temperature^2 (Tables S4). For biting rate, the best fitting models included species, temperature and the number of days between blood meals (Table S5 & S6).

Adult female survival varied significantly between species and among temperatures (Fig. 1; Table S5). Overall, adult female survival declined across most of the temperature range. Across all temperatures, adult mortality was 63% higher for *Ae. albopictus* compared to *Ae. aegypti*. Surprisingly, *Ae. albopictus* had a non-monotonic response to temperature with a small increase at cold temperatures, while *Ae. aegypti* decreased nonlinearly with increasing temperature (Fig. 1). For *Ae. albopictus*, a 4.5°C increase in temperature from 13.5°C to 18°C decreased adult mortality by 63%, while there was no significant change in mortality for *Ae. aegypti* (Fig. 3). Additionally, a 5°C increase from 30.2 to 35.5°C increased adult mortality by ~3-fold for both species.

The cumulative fraction of females taking a second blood meal increased significantly with the number of days between blood meals (Fig. 2; Table S5). Increasing the time between blood meals from 4 to 7 days increased the probability of a female taking a second blood meal by 54% (Fig. 2). *Ae. aegypti* had a higher vectorial capacity than *Ae. albopictus* for all four viruses, and the pathogen with highest vectorial capacity was yellow fever, followed by, Zika, chikungunya, and dengue (respectively; Fig. 3).

DISCUSSION

Vectorial capacity for both species was highest for yellow fever virus, followed by Zika virus, which were both higher than dengue virus which has caused occasional outbreaks in the southern USA and Hawaii (http://www.cdc.gov/dengue/epidemiology/#dengue-in-us). Our results suggest that outbreaks of Zika will be more difficult to control than dengue virus, and extensive travel to areas with Zika transmission (e.g. Brazil, the site of the 2016 Olympics) makes introduction of the virus into many locations in the US a certainty. In the US, in 2015 and 2016, there have been 1,132 imported cases of Zika (http://www.cdc.gov/zika/geo/united-states.html), suggesting that if the transmission potential or basic reproductive ratio (R_0) of this virus is above the threshold of one, there may be local transmission of this virus in the US. The higher vectorial capacity for yellow fever virus suggests that this virus presents an even larger threat if it were introduced. This highlights the importance of reducing the probability of introduction

from an infectious person traveling from regions of Africa where outbreaks are currently occurring (Green 2016).

As other studies have suggested, vectorial capacity was higher for *Ae. aegypti* than for *Ae. albopictus* for all four viruses. This was due to the substantially higher adult survival for this species, which is well known to be highly influential in vectorial capacity (Dye 1992); differences in other vectorial capacity parameters were smaller and did not consistently favor one species (Table S7). These results suggest that where *Ae. aegypti* and *Ae. albopictus* are at similar abundance, as they were at our sites in New Orleans, vector control should focus on *Ae. aegypti*. However, there are many regions where *Ae. aegypti* is rare and *Ae. albopictus* is highly abundant (e.g. the mid-Atlantic) (Green 2016). At these sites control of *Ae. albopictus* will be necessary to keep transmission potential low, and control will be especially important as mosquito abundances increase during warmer temperatures.

CONCLUSION

Between-species differences in temperature-dependent life-history traits and resultant vectorial capacity for Zika, dengue, chikungunya, and yellow fever viruses will strongly influence spatial and temporal variation in pathogen transmission. Although *Ae. aegypti* is a more efficient vector in New Orleans, this species is thus far limited to tropical and subtropical areas. In contrast, *Ae. albopictus* has life history traits that make it a moderately efficient vector for several human pathogens, and its more northern distribution creates the potential for pathogen transmission to

occur at higher latitudes where *Ae. aegypti* is less abundant. The continued emergence of novel and or previously discounted viruses emphasize the importance of understanding the ecology of these key vectors of human disease.

Competing Interests: The authors have declared that no competing interests exist.

Authors' contributions JR and AMK designed the study. JR collected the data. JR and AMK analyzed the data, wrote the first draft, edited, and approved the final version of the manuscript.

Acknowledgements: We thank S. Munch, S. Forde, B. Lyon, and the Kilpatrick lab for comments on the manuscript. Funding was provided by, NIH grant 1R01AI090159 and NSF grant EF-0914866

TABLES

Table 1. Parameters used to estimate relative vectorial capacity. M is relative mosquito abundance in New Orleans based on the numbers of eggs laid in oviposition traps. μ is daily adult mortality, and βis the biting rate ($days^{-1}$). The species are abbreviated aeg (*Ae. aegypti*) and alb (*Ae. albopictus*).

virus	spp	pop	virus_titer	Cv	EIP	Source	M	μ	β
ZIKV	aeg	USA	10^7	0.088	5.5	3	0.55	0.017	0.17
ZIKV	alb	USA	10^7	0.063	5.5	3	0.45	0.024	0.22
DENV	aeg	Florida	10^7	0.0075	10.9	1	0.55	0.017	0.17
DENV	alb	Florida	10^7	0.0075	14.2	1	0.45	0.024	0.22
YFV	aeg	brazil	10^7	0.145	16.4	11	0.55	0.017	0.17
YFV	alb	brazil	10^7	0.145	16.4	11	0.45	0.024	0.22
CHIKV	aeg	polynesia	10^7	0.02	12.8	19	0.55	0.017	0.17
CHIKV	alb	Africa	5x10^6	0.02	11.4	19	0.45	0.024	0.22

FIGURE LEGENDS

Figure 1. Estimated survival for adult female *Ae. aegypti* and *Ae. albopictus* to 9 days post-emergence based on Cox proportional-hazard models (see Fig. S1 for raw survival plot).

Figure 2. Biting rates for *Ae. aegypti* and *Ae. albopictus*. Cumulative fraction of females taking two blood meals. Each line represents a group of females that took one blood meal and then was followed over time until they took their second blood meal. A y-value of 100% means that of the initial females who took their first blood meal, all females within that group went on to take a second blood meal before the study was terminated. Error bars are binomial errors based on the number of individuals within a group.

Figure 3. *Ae. aegypti* and *Ae. albopictus* relative vectorial capacity (a component of R0) for Zika, dengue, yellow fever, and chikungunya viruses at 27°C. Vector competence and extrinsic incubation period were estimated from published literature values.

FIGURES

Fig. 1

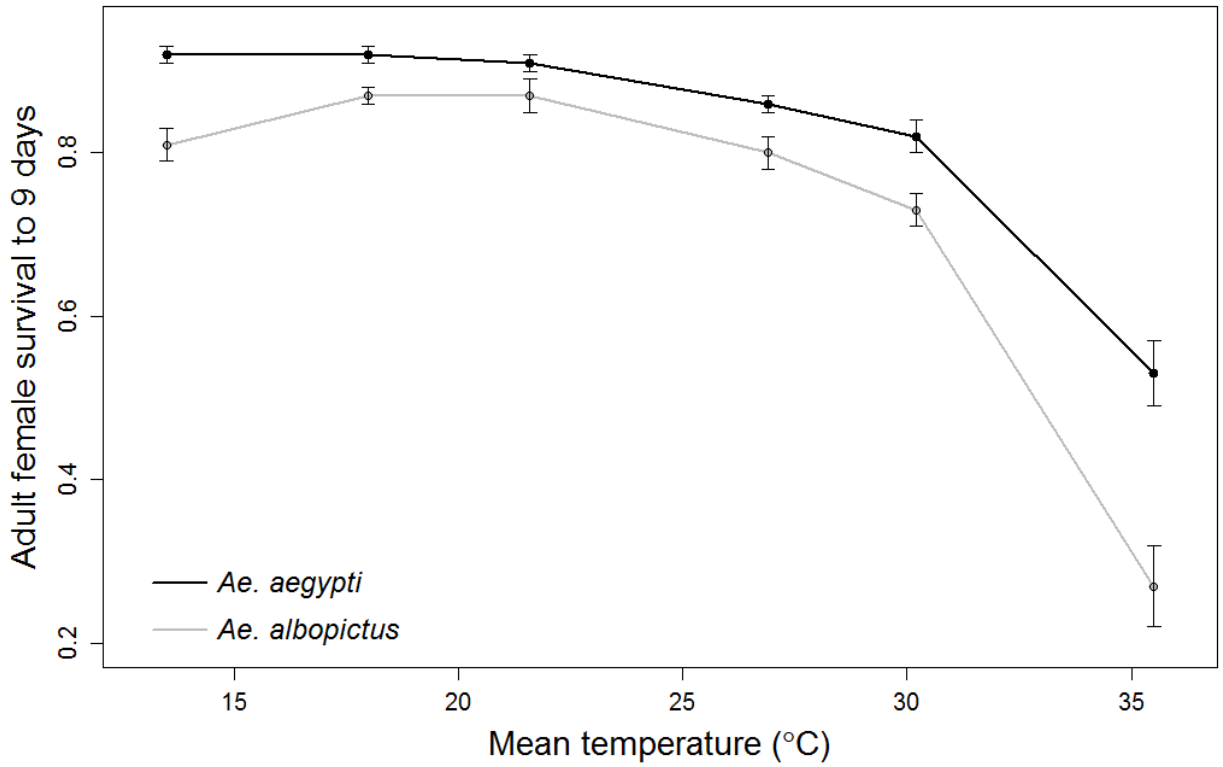


Fig. 2

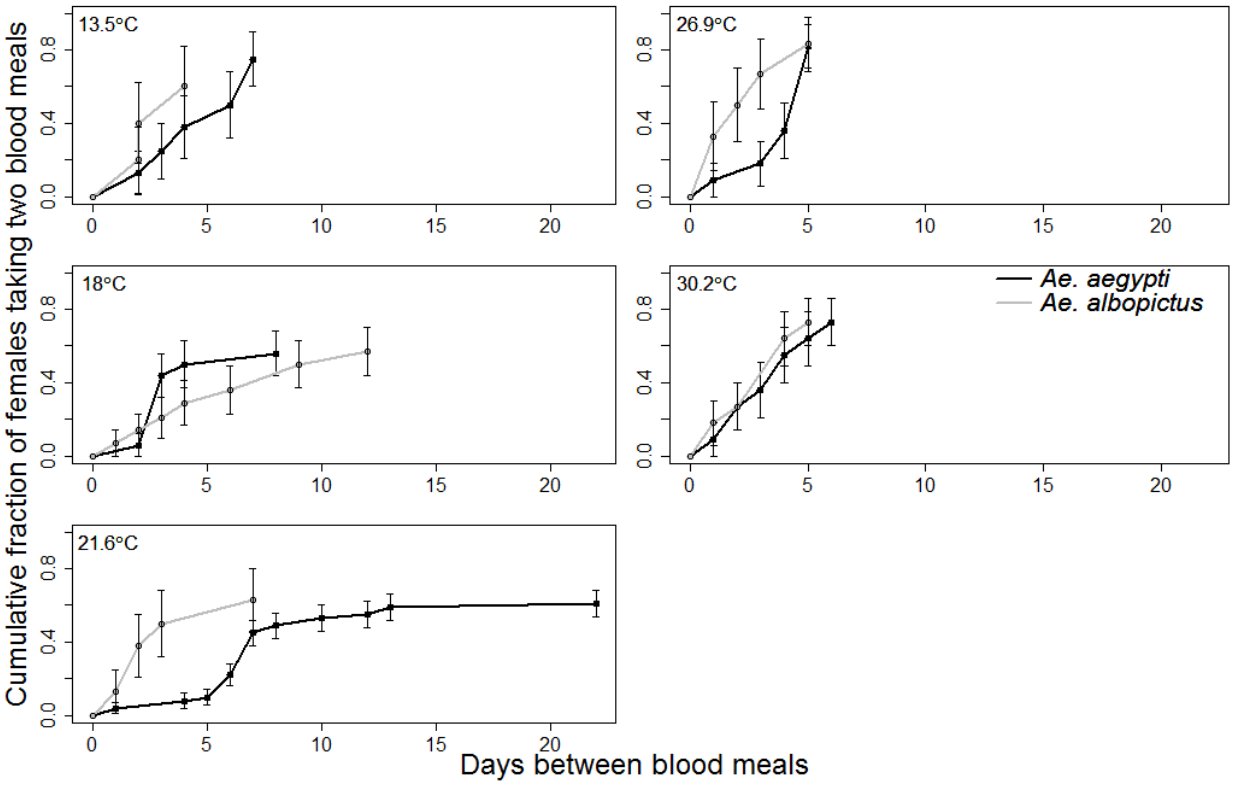
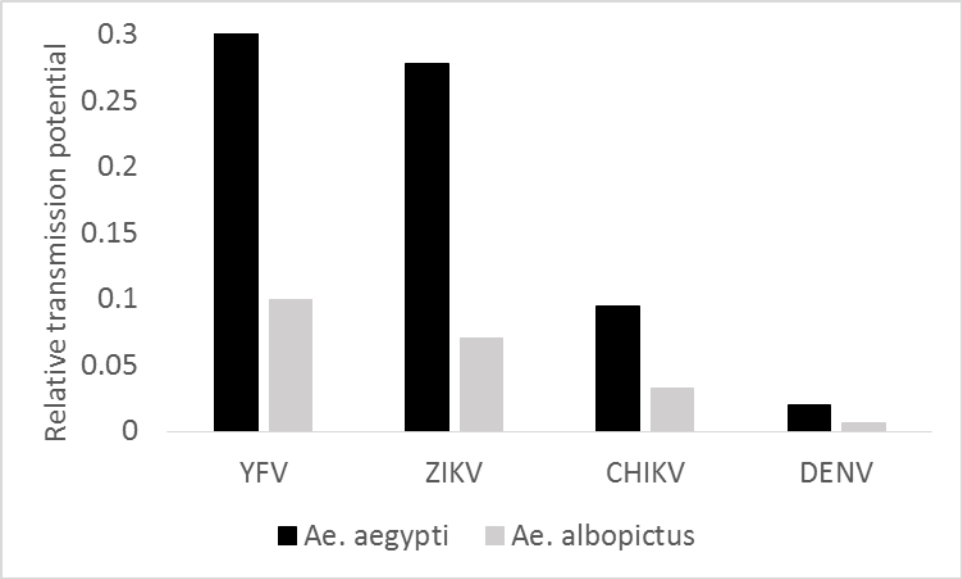

13.5°C
26.9°C
18°C
30.2°C
21.6°C
Ae. aegypti
Ae. albopictus
Cumulative fraction of females taking two blood meals
Days between blood meals
0.0
0.4
0.8
0
5
10
15
20

Fig. 3


Relative transmission potential
0.3
0.25
0.2
0.15
0.1
0.05
0
YFV
ZIKV
CHIKV
DENV
Ae. aegypti
Ae. albopictus

CHAPTER THREE

Adaptation of *Aedes aegypti* to simulated climate change reveals sensitivity to temperature variability

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ABSTRACT

Background: Most studies that predict the impact of climate change on vector-borne diseases use temperature-trait relationships measured in the lab, or previous patterns of incidence. However, evolutionary adaptation could be an important way for mosquito populations to respond to climate change. Here we tested the assumption that mosquito temperature-traits will not evolve fast enough to rising temperatures. In doing so, we are testing a key assumption of the current paradigm for projecting the impacts of global warming on vector borne disease.

Methods: We collected *Aedes aegypti* (Linnaeus) mosquitoes from New Orleans, Louisiana and reared them for one year under six climate change scenarios. We had three time-compressed increasing mean temperature trajectories (+2, +4, and +5°C), two control trajectories (one time-compressed and one not time-compressed) with no increase in mean temperature (+0°C), and one time compressed decreasing mean temperature trajectory (-2°C). All climate treatments incorporated stochastic daily (mean= 8°C, max= 17°C, min= 1°C), seasonal (mean= 17°C, max= 24°C, min= 10°C) and yearly fluctuations in temperature (mean= 23°C, max= 28°C, min= 20°C), as well as seasonal variation in photo-period. At the start and end of the one year climate treatments we measured four life history traits, larval and adult survival, development rate, and biting rate under six diurnally fluctuating (mean= 8.2°C; SD= 1.3) temperature regimes, with mean daily temperatures between 13.5-35.5°C, to determine whether there was variation in these life history traits due to climate treatment.

Results: There was significant variation between the starting population and the six climate treatments in the thermal response curves for the four life history traits. For all populations (starting population and 6 climate treatments), larval development rate and adult survival increased with temperature, while larval survival and biting rate decreased. However, the magnitude of these life history responses was uncorrelated with the climate regime, suggesting that the mosquitoes were adapting more to the increased variance in temperatures due to time compression than the differences in mean temperature.

Conclusion: These results suggest that evolution of mosquitoes in response to climate change may be smaller than would be expected from simple step-change or gradual linear increases in temperature over time. Due to the time compression of the experiment, mosquitoes were forced to survive and reproduce under both hot and cool conditions in each generation, as they do in nature, but they were also subjected to higher variation in seasonal temperature than would be the case in nature. Our results suggest that this increased temperature variation was a stronger selective pressure than the 7°C variation in mean temperatures by the end of the experiment.

Keywords: Thermal adaptation, Zika, Chikungunya, dengue, yellow fever virus, global warming, vector-borne disease, life history traits

INTRODUCTION

Increased temperatures, from anthropogenically-driven climate change, are predicted to alter the transmission of many vector borne diseases (Rogers & Randolph 2000; Kilpatrick & Randolph 2012; Siraj *et al.* 2014). Most studies on this topic assume that traits of the pathogen, hosts and vectors are static and project the impacts of warming based on temperature-trait relationships measured in the lab, or previous patterns of incidence in the field (Rogers & Randolph 2006; Ogden *et al.* 2008; Paaijmans *et al.* 2012). However, predictions of the impact of climate change differ depending on the methods used and assumptions made about the relationships between temperature and various aspects of transmission. Abundant laboratory evidence indicates that increased temperature increases four aspects of pathogen transmission or basic reproductive ratio (R_0) and decreases only one, mosquito mortality (Maharaj 2003; Kilpatrick *et al.* 2008b; Delatte *et al.* 2009). Because mortality enters the R_0 equation two times, it is often predicted that in tropical regions, rising global temperatures will result in increased mosquito mortality, thus limiting pathogen transmission and ultimately resulting in a geographical shift in vector-borne diseases (Rogers & Randolph 2006). This prediction assumes mosquitoes can only respond to warming temperatures via a range shift (movement in *both* the upper and lower range limits of a species). However, there are at least two other potential responses to climate change that would result in significantly different predictions for the geographic distribution of mosquitoes and mosquito-borne disease (Parmesan & Yohe 2003). One possibility is for organisms to maintain their current

geographic range and adjust to the changing environment via phenotypic plasticity or evolution (Chen et al. 2011; Kingsolver & Huey 1998; Charmantier et al. 2008; Franks 2010). The second is that organisms might evolve increased physiological tolerance which could result in maintaining the current geographic ranges or lead to a range expansion (pole-ward movement in *only* the upper range limits of a species) (Alto & Juliano 2001; Grant & Grant 2002; Levitan 2003; Ford & Smolowitz 2006; Franks *et al.* 2007; Tingley *et al.* 2012). Currently, there is a lack of empirical studies quantifying the evolutionary responses of mosquitoes to current and predicted rates of climate change (Egizi *et al.* 2015). Growing evidence suggests that rapid evolutionary responses are frequent (Huey *et al.* 1991; Schoener 2011) and evolution, especially in the form of increased heat tolerance in mosquitoes, would qualitatively change the predicted impacts of warming temperatures in hot areas from a decrease in transmission to no change or possibly even an increase.

Ae. aegypti is the most important mosquito vector for many important human viruses, including: Zika, dengue, chikungunya, and yellow fever (Johnson *et al.* 2002; Alto *et al.* 2014; Vega-Rúa *et al.* 2014; Chouin-carneiro *et al.* 2016; Green 2016). This is partly due to the fact that this species thrive in human environments and take a high fraction of their blood meals from humans (Jansen & Beebe 2010). *Ae. aegypti* is present on all continents except Antarctica, and has a tropical distribution ranging up to 40° latitude (Kraemer *et al.* 2015). The evolutionary response of this species to climate change will partly determine the distribution and transmission intensity of these pathogens.

We collected *Ae. aegypti* from the field and measured life history traits (larval and adult survival, development rate, and biting rate) before and after rearing them for one year in different temperature regimes representing several different rates and directions of temperature change. We hypothesized that mosquitoes in the hottest treatments would evolve increased heat tolerance compared with populations reared in the control and cooling treatments, whereas these latter populations would have higher performance at cooler temperatures.

METHODS

Study population

Each night, for two weeks during September 2013, we set three oviposition traps at 40 sites in New Orleans, LA that spanned 32 square-miles. Oviposition traps consisted of 1 quart plastic containers filled with water and lined with garden seed paper. In total, we collected 1,287 *Aedes aegypti* eggs.

Rearing and Handling

All field collected eggs were hatched at 25°C under a photoperiod of 14:10 hrs (L:D), and pupae were morphologically identified as *Ae. aegypti*. Pupae were pooled together in a 16oz (Solo®) cup filled to approximately ¼ volume with DI water. Adults emerged directly from the pupal cup into a 30.5cm^3 white plastic cage with mesh lining (BioQuip). Adults had constant access to cotton soaked in a 0.3M sugar solution. Every other day adults were offered an anaesthetized mouse (IACUC #Kilpm1207) and had constant access to an oviposition cup (the bottom half of a 16oz Solo® cup) filled with water and lined with absorbent lab paper. Every few days the

egg paper was removed from the oviposition cup, air dried, and then stored in a Ziploc® bag until a total of ~6,000 G_1 eggs were collected. This first laboratory-raised generation of offspring was used for our experiment.

Climate Change Scenarios

We developed climate trajectories from a combination of historic (1986-2005) temperature observations from New Orleans, LA and an ensemble of CMIP5 climate model simulations of the 20th (1986-2005) and 21st centuries (RCP8.5; 2006-2065) (Diffenbaugh & Scherer 2011; Diffenbaugh & Giorgi 2012). We simulated six decades of climate change and condensed these trajectories into six annual cycles in 12 months. As a result, each 60 day period included one "year" of simulated climate change representing each of the next six decades (Fig. S1). We refer to these as "time-compressed" temperature trajectories. This approach produced temperature profiles that had higher seasonal variation than mosquitoes would experience in nature—because they experienced a full "season" in 15 days rather than 3 months.

We developed three time-compressed increasing mean temperature trajectories and two control trajectories (one time-compressed and one not time-compressed) with no increase in mean temperature (+0°C), and one time compressed decreasing mean temperature trajectory (Fig. S1). For all six climate treatments temperature fluctuated diurnally, seasonally, and yearly. Photoperiod also varied with season, but relative humidity was held constant at 70±10%. The only variables that changed among treatments were the time frame in which a season or year occurred and mean temperature increases (or decreases; Fig. S1). The increasing

trajectories simulated environments where temperature increased 2, 4, or 5°C over a 60 year period. The control trajectory with no time compression was a normal year of temperature in New Orleans. The time compressed decreasing trajectory had a -2°C change in mean annual temperature over the 12 months to match the +2°C trajectory, but in the opposite direction.

For all climate change treatments, minimum temperature was held at 15°C, this was due to substantial adult mortality at temperatures below 15°C. Early on in the experiment we saw a substantial (82-90%) declines in mosquito abundance when temperatures dropped below 15°C. It's likely that adult mosquitoes in New Orleans, don't experience extremely low temperatures because they thermoregulate by moving to warmer sites (attics, garages, etc.).

Climate change treatment (population) maintenance

Juveniles and adults were raised inside Percival Biological Incubators (GSI-36VL) for one year. We raised juveniles in plastic Ziploc® trays (17.8cm x 11.4cm x 5.1cm) filled with 0.3 L of DI water. Each of the six climate change populations was initiated with three trays of 150 larvae. Larvae were fed finely ground TetraFin® Goldfish Flakes ad libitum each day. Pupae and adult mosquitoes were maintained as described above. Adult density was kept near 94 ± 50 (mean ± SD) individuals per cage. Approximately one generation occurred in each 60 day simulated annual cycle, in which we added eggs when temperatures reached ~20°C. These eggs hatched and matured through the larval and pupal stage, emerged as adults and then fed and laid eggs until temperatures were so cold that they ceased feeding and egg-laying, adults

that survived the cold temperatures could go on to lay eggs in the next 60-day period. As a result the one year experiment encompassed 6-7 generations between measurements.

Experimental measurements

Four life history traits—larval and adult survival, development rate, and biting rate—were measured inside Percival Biological Incubators (GSI-36VL) on groups of G_1 and G_6-G_7 individuals. We measured the starting population traits at six temperatures with diurnal but no seasonal or stochastic variation in temperature: 13.5°C (min 9.8; max 17.3), 18°C (14.2; 21.8), 21.6°C (16.4; 26.3), 26.9°C (22; 31.4), 30.2°C (27.1; 33.6), and 35.5°C (31.2; 39.6). At the end of the experiment (one year later) we measured the six climate change populations at five temperatures, with diurnal but no seasonal or stochastic variation in temperature: 13.5°C (min 9.8; max 17.3), 18°C (14.2; 21.8), 25.9°C (22.2; 29.7), 30.2°C (27.1; 33.6), and 35.5°C (31.2; 39.6). For the first measurement we had two trays with ~100 G_1 larvae and for the final measurement we had one to two trays with ~150 G_7-G_8 larvae in each incubator. Each day dead larvae and pupae were counted and removed. Surviving pupae were transferred to a single emergence cup (Solo® cup with mesh top). The number of emerged males and females was counted daily and adults were immediately transferred to plastic 1 quart containers with mesh tops. We inspected each cage daily, and counted and removed dead adults. Females had constant access to a 2oz (Solo®) oviposition cup filled with DI water and lined with lab absorbent

paper. Adult females were offered an anesthetized mouse (blood meal) every day for the duration of the experiment.

Our experimental measurements allow us to address two questions using three sets of comparisons. First, comparing the non-time compressed (+0°C) population to the starting population allows us to determine how populations might change over the 12 months simply due to being reared in a laboratory environment. Second, comparing the time-compressed (+0°C) population to the non-time compressed (+0°C) population allows us to determine the impact of rearing mosquitoes under increased temperature variation within a given time frame. Third, comparing among the five different time-compressed trajectories (-2°C, +0°C, +2°C, +4°C, or +5°C) allows us to determine whether mosquitoes adapted to the different warming (or cooling) temperature trajectories.

Statistical analysis

All statistical analyses were done in R v3.2.2 (R Development Core Team 2012). We used a generalized linear model to quantify the effects of population (climate treatments) and temperature on juvenile daily survival, and included two-way interactions between population and temperature to allow for the effect of temperature to vary among populations. We included linear and quadratic terms for temperature because residuals from linear models showed obvious evidence of nonlinearity. We used Cox proportional hazard models with Weibull distributions and right-censored data to analyze how temperature and population influenced female adult survival (Cox *et al.* 2007). We display patterns of adult survival across

temperatures using the fraction of adult mosquitoes alive 9 days after emergence, which coincides with average lifespan of *Ae. aegypti* in the field (Hugo *et al.* 2014). We used generalized linear mixed models to quantify the effects of population and temperature on adult female development time, fraction of larvae emerging as adults, and the probability of a female taking a second blood meal. We used the fraction of larvae emerging as adults to quantify larval survival rather than survival analyses, because larval death included both individuals that were found dead on a known day, and cannibalism, in which larvae disappeared and the date of larval death from cannibalism could not be determined. For female development time and the fraction of larvae emerging as adults, population and temperature were included as fixed effects' while replicates were included as random effects. For the probability of a female taking a second blood meal (biting rate) days between blood meals (time) and temperature were included as fixed effects and individual mosquitoes were random effects. For each life history trait, we compared the full fitted model to models that were each missing one fixed effect predictor, by likelihood ratio tests.

RESULTS

In total, we measured juvenile survival for 5,643 individuals (806 ± 282 (mean ± SD) per population (starting population and 6 climate treatments)), development time for 1,232 females (176 ± 147), adult mortality for 662 females (95 ± 104), and biting rate for 139 females (20 ± 17). The best fitting model by AIC for daily juvenile survival was the full model which included population, temperature, temperature^2, population*temperature, and population*temperature^2 (Table S2). The

best fitting model for adult female survival included population, temperature, temperature^2, and population* temperature^2 (Table S4). For female development time and the fraction of larvae emerging as adults (juvenile stage survival), the best fitting models included population and temperature (Tables S1 & S3). For biting rate, the best fitting model only included temperature and the number of days between blood meals (Table S5).

Female development time varied significantly with temperature and population, but did not correlate with climate change treatment (Fig. 1; Table S1). Across all temperatures the non-time compressed (+0°C) population had the slowest development time, specifically, the non-time compressed population developed significantly, 26% (6 days), slower than the starting population (Fig. 1; Table S1). The time compressed populations had a significantly faster development time (37% (10 days) faster) compared to the non-time compressed (+0°C) population (Table S1). Additionally, the +4°C population developed significantly, 36% (8 days), faster than the decreasing (-2°C) population (Fig. 1; Table S1).

Daily juvenile mortality varied significantly with temperature and population, but did not correlate with climate treatment (Fig. 2A; Table S2). No larvae developed into pupae at 13.5°C, and at 18°C no larvae developed into pupae for the medium increasing (+4°C) population. There was a significant difference in daily juvenile mortality for the initial population and the non-time compressed (+0°C) populations; at 18°C the non-time compressed population had 44% lower mortality compared to the initial population, but at 35.5°C the initial population had 80% lower mortality

than the non-time compressed population (Fig. 2A; Table S2). The time compressed (+0°C) population had significantly (39%) higher daily mortality compared to the non-time compressed (+0°C) population (Fig. 2A; Table S2). There was also a significant difference in juvenile mortality for the +5°C increasing and decreasing (-2°C) populations; at 18°C the +5°C population had 63% higher mortality compared to the -2°C population, and at 35.5°C the -2°C population had 24% higher mortality compared to the +5°C population (Fig 2A; Table S2). For juvenile stage mortality (the product of average daily mortality and pupal development time) there were no significant differences among populations, instead temperature was the best predictor of juvenile stage mortality (Fig. 2B; Table S3). An 8°C increase in temperature (from 18 to 26°C) decreased stage mortality by 40% while a 10°C increase in temperature (from 26 to 35.5°C) increased stage mortality by 52% (Fig. 2B). Additionally, larval cannibalism varied substantially with temperature and was responsible for 6% of juvenile mortality (Fig. S2).

Adult female survival varied significantly with temperature and population (Fig 3; Table S4 & S5). There was a significant difference in adult female mortality for the non-time compressed (+0°C) and initial populations; across all temperatures, the initial population had 0.06% lower survival compared to the non-time compressed population (Fig. 3; Table S4). There was a slightly significant difference in adult survival for the non-time compressed (+0°C) and time compressed (+0°C) populations, with the non-time compressed population having 7% lower survival compared to the time compressed population (Table S4). Surprisingly, there were no

significant differences among any of the time compressed populations with temperature trajectories (Fig. 3; Table S4).

The cumulative fraction of females taking a second blood meal increased with the number of days between blood meals (Fig. 4). There were no significant differences in biting rates (fraction of females taking their second blood meal over time) between populations, however temperature and the number of days between blood meals significantly influenced biting rate (Table S5). Surprisingly, at 18°C a significantly higher fraction of females took their second blood meal compared to females at 30.2°C (Fig. 4; Table S5). However, biting rate At 18°C, increasing the time between blood meals from 4 to 7 days increased the probability of a female taking a second blood meal by 48%, while it only increased by 39% for females at 30.2°C (Fig. 4).

There was evidence that climate treatment altered the variance of population traits; though this was often idiosyncratic (Fig. S8). Variation in female development time and adult survival often varied idiosyncratically with temperature and population. At 18°C all climate treatment populations showed a significant decrease in variation ($F= 0.19$, $df=49$, p-value= <0.001) in female development time relative to the initial population; however, at 35.5°C the starting population and all climate change treatments showed relatively similar variation in development time (Fig. S8). For adult female longevity there was a no significant decrease in variance for any population at any assay temperature (Fig. S10).

There was a strong tradeoff between larval development rate and adult survival across experimental temperatures, but little evidence for a tradeoff across treatments (Fig. S11A). Cooler temperatures increased larval development time and adult longevity and hotter temperature decreased development time and longevity (Fig S11A).

DISCUSSION

Our climate change experiments caused significant changes in mosquito life history traits, however, changes did not correspond to variation in temperature trajectories across the 7°C difference between our fastest increasing and decreasing climate change scenarios. Observed changes in life history traits were primarily driven by adaptation to our time-compressed temperature trajectories rather than laboratory conditions. There were significant differences in life history traits for the starting population versus the non-time compressed (+0°C) population, but there were also significant differences between the time compressed and non-time compressed (+0°C) populations. Additionally, the variation among the time-compressed treatments was more consistent with population drift than a response to selection to adapt to the different mean temperatures.

Mosquitoes in New Orleans, Louisiana spend 10x as much time at cooler temperatures (below 20°C) compared to warmer temperatures (above 30°C), whereas in our experiment mosquitoes only spent 2x as much time at cooler temperatures relative to warmer temperatures.

These results provide only limited insight into the potential responses of mosquitoes to climate change, but demonstrate the importance of, and challenges in, designing experiments to examine the evolutionary potential of species to adapt to decadal climate change. No reasonable length experiment can simulate the relatively slow trajectory expected under actual climate change over the next several decades. As a result, one faces tradeoffs in selecting temperature regimes to examine this question. Several studies have reared sets of populations across a range of constant temperatures and then briefly exposed them to warmer (or cooler) temperatures and observed rapid adaptation to changing climates (Huey *et al.* 1991; Huey & Kingsolver 1993; van Heerwaarden *et al.* 2016). However, these experiments represent step changes in the temperatures experienced by organisms that have to adapt to variable temperatures in the field. Our design lies at the opposite extreme – we reared populations in gradually changing environments that incorporated the full daily, seasonal, and stochastic variation in temperatures that organisms experience in nature, which is important in determining individual performance (Paaijmans *et al.* 2010; Lambrechts *et al.* 2011) and has strong effects on species traits. Rearing mosquitoes under these variable temperature trajectories forced mosquitoes to survive and reproduce under both hot and cool conditions in successive generations as they do in nature, but also subjected them to higher variation in temperature within a generation than would normally be the case. Our results suggest that this increased variation was a stronger selective pressure than the 7°C variation in mean temperatures by the end of the experiment.

The lack of temperature adaptation we found is initially surprising given the large (7°C) difference between our treatments. Several potential explanations for this exist. First, although 7°C is a relative large, and certainly a biological meaningful difference in temperature, it is actually small compared to the combination of daily, annual, and stochastic variation mosquitoes experience in both our experiment and in nature (Fig. S1). Second, our experiment only encompassed a relatively limited number of generations (6-7), and we did not specifically select on individual traits of mosquitoes which may have limited the evolutionary response. However, we did see significant and relatively large differences between the no-time compression (+0°C) and starting populations, indicating that there was a sufficient number of generations for changes in population traits to occur. Instead our results indicate that the increased variation in temperature was a much stronger selective pressure than the differences among the mean trajectories.

Our results leave open the question of to what extent mosquitoes will respond to climate change and how this will alter transmission of vector borne pathogens. Future studies could measure the response of multiple mosquito species to climate change to provide an estimate of variation among species in their response to climate change. Our results suggest that it is unclear whether 2-4°C of warming will exert a significant selective pressure, relative to the large daily and seasonal temperature fluctuation of 15-30°C. Thus future studies could examine the response to selection with treatments that vary both the mean rate of increase and variation around the

mean. These studies will help determine whether climate change driven evolution by mosquitoes will lead to increases in the distribution of vector borne diseases.

Competing Interests: The authors have declared that no competing interests exist.

Authors' contributions JR, SBM, ND, DH, and AMK designed the study. JR collected the data. JR and AMK analyzed the data and wrote the first draft. All authors edited, read, and approved the final version of the manuscript.

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FIGURE LEGENDS

Fig. 1. *Ae. aegypti* adult female development time (egg hatch to female emergence). No larvae developed into pupae at 13.5°C. Error bars show standard deviations. Points are jittered along the x-axis to facilitate presentation. **A.** Adult female development rate (1/days). **B.** Adult female development time (days).

Fig. 2. *Ae. aegypti* larval survival. No larvae developed into pupae at 13.5°C. Error bars show binomial errors. Points are jittered along the x-axis to facilitate presentation. **A.** Daily juvenile survival. Error bars show standard errors for individual mosquitoes. **B.** Fraction of larvae that survived to emerge as adults.

Fig. 3. Estimated survival of *Ae. aegypti* adult females to 9 days post-emergence based on Cox proportional-hazard models (see Fig. S1 for raw survival plot). Points are jittered along the x-axis to facilitate presentation. **A.** Shows full temperature range (x-values from 20-40 °C). **B.** Shows cooler temperatures (x-values from 16-30 °C) and y-axis is truncated to 0.85-1. **C.** Shows hotter temperatures (x-values from 32-40 °C) and y-axis is ranges from 0.3-1.

Fig. 4. Adult female *Ae. aegypti* biting rate. Cumulative fraction of females taking two blood meals. Each line represents a group of females that took one blood meal and then was followed over time until they took their second blood meal. A y-value of 100% means that of the initial females who took their first blood meal, all females within that group went on to take a second blood meal before the study was terminated. Error bars are binomial errors based on the number of individuals within a group.

FIGURES

Figure 1

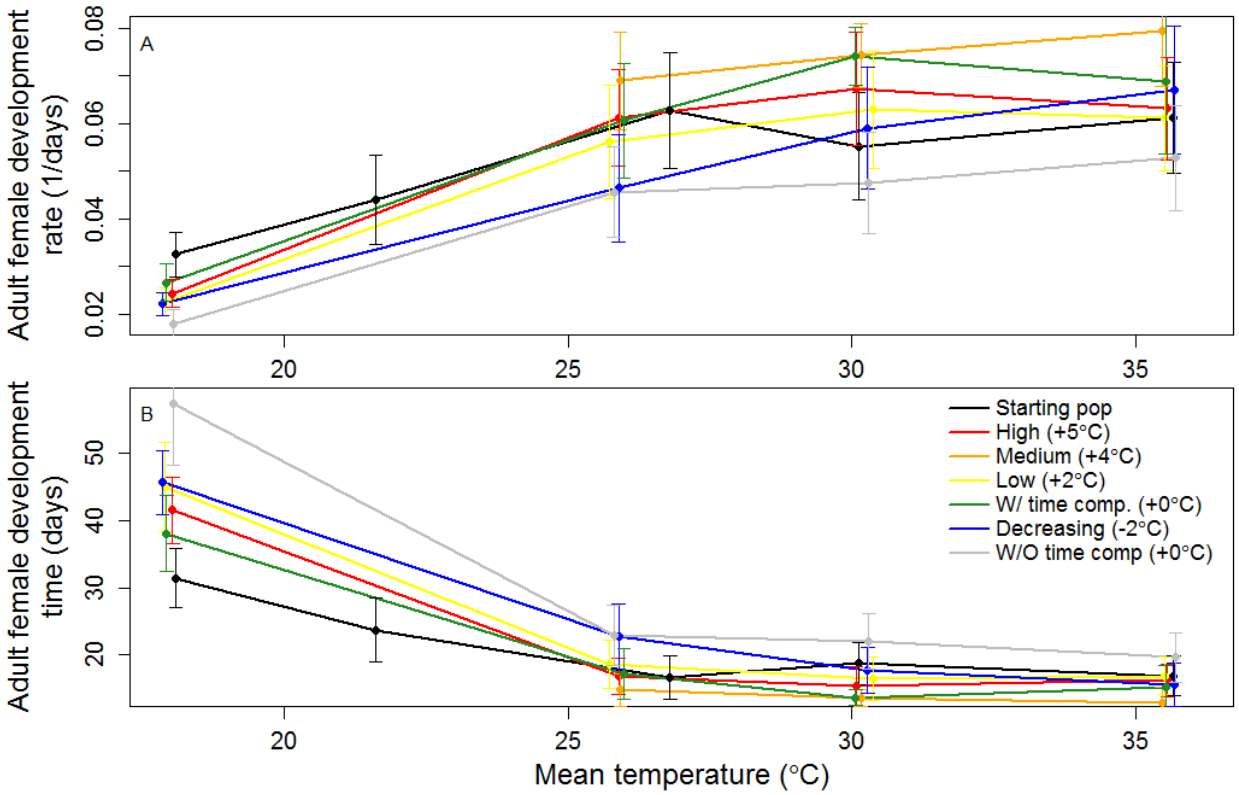


Figure 2

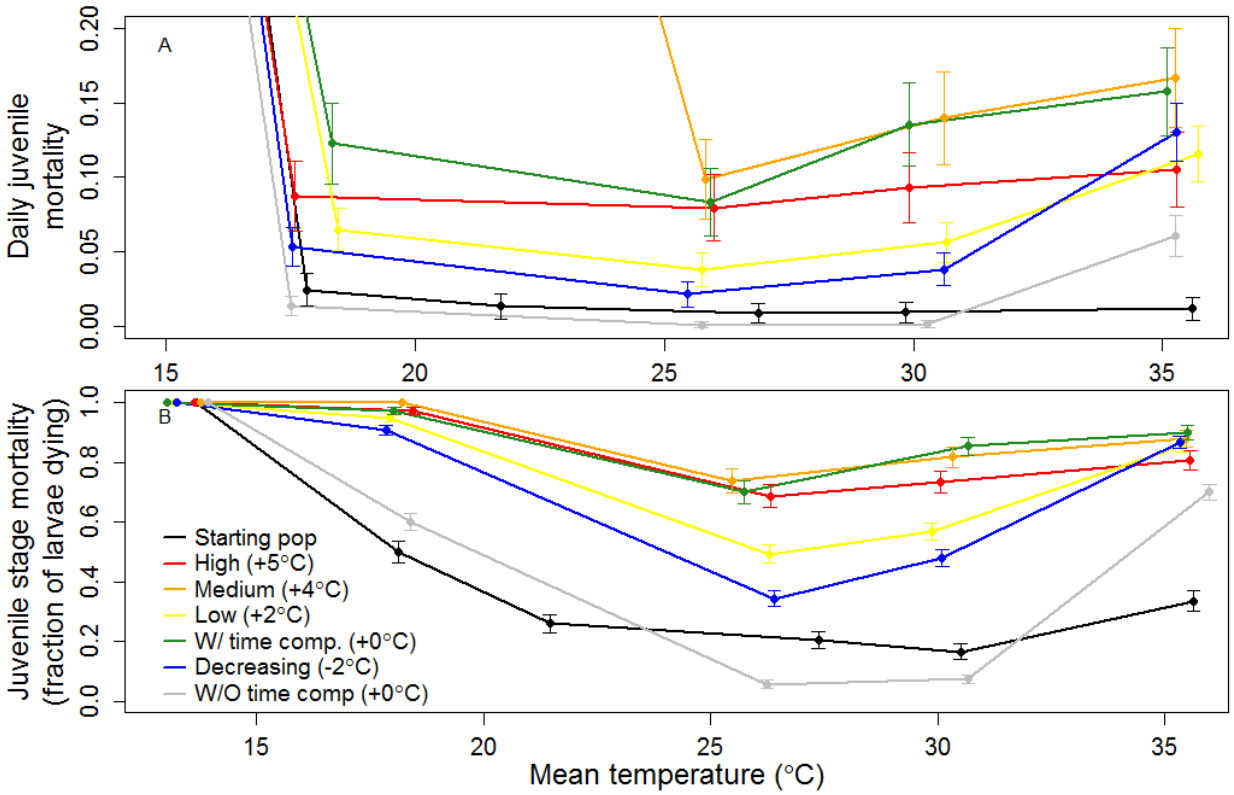

A
Daily juvenile mortality
0.00
0.05
0.10
0.15
0.20
15
20
25
30
35
B
Juvenile stage mortality (fraction of larvae dying)
0.0
0.2
0.4
0.6
0.8
1.0
Starting pop
High (+5°C)
Medium (+4°C)
Low (+2°C)
W/ time comp. (+0°C)
Decreasing (-2°C)
W/O time comp (+0°C)
15
20
25
30
35
Mean temperature (°C)

Figure 3

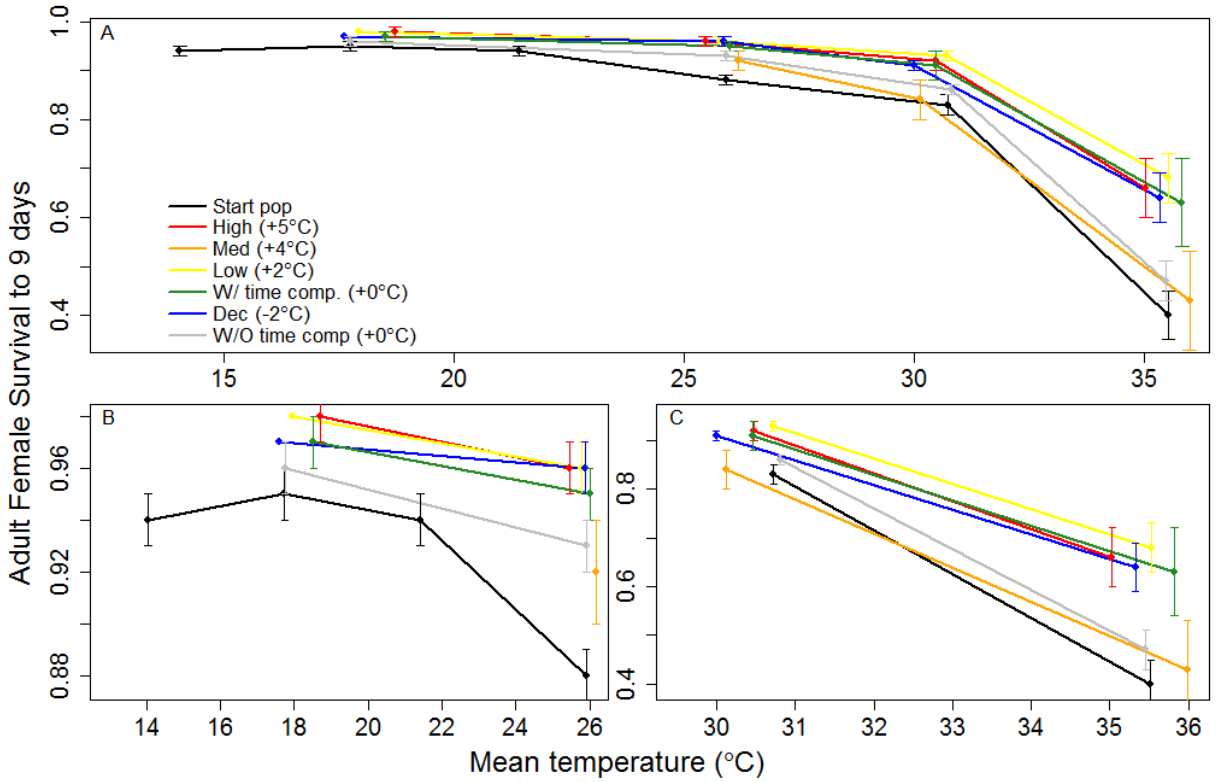
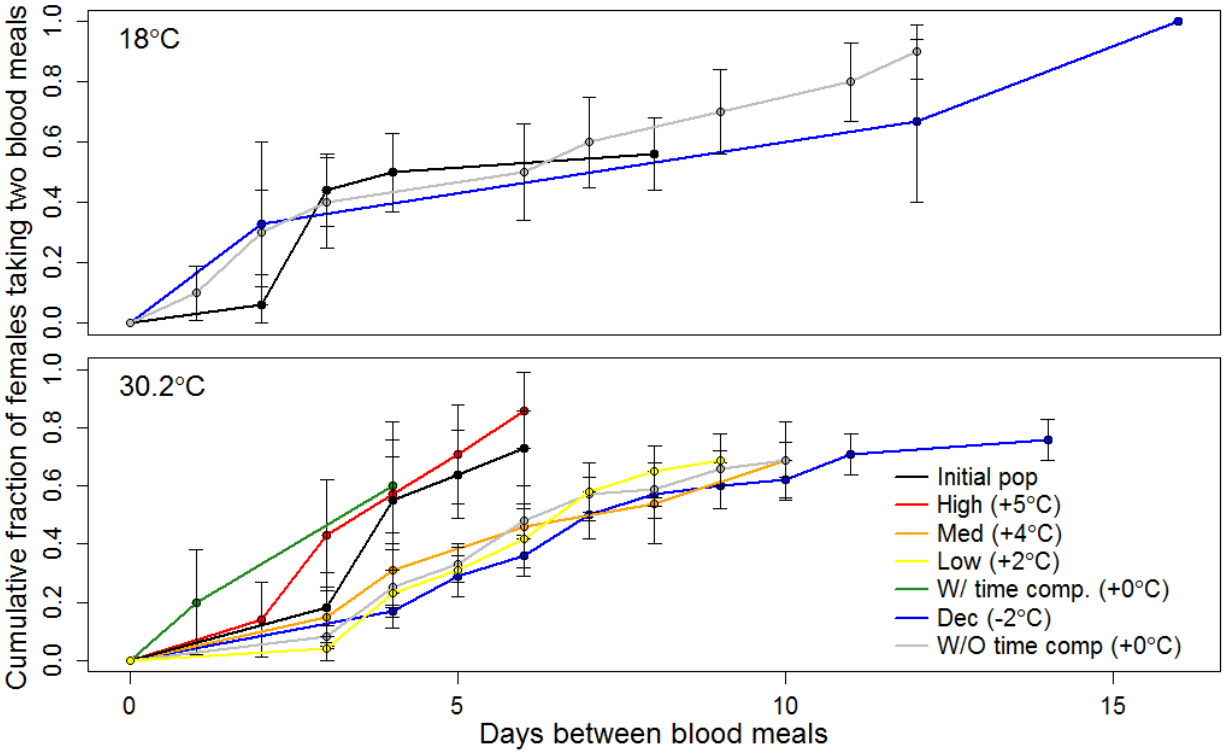

A
B
C
Start pop
High (+5°C)
Med (+4°C)
Low (+2°C)
W/ time comp. (+0°C)
Dec (-2°C)
W/O time comp (+0°C)
Adult Female Survival to 9 days
Mean temperature (°C)

Figure 4


18°C
30.2°C
Cumulative fraction of females taking two blood meals
0.0
0.2
0.4
0.6
0.8
1.0
0
5
10
15
Days between blood meals
Initial pop
High (+5°C)
Med (+4°C)
Low (+2°C)
W/ time comp. (+0°C)
Dec (-2°C)
W/O time comp (+0°C)

SYNTHESIS

This dissertation research was motivated by the desire to understand how mosquitoes will evolve in response to climate change and how this would influence vector-borne pathogen transmission. We examined three species of mosquitoes *Culex pipiens* (Linnaeus), *Aedes aegypti* and *Aedes albopictus* (Linnaeus), and all three species are dominant vectors of pathogens that cause substantial morbidity and mortality in humans including Zika, dengue, yellow fever, and chikungunya virus. We utilized common garden experiments and a novel time-compressed climate change simulations to quantify variation in four temperature dependent life history traits (larval and adult survival, development rate, and biting rate).

In chapter one **Geographic Variation in the Response of *Culex pipiens* Life History Traits to Temperature**, our results show that the impact of climate change on mosquitoes will be more variable than previous predictions due to the substantial variation that exists in the response of populations to temperature. At the same time, these differences among populations are likely to contribute to spatial variation in transmission, and will be an important source of variation for selection to act on as climate warms (Rohr *et al.* 2011; Egizi *et al.* 2015). The combination of standing variation and mosquitoes' evolutionary response will determine the impact of changing climates on vector borne disease.

In chapter 2 **Variation in vectorial capacity for Zika, dengue, chikungunya, and yellow fever virus for North American *Aedes aegypti* and *Aedes albopictus* mosquitoes**, between species differences in temperature dependent life-history traits and vectorial capacity for Zika, dengue, chikungunya, and yellow fever viruses are likely to contribute to spatial and temporal variation in pathogen transmission. Although *Ae. aegypti* was a more efficient vector, this species is thus far limited to tropical and subtropical areas. In contrast, *Ae. albopictus* has life history traits that make it a moderately efficient vector for several human pathogens, and its more northern distribution creates the potential for pathogen transmission to occur at higher latitudes where *Ae. aegypti* is less abundant. The seemingly never-ending emergence of novel or mostly forgotten viruses emphasize the importance of understanding the ecology of these key vectors of human disease.

In chapter 3 **Adaptation of *Aedes aegypti* to simulated climate change reveals sensitivity to temperature variability**, our results leave open the question of to what extent mosquitoes will respond to climate change and how this will alter transmission of vector borne pathogens. Future studies could measure the response of multiple mosquito species to climate change to provide an estimate of variation among species in their response to climate change. Our results suggest that it is unclear whether 2-4°C of warming will exert a significant selective pressure, relative to the large daily and seasonal temperature fluctuation of 15 - 30°C. Thus future studies could examine the response to selection with treatments that vary both the mean rate of increase and variation around the mean.

In summary, this research has advanced our understanding of how life history traits of populations and species of mosquitoes vary spatially in response to temperature, and how mosquitoes may respond to climate change.

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