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The Role of Land Use in *Aedes* abundance and Insecticide Resistance in Central Africa and an Analysis of Arbovirus Transmission Potentials

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

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Matthew J. Montgomery

March 2023

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Abstract
The Role of Land Use in *Aedes* abundance and Insecticide Resistance in Central Africa and an Analysis of Arbovirus Transmission Potentials
Matthew J. Montgomery

Globally, mosquito-borne diseases are a major source of mortality, with the burden focused primarily in the poorest nations. Central to reducing mosquito borne disease is an understanding of the biology of those species responsible for transmitting disease to humans. Accordingly, this work focuses on the ecology and evolutionary biology of two vectors of global importance: *Aedes aegypti* and *Aedes albopictus*.

The first chapter examines the role of urbanization and species interactions in determining *Aedes* abundance across a 900km latitudinal range representing vast climatic gradients and the major ecotypes of Central Africa. Additionally, we performed pathogen screening on over 9,000 female mosquitoes for dengue, chikungunya, and Zika viruses.

The second chapter focuses on the distribution and potential causes of insecticide resistance in *Aedes* across the same broad climatic and ecotype gradient in Cameroon. In this chapter we detected significant variation in insecticide resistance between cities, habitat types, species, and insecticide class. We applied land use data to show the significant effect that urbanization can have on determining insecticide efficacy across an area as small as a single city and further revealed the underlying genetic and metabolic mechanisms for insecticide resistance.

The final chapter investigates viral fitness among the primary arboviruses transmitted by *A. aegypti* and *A. albopictus*: chikungunya, dengue (serotypes 1-4),

yellow fever, and Zika. Our innovative approach to comparing relative viral fitness is multi-facted, combining field observations of human infection rates with laboratory studies of host and vector competence.

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Firstly, I would like to thank my advisor Dr. Marm Kilpatrick for the tremendous help he has given me throughout this process and for allowing me the privilege to be a part of the Kilpatrick Lab. He is one of the most brilliant minds I have ever encountered and I have learned so much from him over the course of my graduate studies. As part of the Kilpatrick Lab, I also had the benefit of working with fellow graduate students Christa Seidl, Billy Gardner, and Nikka Malakooti who provided constructive input and advice throughout our time together. I also must thank my committee members, Dr. Bruce Lyon, Dr. Chris Barker, and Dr. Jean Tsao for not only serving on my committee, but for the expertise they provided in shaping my research projects at their early stages and guiding me throughout the process.

The Center for Research in Infectious Disease (CRID) in Yaounde, Cameroon performed the vast majority of actual field and laboratory work that composed the first two chapters. Their scientific expertise was invaluable to the completion of the research.

My second chapter, Spatial distribution of insecticide resistant populations of *Aedes aegypti* and *Ae. albopictus* and first detection of V410L mutation in *Ae. aegypti* from Cameroon, utilizes previously published materials with the permission of my co-authors (see Appendix) and my advisor who is an additional co-author on the published article. This work was based on a Department of Defense funded grant for a study which I designed and was the Principal Investigator. All field and lab work was performed by CRID via a contract funded by the aforementioned grant. I

performed the statistical analysis and wrote and created the figures for the majority of the manuscript.

On a personal note, I could not have completed this dissertation without the help of my family. My brother Tim selflessly offered his skills in GIS for calculating the urbanization indices in Chapter 2. I also must thank the two most important women in my life. My late mother, Lynda, always instilled in me the value of higher education and she would be so happy to know that I have earned my doctoral degree. Lastly my wife, Lynné provided invaluable support and encouragement throughout these years.

Introduction

Globally, mosquito-borne diseases are a major source of mortality, with the burden focused primarily in the poorest nations. In the early half of the 20th century major progress was made towards reducing mosquito-borne disease burden through improved vector control, vaccines, and the development of new medications. However, in recent years emerging pathogens have swept across the globe in explosive epidemics, as demonstrated by West Nile Virus in 1999, the 2013 chikungunya outbreak, Zika virus in 2015, and dengue's rapidly increasing global burden (Lanciotti et al. 1999, Shragai et al. 2017, Wahid et al. 2017, WHO 2020). Central to reducing mosquito borne disease is an understanding of the biology of those species responsible for transmitting disease to humans. Accordingly, my dissertation work focuses on the ecology and evolutionary biology of two vectors of global importance: *Aedes aegypti* and *Aedes albopictus*. Both of these species are found in tropical to temperate regions on six continents, and they are the primary vectors of chikungunya, dengue, Yellow Fever, and Zika viruses as well as other human pathogens.

The first two chapters of my dissertation are focused on the biology of *A. aegypti* and *A. albopictus* in Cameroon, in central Africa. While these studies take place in a single nation, they were conducted over a large longitudinal gradient and across a range of different ecotypes and climates representative of a wide area of the African continent, with habitats ranging from tropical equatorial forests to the dry Sahel. The final chapter takes a global approach to the major viruses spread primarily

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by these species and investigates how our understanding of the factors which predict a mosquito-borne disease epidemic correlate with empirical observations of human infections across the globe.

The first chapter examines the role of urbanization in determining *Aedes* abundance. Urbanization, defined as converting land from natural or agricultural land uses to areas built upon by humans, is among the most drastic and consequential form of human land use and plays a pivotal role in shaping the biotic and abiotic conditions faced by mosquitoes (Gubler et al. 2011). Previous research has shown that urbanization can influence mosquito abundance and distributions, and pathogen transmission to humans (Alirol et al. 2011). Consequently, it is imperative that the ecological factors leading to increased mosquito populations and thus vector borne disease risk be better understood.

The chapter begins with a literature review of the effects of urbanization on *Aedes aegypti* and *A. albopictus* abundance from studies outside of Africa. These studies show conflicting patterns in the response of *A. albopictus* to urbanization (and consistent increases in abundance for *A aegypti)*. We sought to investigate this highly varied response to urbanization by *A. albopictus* on the African continent. Additionally, we wanted to determine if *A. aegypti*'s abundance in response to urbanization would vary based on climatic factors or the presence of a competitor species. To address these questions, we obtained data on mosquito abundance from 231collections across six cities spanning nearly 1,000km of latitudinal extent over a one year period. The six surveilled cities varied vastly in climate and ecotype, ranging

from coastal equatorial forests to the Sahel. Our results showed that urbanization increases abundance for *A. aegypti* throughout Cameroon, consistent with global patterns. Surprisingly, the slope of *A. aegypti* abundance in response to urbanization remained consistent across all cities even though the intercepts varied. Yet for *A. albopictus* there was heterogenous response to urbanization even within a single country: *A. albopictus* abundance increased with urbanization in one city, decreased with urbanization in another and showed no trend in a third city. To better understand the mechanisms of urbanizations effect on adult mosquito abundance we performed further analysis on climatic factors, larval habitat, structures, host abundance, and available host community composition.

Chapter 1 also included a wide scale effort to detect and identify the major viruses transmitted by *Aedes* mosquitoes (chikungunya, dengue, and Zika) across their global range in field caught populations in Cameroon and how urbanization could effect mosquito viral infection rates. Our extensive surveillance resulted in only a single detection of Zika virus among hundreds of pools containing many thousands of individual specimens. As such, our data were unable to address any hypotheses on how urbanization could influence disease distribution. This study supports numerous prior instances showing that field-caught mosquitoes generally have exceedingly low infection rates, even during periods where surveillance was concurrent with active human outbreaks. Reassuringly, our study shows that infection rates for chikungunya, dengue, and Zika were exceedingly low even in the absence of active public health vector control measures.

The second chapter focuses on the distribution and potential causes of insecticide resistance in *Aedes* across the same broad climatic and ecotype gradient in Cameroon. Public health efforts to mitigate vector borne disease often include insecticide use to reduce vector populations. However, mosquitoes can rapidly develop resistance to insecticides (Hamdan et al. 2005), which reduces their effectiveness (Ranson et al. 2011). Furthermore, there are multiple physiological and genetic mechanisms to insecticide resistance and these mechanisms can vary both between species and within geographically dispersed populations of a single species (Kamgang et al. 2011; Dusfour et al. 2019). Lastly selective pressure against resistance may occur through reduced mating performance, fecundity, or survival (Dusfour et al. 2019; Rigby et al. 2021). This may lead to fluctuations in insecticide resistance depending on insecticide usage in a region (Dusfour et al. 2019). Understanding the regional and species-specific susceptibility of mosquitoes to insecticides of public health importance is vital for converting knowledge of disease risk into effective mitigation strategies (Ranson et al. 2011;World Health Organization 2012; Lima et al. 2015; Bouzid et al. 2016; Alvarado-Castro et al. 2017).

In this chapter we detected significant variation in insecticide resistance between cities, habitat types, species, and insecticide class. We applied land use data to show the significant effect that urbanization can have on determining insecticide efficacy across an area as small as a single city and further revealed the underlying genetic and metabolic mechanisms for insecticide resistance. Our analysis also

includes temporal changes of allele frequency for a particular resistance mutation which shows increase in resistance allele frequency and distribution over rapid time scales across Cameroon, showing the high selective pressure for insecticide resistance in *A. aegypti*. We also detected the first instance of a particular resistance mutation, V410L, in central Africa. This mutation is found on other continents, but was only detected in Africa in distant (>1,400km) Angola in 2016, which indicates the high degree of gene flow between populations or far-reaching human-facilitated introduction of populations on the African continent (Ayres et al. 2020).

The final chapter investigates viral fitness among the primary arboviruses transmitted by *A. aegypti* and *A. albopictus*: chikungunya, dengue (serotypes 1-4), yellow fever, and Zika. Our innovative approach to comparing relative viral fitness is multi-facted, combining field observations of human infection rates with laboratory studies of host and vector competence.

We first determined the relative transmission intensity of co-occurring arboviruses in human populations through the presence of arbovirus antibodies in human serosurveys. To obtain this data, we conducted a literature review and compiled data from historical serosurveys, which are studies where human blood samples are tested for specific disease antibodies. After identifying 213 unique serosurveys that simultaneously tested for at least two of our study pathogens among over 95,000 individuals across 5 continents, we were able to compare relative infection rates among seven arboviruses. Our results show that there are significant

differences in transmission intensity of these co-occurring viruses within the same human populations.

Next, we compiled estimates of relative viral transmission efficacy calculations generated from mathematical models using a vast trove of host and vector competence data for simultaneous calculations via the calculation of the relative reproductive number R_0 for each pathogen, which describes the number of secondary cases arising from an initial primary case in a fully susceptible population (Dye 1992).
We hypothesized that among these co-occuring pathogens, those with higher relative R_0 values should infect a larger fraction of a given host population and should have higher relative seroprevalence. Surprisingly, our results showed no significant correlation between relative R_0 and transmission intensity across the global dataset, and within continents relative R_0 was only significantly correlated with greater seroprevalence in Asia. These results indicate a stark disconnect between estimates of pathogen fitness and field observations of pathogen infection rates.

In this body of research I hope to show how human modifications of the environment through urbanization and insecticide use shapes the disease vector landscape that we face as humans and to calculate and compare arbovirus fitness while simultaneously presenting a new way to evaluate predictions of viral fitness using real world observations of human infection rates.

Chapter 1: The effects of urbanization on *Aedes aegypti* and *A. albopictus* abundance across a broad latitudinal gradient in Central Africa

Abstract

Urbanization shapes the biotic and abiotic conditions faced by many species. The effects of urbanization on disease vectors may increase or decrease their abundance and disease risk by altering larval habitat, microclimates, and host abundance. We investigated the effect of urbanization and species interactions on the abundance and disease risk posed by two cosmopolitan mosquito vectors of global health concern, *A. aegypti* and *A. albopictus,* in Cameroon, Central Africa. We collected adult mosquitoes during both the rainy and dry seasons at 63 sites on a rural to urban gradient from six cities spanning a 900 km latitudinal range from equatorial forest to the Sahel. We also measured larval habitat, host abundance, multiple measures of urbanization, and climate data. Urbanization increased larval habitat, the number of structures, and host availability, but a GIS urbanization index was the best predictor of mosquito abundance. *A. aegypti* abundance increased with rainfall and decreasing temperatures and increased 2.7% with each 1% of additional urbanization in all six cities. In contrast, *A. albopictus* abundance increased, decreased or showed no influence of urbanization in the three cities where this species was present; abundance also increased with rainfall and humidity. We found no evidence of interspecific competition among these species using adult abundance data, but analyses were limited spatially. We screened over 9,000 female mosquitoes for dengue,

chikungunya, and Zika viruses but only detected one virus (Zika) in a single mosquito pool. Our results show that urbanization consistently increases *A. aegypti* abundance across a broad range of habitats, while urbanization effects on *A. albopictus* abundance varied markedly over a relatively small geographic area. Urbanization is thought to increase mosquito abundance through mediating availability of larval habitat, temperature, humidity, number of structures, and host availability, yet our results showed temperature and humidity at the time of collection were not significantly correlated with urbanization and larval habitat, host availability, and number of structures were either not significantly correlated with abundance or were less effective predictors of abundance than the urbanization index. These results indicate that the precise mechanisms of urbanization effect on adult *A. aegypti* and *A. albopictus* abundance are not fully understood.

<u>Introduction</u>

Urbanization, the conversion of natural and agricultural habitats to urban areas, alters the biotic and abiotic conditions faced by organisms and is increasing globally (Grimm et al. 2008; Alig et al. 2004; Chen et al. 2022). Urbanization often reshapes community composition, with introduced and human commensal species replacing native species (McKinney 2006). Urbanization also alters microclimates, including increasing temperatures through the Heat Island effect (Chapman et al. 2017, Deilami et al. 2018), and reducing humidity (McCluney et al. 2017). These changes in the biotic and abiotic environment also influence the transmission of pathogens (Bradely & Altizer 2007, Gottdenker et al. 2014, Hassel et al. 2017), and

especially vector-borne pathogens (Kilpatrick & Randolph 2012). However, the effect of urbanization on vector-borne disease depends on both the response of the vectors to urbanization and the microclimate effects on pathogen development in the vector, and both these factors can increase or decrease with urbanization depending on the vectors, the pathogen, and the local climate (Roger & Randolph 2000, Mordecai et al. 2013, Kraemer et al. 2015, Mordecai et al. 2017). Africa is a key area for understanding the effect of urbanization on vector borne disease because it has the world's highest rates of urban growth (OECD/SWAC 2020). *Aedes*-borne arboviruses, including dengue, chikungunya, and zika viruses, are an especially important group of pathogens because of recent increases in incidence and outbreaks (Bhargavi & Moa 2020, ECDC 2022, WHO 2022).

Urbanization can increase larval habitat, especially for key anthropogenic species like *Aedes aegypti* and *Aedes albopictus*, either through more impervious surfaces or through increased refuse, containers, and other objects which retain water (Braks et al. 2003; Morrison et al. 2004; Higa et al. 2010; Gubler et al. 2011; Li et al. 2014; Arduino et al. 2020). Furthermore, urbanization is often associated with storing water use throughout the year, which can provide mosquito larval habitat in periods with low rainfall, especially in arid climates (Faeth et al. 2005, Gubler et al. 2011; McCluney et al. 2017). Urbanization in poorer countries may generate more larval habitat compared to more developed nations due to poor sanitation, increased refuse, and more personal water storage, thus increasing mosquito abundance and disease risk (Service 1992; Gubler et al. 2011; Obenauer et al. 2017).

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Temperature increases associated with urbanization can alter mosquito community composition and pathogen transmission through multiple mechanisms. Temperature tolerance (both minimum and maximum, as well as daily variation) vary considerably between mosquito species (Reinhold et al. 2018) and temperature influences survival (Bar-Zeev 1957; Couret et al. 2014), biting rate (Christophers 1960; Scott et al. 2000), development rate (Bar-Zeev 1958; Delatte et al. 2009), fecundity (Delatte et al. 2009; Carrington et al. 2013), vector competence (Lambrechts et al. 2011; Reinhold et al. 2018) and the extrinsic incubation period (Watts et al. 1987; Kilpatrick et al. 2008; Rohani et al. 2009; Reinhold et al. 2018). Increased temperature caused by urbanization can decrease larval development time and adult survival which can have opposing effects on transmission (Costa et al. 2010, Kilpatrick & Randolph 2012, Mordecai et al. 2013). In contrast, decreased humidity generally reduces mosquito survival without any positive effects on transmission (McCluney et al. 2017). Urbanization can also alter interspecific competition among mosquito larvae. For example, larval habitat that entirely dries out favors *A. aegypti* over *A. albopictus*, whereas the inverse is true in larval habitat that never entirely dries out (Costanzo et al. 2005).

The effect of urbanization on mosquito abundance can vary among species. *A. aegypti* abundance consistently increases with urbanization across its global range, with very few exceptions (Table S1). In contrast, *A. albopictus* abundance increased with urbanization in half of 15 previous studies, decreased in six, and had no effect in one (Table S2). Specifically, *A. albopictus* abundance was higher in urban areas than

rural areas in China (Li et al. 2014), and Mayotte (Bagny et al. 2012), but abundance was highest in suburban/transitional areas in Virginia (Barker et al. 2003) and Thailand (Tsuda et al. 2006) and rural areas in Brazil (Honorio et al. 2009) and Florida (Hornby et al. 1994). A key area where the effect of urbanization on *A. albopictus* has not been studied is the continent of Africa.

Our goal was to examine the effect of urbanization on the abundance and arbovirus infection rates of *A. aegypti* and *A. albopictus* mosquitoes across a broad latitudinal gradient in central Africa. We examined adult mosquito abundance, microclimate, and larval habitat at ten sites in each of six cities spanning the 900km latitudinal extent of Cameroon and tested *Aedes* mosquitoes for dengue, chikungunya, and Zika viruses which are important arboviruses and all circulate in this region (Kuniholm et al. 2006). We studied adult mosquitoes rather than mosquito larvae, which have been the focus of some earlier work (Bagny et al. 2012; Higa et al. 2010; Honorio et al. 2009; Tsuda et al. 2006), so we could detect pathogens and because adult mosquito abundance is often a better predictor for outbreaks than larval indices (Leandro et al. 2022). We hypothesized that *A. aegypti* would increase with urbanization, but we predicted the relationship would vary among cities because the benefits of urbanization might be counterbalanced in areas that were very hot and dry. We hypothesized that *A. aegypti* abundance might also be influenced by *A. albopictus*, which itself might show increasing or decreasing relationships with urbanization. While *A. aegypti* is native to Cameroon, *A. albopictus* is an introduced species that was first detected in 2000 (Simard et al. 2005). In some regions of the

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world *A. albopictus* competes with and can displace *A. aegypti* populations through larval competition (Shagrai et al. 2017) and reproductive interference (Tripet et al. 2011). We examined multiple scales of urbanization on mosquito abundance and hypothesized that small-scale measures of urbanization (100-200m rather than >1km) would most closely correlate with abundance, based on the short-distance dispersal distances of *A. aegypti* and *A. albopictus* in mark-recapture studies (Medeiros et al. 2017, Juarez et al. 2020) .

Methods

Study area. We collected mosquitoes in six cities spanning a 900 km latitudinal gradient in Cameroon (Figure 1) representing five African ecoregions: Atlantic equatorial coastal forest (Douala and Kribi), Congolian lowland forest (Yaounde), Northern Congolian Forest Savannah mosaic (Ngaoundere), East Sudanese Savannah (Garoua), and Sahelian (Maroua) (World Data Base on Protected Areas 2022). Average annual temperatures and relative humidity varied between 18°C to 28°C and 85% to 45%, respectively for our study cities. Cities range in altitude from 2m to 1200 m (Olivry, 1986). Within each city we surveyed at a minimum of ten sites across an urbanization gradient from forest to agrarian to dense urban centers.

Land use analysis. For each site we quantified urbanization at 100m, 200m, 500m, 1km, and 2km radii using an Urbanization Index (UI) (Gomez et al. 2008), where UI = (100% – % vegetation cover + % impervious surface)/2.

We performed spatial analysis using ArcGIS Pro version 2.8.7. We imported GPS field coordinates into ArcGIS Pro and spatial landcover data was obtained from the European Space Agency's (ESA) Climate Change Initiative (CCI) – S2 Prototype Land Cover 20M map of Africa which included 10 classes of land surface data at a 20 meter resolution and were classified as follows: "trees cover areas", "shrubs cover areas", "grassland", "cropland", "vegetation aquatic or regularly flooded", "lichen and mosses / sparse vegetation", "bare areas", "built up areas", "snow and/or ice" and "open water" (Figure S1). We used satellite imagery (Google Earth Pro version 7.3.4.8642) to visually quantify the number of structures at the 100m radius for each surveillance site.

Larval habitat survey. At each of the ten surveillance sites (with three exceptions) in five of the six cities (all except Yaounde) we recorded potential larval habitat within 50m of adult collection sites. We categorized and sampled any container with mosquito larvae present, and any larvae collected were reared to adulthood and identified to species for *A. aegypti* and *A. albopictus*.

Adult mosquito collection. We collected adult mosquitoes outdoors using two stationary human collectors using Modified CDC Backpack Aspirators (John Hock W. Hock Company, Gainesville, Florida). We used human landing catches, rather than passive traps, to increase the number of adult *Aedes* captured and thereby to increase our chances of detecting and accurately measuring arbovirus infection rates

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(Schoeler et al. 2004; Scott & Morrison 2009). We conducted collections for 3 hours during daytime at consistent three hour periods (8am-11am and 3pm-6pm) and we recorded temperature and humidity at the starting time of each collection. We chose collection times based off of previous work in Africa showing both species were diurnal and that *A. aegypti* and *A. albopictus* activity peaked in both the morning and early evening (McClelland 1959; Trpis et al. 1973; Delatte et al. 2010). Collections took place over a one week period in each city once during both the dry and rainy seasons from September 2020-August 2021. When possible, we repeated collections a second time at each site during the same collection week. In Yaounde, we visited sites more frequently (ten times between September 2020 and October 2021). At the end of each collection period, we knocked down adult mosquitoes at -20°C or using a chloroform solution and identified using them morphological identification keys (Edwards, 1941; Jupp, 1996) under a microscope. We grouped *Aedes* females by species and pooled in groups of up to 30 individuals and stored at -80°C in RNA*later* following the manufacturer's instructions (LifeTechnologies, 2011) for viral detection. The rest were stored at -20°C in silicagel.

We analyzed mosquito abundance using generalized linear models (GLM) with a negative binomial distribution and log link using the *lme4* package in R version 3.6.1. We compared models with different urbanization radii, closely correlated climatic parameters (e.g. monthly rainfall versus number of monthly rainy days), and interactive versus additive effects of variables using Akaike's Information Criterion (AIC).

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Pathogen detection. We pooled female mosquitoes in Eppendorf tubes and first ground them in 200 µL of Leibovitz L15 medium equilibrated at room temperature. Then, we centrifuged the sample for 15 min at 15,000 rpm and transferred the supernatant to a new Eppendorf tube. We extracted potential total viral RNA present in each pool using the Qiagen extraction kit (QIAamp-viral RNA mini kit, Qiagen) following the manufacturer's instructions and stored at -80°C.

We retrotranscribed extracted RNAs into cDNAs using the High-Capacity cDNA Reverse Transcription kit, 1000 reactions (Applied Biosystems, Foster city, California, USA). We prepared a mixture of 50 µL final volume, including 25 µL of RNA sample, 5 µL of 10X reverse transcription buffer, 2µL of 100 mM dNTPs, 5µL of 10X hexa random primers 10.5µL, 2.5 µL of reverse transcriptase, and 10.5 µL of RNAse-free H_2O. We incubated the samples for 10 min at 25°C and 1 h at 37°C.

We performed quantitative real-time PCR using TaqMan Universal PCR Master Mix reagents (Applied Biosystems, Foster city, California, USA) to amplify UTR genes for Zika (Conceição *et al.*, 2010; Grard *et al.*, 2014) and Dengue viruses (Leparc-Goffart *et al.*, 2009), and the E1 envelope protein for chikungunya virus (Ngoagouni *et al.*, 2017) (Table S3). Each PCR was performed in a 25 µL reaction mixture containing 12.5µL of 2x PCR TaqMan Universal PCR Master Mix, 1µL of each primer, 1 µL of each TaqMan probe (Applied Biosystems, Foster city, California, USA), 4.5 µL of RNAse-free H_2O, and 5 µL template of cDNA. The amplification program consisted of a pre-activation heat step of 2 min at 50°C followed by 10 min at

95°C, and the amplification and fluorescence quantification steps of 45 cycles of 15 sec at 95°C and 1 min at 60°C. All assays were done on the Stratagene MX3005P qPCR machine (Agilent Technologies, Santa Clara, California, USA).

Host availability. In September of 2021 in Douala, Kribi, and Yaounde we counted potential hosts using two stationary observers between 8am-11am and 3pm-6pm on two separate days at each established surveillance site. We recorded all humans, chickens, goats, cows, and dogs visible over a 5 minute period. We counted human hosts to a maximum of 100 due to extremely large crowds in some sites rendering more accurate counts unfeasible.

Indoor vs outdoor mosquito abundance surveys. We conducted paired surveillance of an outdoor site with simultaneous mosquito collections inside 10 nearby homes at 10 sites in Yaounde in October of 2021 to investigate whether outdoor collections correlated with indoor household vector abundance. Outdoor mosquito collection took place for 3 hours as described above, while we surveyed the interior of willing households within 50 meters of the outdoor sites using the same Modified CDC Backpack Aspirators.

Competition between *A. aegypti* and *A. albopictus*. We calculated monthly population growth rates, ($\lambda = N_{t+1}/N_t$), for each species for the two sites in Yaounde from which adequate numbers of both species were simultaneously collected. We then examined the potential effects of interspecific competition between *A. aegypti*

and *A. albopictus* by examining the effect of the abundance of *A. albopictus* on the

population growth rate $\log_{10}(\lambda)$ over the following month for *A. aegypti*, and vice

versa. We analyzed the effect of mosquito abundance on population growth rates

using generalized linear models (GLM) with a gaussian distribution and log link

using the *lme4* package in R version 3.6.1.

Results

Larval Habitat Survey. The number of containers increased with urbanization and

the urbanization index at 100m (UI_100m) was the best correlate of both containers

($\log_{10}$(containers)=3.04+0.0067 (± 0.0021) * UI_100m, P = 0.0020; Figure 2) and

larvae-positive containers ($\log_{10}$(positive_containers)=2.34+0.0078 (± 0.0024) *

UI_100m, P = 0.0012). On average a site with UI of 0 would be expected to have 21

larval containers while a site with a UI of 100 would have 41. There was no

significant relationship between the Container Index (larvae occupied

containers/available containers) and UI (logit(CI)=-0.05+0.0028 (± 0.0051) *

UI_100m, P = 0.57).

Host Availability. Human abundance increased significantly with UI and 100m was

the best correlate ($\log_{10}$(Humans)=2.83+0.0097 (± 0.00085)*UI_100m, P ≤ 0.001),

while animal abundance was negatively correlated with UI ($\log_{10}$(Animals)=1.7-

0.025 (± 0.0035)*UI_100m, P ≤ 0.001). On average a site with UI of 0 would be

expected to have 17 human hosts present while a site with a UI of 100 would have 45 (Figure S2).

Mosquito Abundance. We collected 25,853 adult mosquitoes from 231 two-person collection sessions from 63 sites across 6 cities (Table S4). Of these mosquitoes, 15,008 were *A. albopictus* and 2,633 were *A. aegypti*. No *A. albopictus* were found in our three study cities above 6° latitude (Ngaoundere, Garoua, and Maroua) (Table S5).

A. aegypti abundance increased with the UI in all cities (Figure 3), increased with rainfall, and decreased with temperature (Figure 4); humidity had no significant effect (Table S6). The best fitting model used the UI at 1km (UI_2km: ΔAIC = 4.080; UI_500m: ΔAIC = 10.30; UI_200m: ΔAIC = 23.13; UI_100m: ΔAIC = 18.082). The slope of the response to urbanization was consistent across cities (an additive model was preferred over a city * urbanization interactive model; ΔAIC 2.90), with an average 2.7% increase in *A. aegypti* females for each corresponding 1% increase in urbanization. The intercepts for average *A. aegypti* abundance varied considerably between cities, with the greatest average abundance in Douala and the lowest in Yaounde. Abundance also increased with rainfall in the month prior (Log_{10}(Abundance)=2.69+0.0019 ($\pm$ 0.00071) * Rainfall, P = 0.0056) (Figure 5) and in the city of Yaounde, *A. aegypti* abundance peaked during the month of highest rainfall (Figure S3).

A. aegypti abundance was significantly correlated with the number of containers (Log_{10}(*A. aegypti* Abundance)=0.43+0.017 ($\pm$ 0.0059)*Containers, P = 0.0032) and larvae-positive containers (Log_{10}(*A. aegypti* Abundance)=1.13+0.041 ($\pm$ 0.010)*+Containers, P $\leq$ 0.001), but not the number of structures in a 100m radius (Log_{10}(*A. aegypti* Abundance)=4.85-0.0057 ($\pm$ 0.0035)*Structures, P = 0.10). *A. aegypti* abundance also increased with human abundance (Log_{10}(*A. aegypti* Abundance)=0.91+0.021($\pm$ 0.007) * Humans, P = 0.018). The time of collection had no significant effect (Log_{10}(*A. aegypti* Abundance)=1.17 -0.58 ($\pm$ 0.36) * Time, P = 0.10)

A. albopictus showed heterogeneity in its response to urbanization in the three cities where it was found (Figure 6). The 2km UI was the best performing measure of urbanization (UI_1km: ΔAIC = 4.86; UI_500m: ΔAIC = 10.40; UI_200m: ΔAIC = 8.24; UI_100m: ΔAIC = 4.13). In Yaounde, *A. albopictus* increased with urbanization, but it decreased with urbanization in Douala and there was no significant pattern in either direction in Kribi. *A. albopictus* abundance also increased with rainfall, and humidity (Figure 7); temperature had no significant effect (Table S7).

A. albopictus abundance was not significantly correlated with the number of larval containers (Log_{10}(*A. albopictus* abundance)=-6.74+0.0027 ($\pm$ 0.0040)*Containers, P = 0.49), larvae positive containers (Log_{10}(*A. albopictus* abundance)=-6.55+0.0038 ($\pm$ 0.0074)*+Containers, P = 0.60) or the number of structures in a 100m radius (Log_{10}(*A. albopictus* abundance)=3.76-0.0025 ($\pm$

0.0024)*Structures, P=0.29). *A. albopictus* abundance increased with human density (Log$_{10}$(*A. albopictus* abundance)=3.34+0.014 ($\pm$ 0.0027)*Humans, P $\leq$ 0.001). The time of collection had no significant effect (Log$_{10}$(*A. albopictus* Abundance)=3.022 +0.059 ($\pm$ 0.22) * Time, P = 0.79).

Combined abundance of *A. albopictus* and *A. aegypti* positively correlated with increased urbanization at the 2km radius in the three cities where both species coexist (Log$_{10}$(*Aedes* abundance)=-2.99+0.021 ($\pm$ 0.0035)*UI_2km, P $\leq$ 0.001). UI at 2km was the most parsimonious measurement for predicting the response of both species' abundance with urbanization (UI_1km: ΔAIC = 2.25; UI_500m: ΔAIC = 4.015; UI_200m: ΔAIC = 3.65; UI_100m: ΔAIC = 1.89).

The Urbanization Index at any radii was not significantly correlated with temperature (Table S8) or humidity (Table S9) in any of our study cities.

Pathogen Detection. We tested 1,660 *A. aegypti* and 7,771 *A. albopictus* females in 402 pools for chikungunya, dengue, and Zika viruses. Zika virus was found in one pool of 30 *A. albopictus* from Yaounde from June of 2021. No chikungunya or dengue virus was detected.

Indoor/Outdoor Abundance. No *A. aegypti* or *A. albopictus* were caught during indoor collections, while simultaneous outdoor collections caught a total of 28 and 397 respectively across all 10 outdoor surveillance sites.

Competition between species. There was no significant effect of *A. albopictus* abundance on the population growth rate $\log_{10}(\lambda)$ of *A. aegypti* nor was there a significant effect of *A. aegypti's* abundance on $\log_{10}(\lambda)$ for *A. albopictus* over the next month (Figure S4). These results remained qualitatively consistent when season and rainfall were added as predictors.

Discussion

We found that the effect of urbanization on mosquito abundance varied depending on the species of mosquito, but urbanization had a positive effect on combined *A. aegypti* and *A. albopictus* abundance. For *A. aegypti,* urbanization increased adult mosquito abundance consistently across the extent of Cameroon, which is broadly representative of the major ecotypes of sub-Saharan Africa and has vast climatic differences between the different cities in our study. We had predicted that the increase with urbanization would be smaller in the hottest, most arid environments, this hypothesis was not supported. We had based our hypotheses that *A. aegypti* abundance would differ in response to urbanization across cities based off of our expectation of finding a heat-island effect which would limit the benefits of urbanization in more northern cities, however we found no relationship between UI and temperature at the time of collection. We also found no support for competition among *A. aegypti* and *A. albopictus*, although our analysis was limited to just two sites in the city of Yaounde.

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We investigated several mechanisms by which urbanization could influence mosquito abundance, including larval habitat, host abundance, and microclimates. Urbanization increased both larval habitat and host abundance, but the GIS urbanization index was more strongly correlated with *A. aegypti* and *A. albopictus* abundance than all of these predictors, indicating that urbanization influenced abundance through additional pathways beyond those we measured. Furthermore, adult *A. albopictus* abundance was not significantly correlated with either metric of larval habitat measured in our study. Thus the variability of *A. albopictus's* varied response to urbanization across cities cannot be explained by differences in larval habitat or host availability between cities, nor can climatic factors explain this variation given that Kribi and Douala share a comparable coastal climate. In fact we have no mechanistic explanation for this pattern despite our study's robust collection of parameters thought to be associated with urbanization or mosquito abundance.

The spatial scale of urbanization that best correlated with mosquito abundance was relatively large, and varied between species (1km for *A. aegypti* and 2km for *A. albopictus*). This ran counter to our expectation that the 100m UI would correlate most strongly with abundance. These results indicate that urbanization affects populations on a scale larger than the typical dispersal distance of an individual mosquito. The spatial scales best correlated with other correlates of mosquito abundance varied as well, with containers and larvae positive containers best predicted by urbanization at 200m, while host availability was best correlated with urbanization at 500m.

During our study no Chikungunya or dengue virus was detected in either mosquito species, despite 41 documented concurrent human dengue cases in at least one city, Douala, at the time our study was conducted (Tchetgna et al. 2021). Zika virus was detected in a single pool from an urban area of Yaounde. A single detection precluded us from conducting any analysis on species infection rates or the possible effects of urbanization on disease positivity rates. Cameroon does not have active surveillance for human Zika cases. The most recent data on human incidence of Zika was a nationwide serological survey of 1,084 Cameroonian blood donors conducted in 2015 which identified Zika antibodies in 5% of the total population, with the city of Douala having the highest seroprevalence of 10% (Grake et al. 2015). Zika infection rates from our study were compared with Minimum Infection Rates (MIR) ([number of positive pools / total specimens tested] x 1,000) from prior entomological Zika surveillance studies of *A. aegypti* and *A. albopictus* (Table S10). Our results show an exceedingly low prevalence among Cameroonian *Aedes* compared to studies which detected Zika virus circulation in other regions.

Low rates of detection of entomological infection are typical for these pathogens and make studies of human disease risk difficult (Kuno et al. 1997; Scott & Morrison 2004). We tested a sizable sample of over 9,000 mosquitoes in 465 pools, which was a considerably greater number of mosquitoes tested compared with other studies which have used ~280-5,000 samples to detect dengue or chikungunya virus in *A. aegypti* and *A. albopictus* (Chow et al. 1998, Chung et al. 2002, Mendez et al. 2006, Edina et al. 2015, Vikram et al. 2015, Cevallos et al. 2018). Our large sample size and

no virus detection for Chikungunya or dengue and only one pool positive for Zika suggests that these viruses were either absent or circulating at low rates during the times and places we surveyed.

In Yaounde, we found no *A. aegypti* and *A. albopictus* during household surveys while simultaneous outdoor surveys captured numerous specimens. This supports prior research describing both species as exophilic (Kamgang et al. 2012). This calls for public health vector control measures that reduce human outdoor exposure to these mosquitoes in order to prevent arbovirus transmission. Based off of our findings, indoor measures to reduce mosquitoes feeding on humans, such as ITNs, are unlikely to have a significant effect on reducing human infections from *Aedes* transmitted arboviruses.

Africa is one of the most rapidly urbanizing regions on earth (Saghir & Santoro 2018). Our results, reflective of a broad range of habitats and climates across the African continent, suggest that *A. aegypti* populations will increase, while *A. albopictus* abundance will likely increase in some places and decrease in others. Furthermore, our results provide a more detailed investigation of the possible mechanisms underpinning urbanizations relationship to abundance. We found that while temperature and humidity could be important predictors of abundance, they were not significantly correlated with urbanization. Although increased larval habitat, host abundance, and the number of structures were significantly correlated with greater urbanization, they were less powerful predictors of adult mosquito abundance than the GIS based urbanization index. As such our results lead us to conclude that

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urbanization likely influences adult mosquito through mechanisms that have yet to be fully determined.

<u>Financial Support</u>
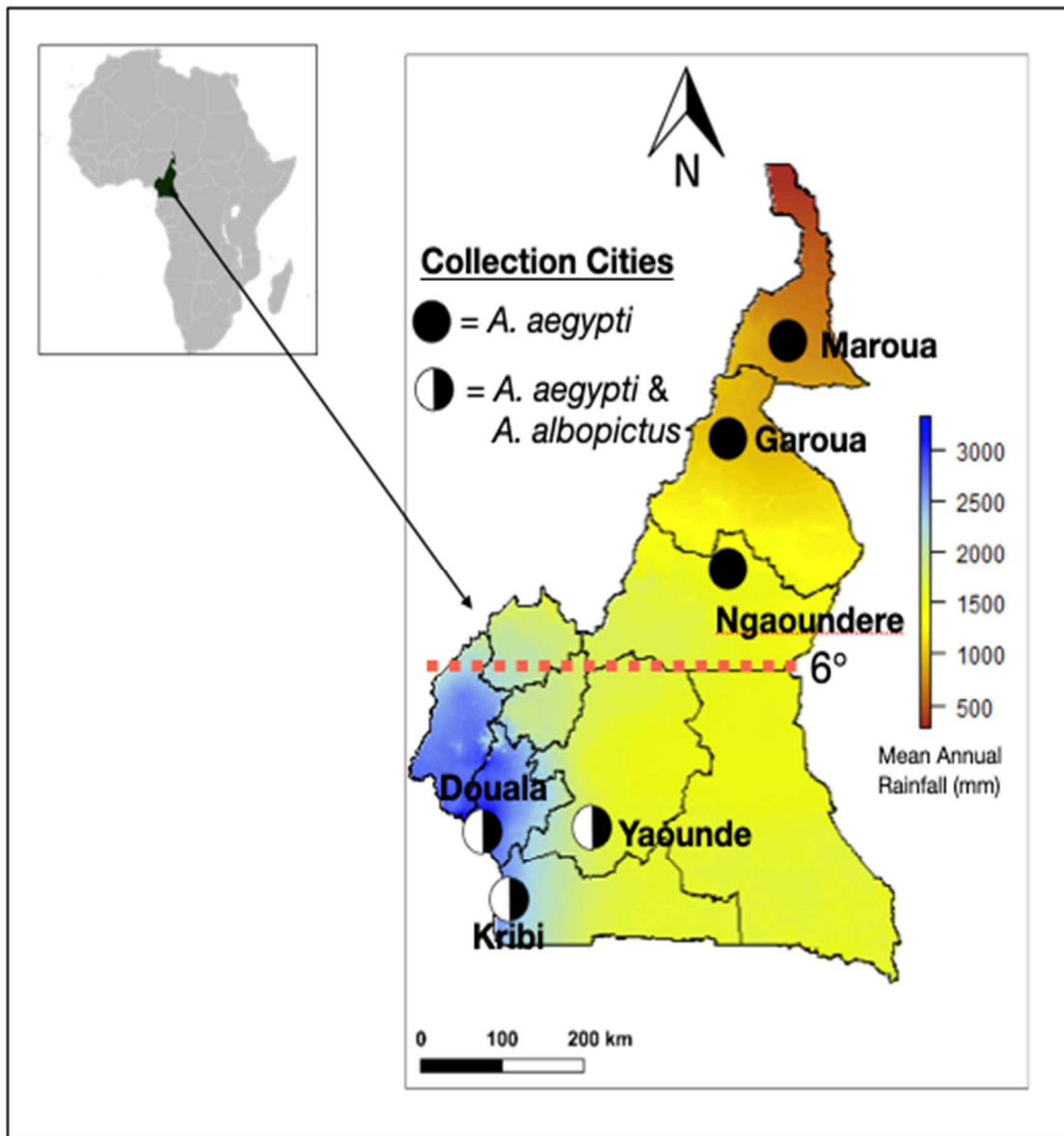
Figure 1.1. Cities surveyed for adult mosquitoes in Cameroon. Above approximately 6° latitude *A. albopictus* is not found.
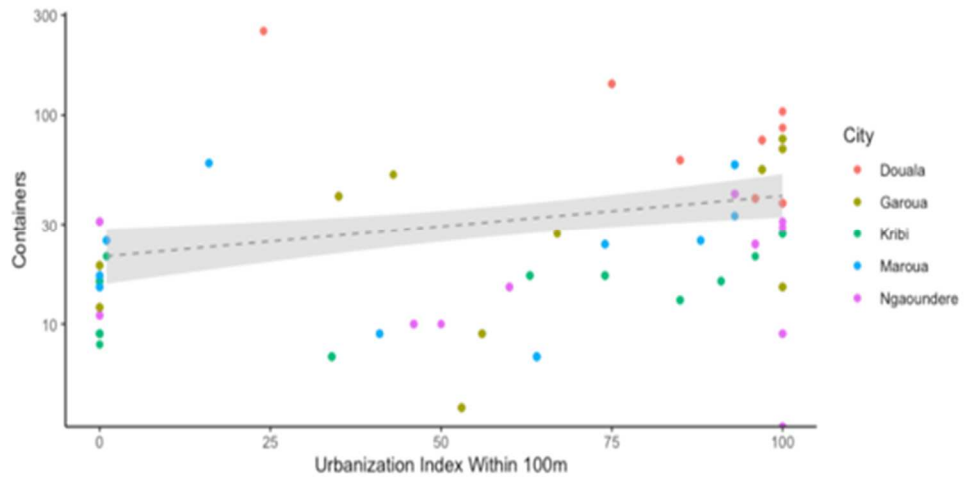
Figure 1.2. Larval habitat plotted against UI at 100m for five of the study cities.

Line and ribbon shows fitted models with UI at 100m as a predictor and 95%

CI, respectively.
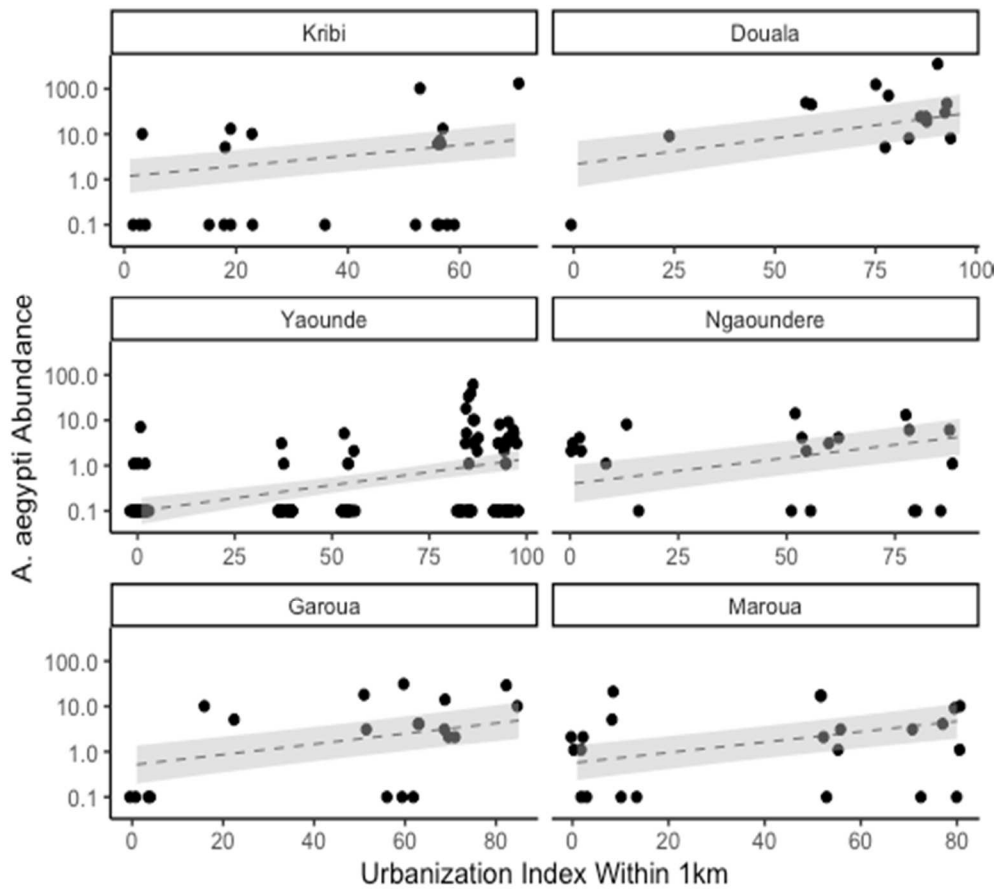
Figure 1.3. *A. aegypti* **abundance on a log scale plotted against the urbanization index in a 1km radius around the study site for ten sites in each of six cities. Collections resulting in zero specimens were plotted by adding 0.1. Lines and ribbons show fitted models with UI at 1km as a predictor and 95% CI, respectively.**
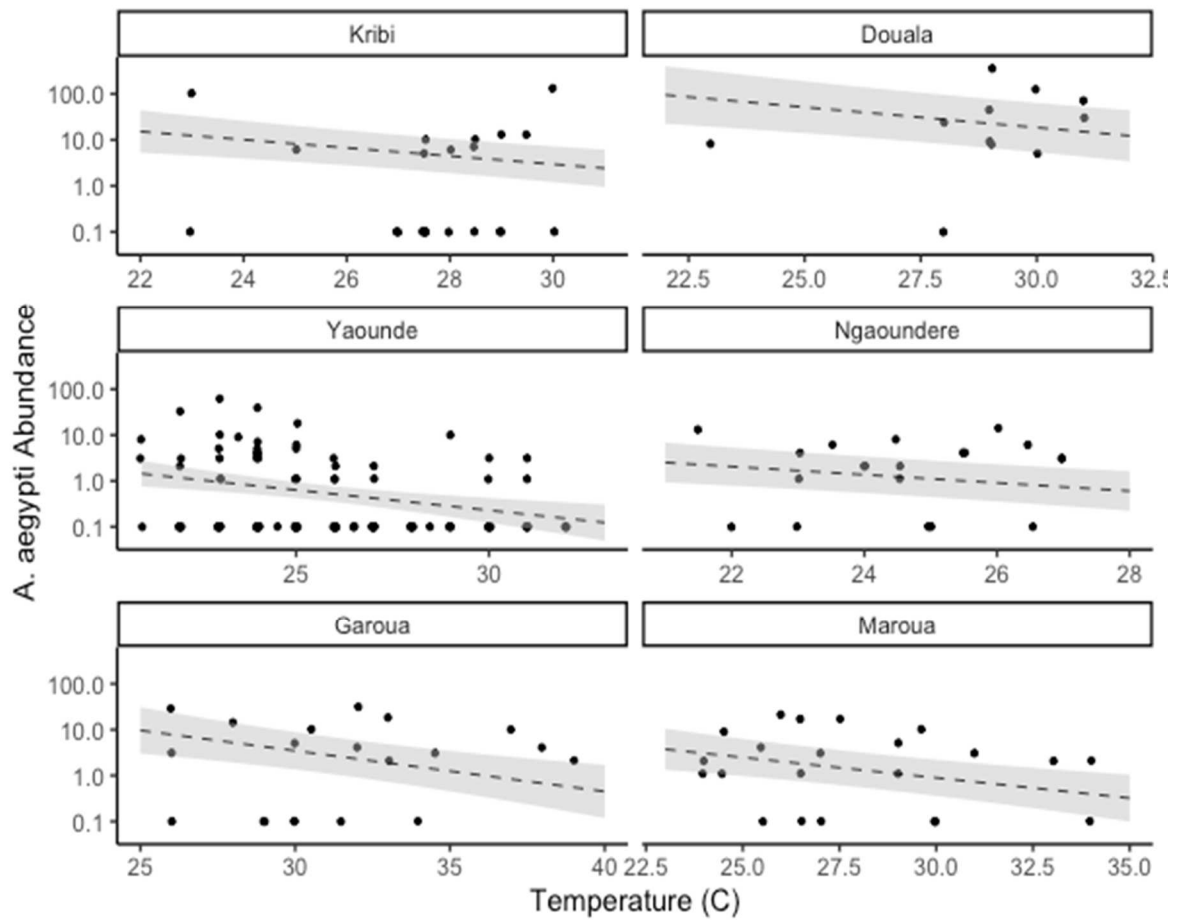
Figure 1.4. *A. aegypti* **abundance on a log scale plotted against temperature at the time of collection. Collections resulting in zero specimens were plotted by adding 0.1. Lines and ribbons show fitted models with temperature as predictor and 95% CI, respectively.**
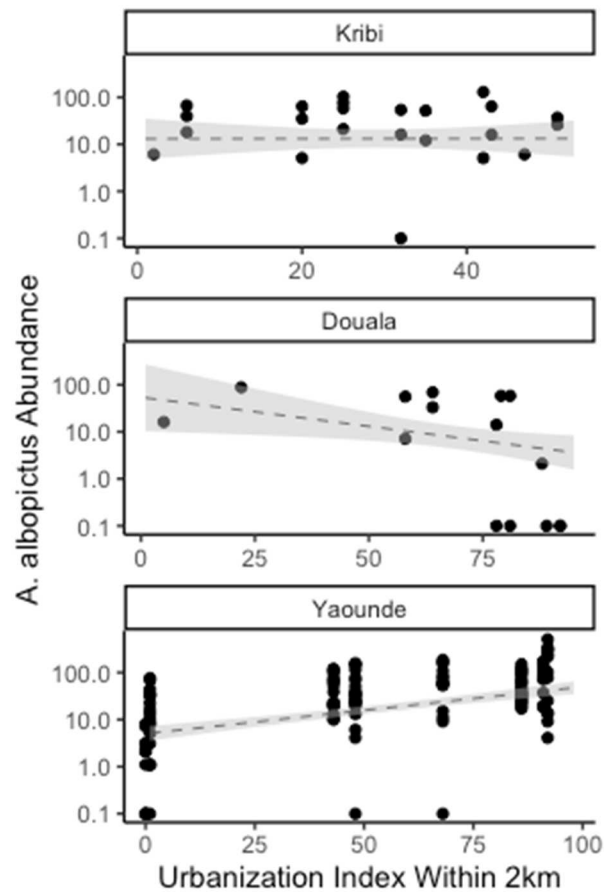
Figure 1.5. *A. albopictus* **(95% CI) abundance on a log scale plotted against the urbanization index in a 2km radius around the study site for ten sites in each of three cities where** *A. albopictus* **was found. Lines and ribbons show fitted model with UI as predictor and 95% CI, respectively. The slope of urbanization in Kribi was not significantly different from zero. Collections resulting in zero specimens were plotted by adding 0.1.**
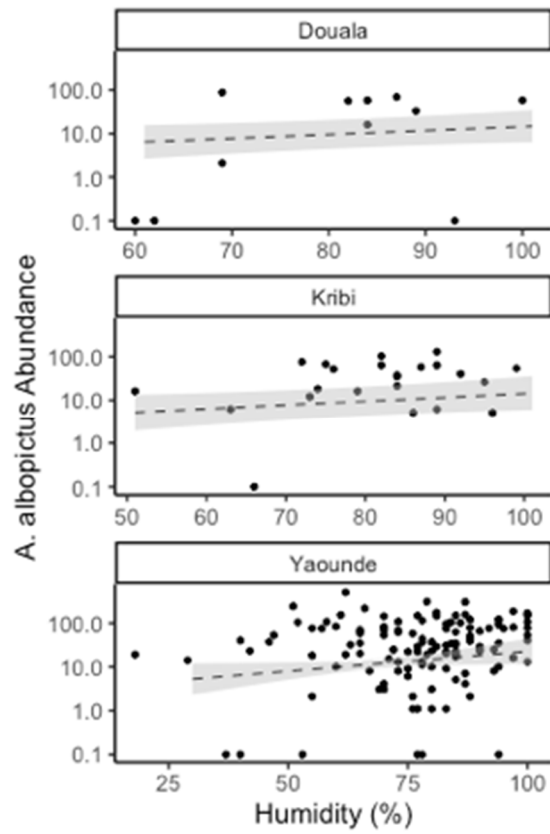
Figure 1.6. *A. albopictus* **abundance on a log scale plotted against relative humidity at the time of collection. Collections resulting in zero specimens were plotted by adding 0.1. Lines and ribbons show fitted models with humidity as predictor and 95% CI, respectively.**
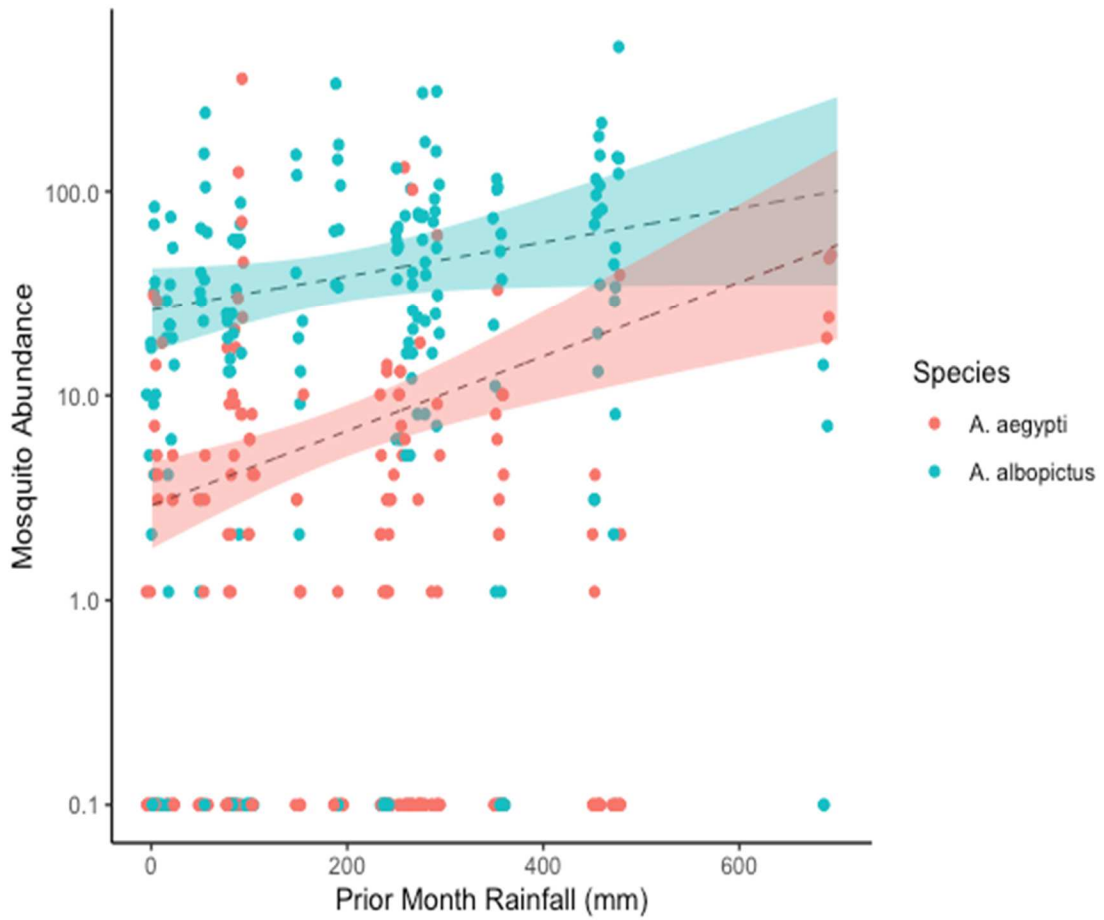
Figure 1.7. Total *A. aegypti* and *A. albopictus* abundance in response to prior month's rainfall across all sites. Collections resulting in zero specimens were plotted by adding 0.1. Lines and ribbons show fitted models with prior month rain interacting with species as predictor and 95% CI, respectively.

Figure 1.8. Locations of studies of *A. albopictus* abundance in response to urbanization. Numbers correspond to study referenced in Table S2.

Spatial distribution of insecticide resistant populations of *Aedes aegypti* and *Ae. albopictus* and first detection of V410L mutation in *Ae. aegypti* from Cameroon

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Abstract

Background: Dengue (DENV), chikungunya (CHIKV) and Zika virus (ZIKV), are mosquito-borne viruses of medical importance in most tropical and subtropical regions. Vector control, primarily through insecticides, remains the primary method to prevent their transmission. Here, we evaluated insecticide resistance profiles and identified important underlying resistance mechanisms in populations of *Aedes aegypti* and *Ae. albopictus* from six different regions in Cameroon to pesticides commonly used during military and civilian public health vector control operations.

Methods: *Aedes* mosquitoes were sampled as larvae or pupae between August 2020 and July 2021 in six locations across Cameroon and reared until the next generation, G1. *Ae. aegypti* and *Ae. albopictus* adults from G1 were tested following World Health Organization (WHO) recommendations and *Ae. aegypti* G0 adults screened with real time melting curve qPCR analyses to genotype the F1534C, V1016I and V410L *Aedes kdr* mutations. Piperonyl butoxide (PBO) assays and real time qPCR were carried out from some cytochrome p450 genes known to be involved in metabolic resistance. Statistical analyses were performed using Chi-square test and generalized linear models.

Results: Loss of susceptibility was observed to all insecticides tested. Mortality rates from tests with 0.25% permethrin varied from 24.27 to 85.89% in *Ae. aegypti* and from 17.35% to 68.08% in *Ae. albopictus*. Mortality rates for 0.03% deltamethrin were between 23.30% and 88.20% in *Ae. aegypti* and between 69.47 and 84.11% in *Ae. albopictus*. We found a moderate level of resistance against bendiocarb, with mortality rates ranging from 69.31% to 90.26% in *Ae. aegypti* and from 86.75 to 98.95% in *Ae. albopictus*. With PBO pre-exposure, we found partial or fully restored susceptibility to pyrethroids and bendiocarb. The genes *Cyp9M6F88/87* and *Cyp9J10* were overexpressed in *Ae. aegypti* populations from Douala sites resistant to permethrin and deltamethrin. *Cyp6P12* was highly expressed in alphacypermethrin and permethrin resistant *Ae. albopictus* samples. F1534C and V1016I mutations were detected in *A. aegypti* mosquitoes and for the first time V410L was reported in Cameroon.

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Conclusions: This study revealed that *Ae. aegypti* and *Ae. albopictus* are resistant to multiple insecticide classes with multiple resistance mechanisms implicated. These findings could guide insecticide use to control arbovirus vectors in Cameroon.

Keywords: Arbovirus, *Aedes aegypti, Aedes albopictus*, Insecticide resistance, Knock down resistance, Cytochrome P450 monooxygenases, Cameroon

Background

Aedes-borne viral diseases such as dengue, chikungunya and Zika are increasingly reported in different regions across the world, including Africa [1]. For dengue, approximately half the world's population is at risk with an estimated 100–400 million new infections reported each year [1]. Vector control remains the cornerstone to prevent and fight against transmission. Use of insecticide based-control strategies is a common approach in response to arboviral disease outbreaks in order to quickly lower vector density and ongoing transmission [2], and is often the primary strategy to mitigate the threat of dengue, chikungunya, and Zika during military operations [3]. However, the increased use of insecticides may result in the selection of mosquitoes that carry genetic traits associated with insecticide resistance. Indeed, resistance to multiple insecticides used in public health has been documented in *Aedes aegypti* and *Ae. albopictus* in different locations across the world [4].

Insecticide resistance is usually achieved through two mechanisms: increased detoxification (metabolic resistance) and target insensitivity (target site resistance). Metabolic resistance through upregulation of detoxification genes is a common resistance mechanism in *Ae. albopictus* and *Ae. aegypti*, caused primarily by three main enzyme families, the monooxygenases (cytochrome P450s), glutathione S-transferases (GSTs) and carboxylesterases (COEs) [5, 6]. Target site resistance is caused by mutations in target genes such as acetylcholinesterase (*Ace-1*), the *GABA* receptor, and the voltage-gated sodium channel (*VGSC*) causing knockdown resistance (*kdr*) [5, 6].

Knockdown resistance is one of the main target site adaptations for both pyrethroids and dichlorodiphenyltrichloroethane (DDT). In *Ae. albopictus*, the *kdr* mutation is less prevalent with only four voltage-gated sodium channel (VGSC) mutations detected affecting two codons (1532 and 1534). In *Ae. aegypti*, many *kdr* mutations have been reported [4, 7, 8]. Among these mutations, the V1016I, F1534C and V410L mutations have been extensively investigated in pyrethroid-resistant *Ae. aegypti* populations from Asia, South America and, to a lesser extent, Africa. In Cameroon F1534C and V1016I mutations have been previously reported in *Ae.*

aegypti [9, 10]. V410L is a novel *kdr* mutation, located in domain I of segment 6 of the VGSC. It was described for the first time in a pyrethroid-resistant *Ae. aegypti* laboratory strain originating from Rio de Janeiro, Brazil [7] and several years later in Africa [11]. Considering that this mutation has shown a potential to reduce the sensitivity of sodium channels to type I and II pyrethroids and to increase resistance when associated with F1534C [12], it is imperative to determine the current distribution of this mutation in natural *Ae. aegypti* populations in Cameroon. This is due to the fact that its presence can greatly impair the usefulness of a wide variety of pyrethroid insecticides, which currently constitute the major class of insecticides used in *Aedes* control [13, 14]. In addition, several cytochrome P450 genes have been found overexpressed in *Aedes* spp. populations tested in the field and demonstrating pyrethroid resistance, with evidence indicating a role in pyrethroids metabolism by *CYP9J28*, *CYP9J10, CYP9J26, Cyp9M6F88/87* and *CYP6P12* [4, 15–19].

Our goal was to quantify the insecticide resistance profile and mechanisms involved in the resistance of populations of *Ae. aegypti* and *Ae. albopictus* from six cities that span most of the 1000 km north–south extent of Cameroon. In Cameroon, *Ae. aegypti* is present across the country, while the distribution of *Ae. albopictus* is limited to the south, under 6°N [20, 21]. We selected insecticides commonly used for public health and military force health protection applications to mitigate disease threats.

Methods

Mosquito sampling and rearing

Aedes mosquitoes were sampled as larvae or pupae between August 2020 and July 2021 in six locations across Cameroon: Douala (N 04° 02′ 729″, E 09° 42′ 142″), Maroua N 10° 37′ 284″, E 14° 18′ 381″), Garoua (N 09° 17′ 7776″, E 13° 23′ 288″), Ngaoundéré N 07° 35′ 414″, E 13° 57′ 365″, Yaoundé (N 03° 51′ 993″, E 011° 27′ 688″), and Kribi (N 02° 56′ 862″, E 09° 54′ 003″) (Fig. 1). Immature stages (field generation, G0) were collected from different potential larval habitats: domestic (e.g., jars, tanks), peri-domestic (e.g., used tires, discarded tanks), and natural (e.g., tree holes). In each location, larvae, or pupae from 20 positive larval habitats were collected, stored in plastic boxes, and transferred to insectary then pooled

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according to the location and land use category (urban and suburban) and reared to adult stage for identification using taxonomic keys [22, 23]. Mosquitoes identified as *Ae. albopictus* or *Ae. aegypti* were blood-fed and allowed to reproduce to generation G1 for adult bioassays. Mosquito populations were maintained at insectary conditions (temperature 27 ± 2 °C; relative humidity $80\% \pm 10\%$), and females were blood fed using hemotek membranes to complete their gonotrophic cycle. Two susceptible lab strains were used for this study: Vector Control Research Unit (VCRU) strain for *Ae. albopictus* and Benin lab stain for *Ae. aegypti*.

Insecticide resistance bioassays
Bioassays were carried out according to the World Health Organization (WHO) protocol using 3–5 days old G1 generation mosquitoes. Four replicates of 20–25 females per tube were exposed to 0.03% and 0.05% deltamethrin, 0.05% alphacypermethrin, 0.1% bendiocarb, and 0.25% or 0.75% permethrin for 1 h. Mortality was recorded 24 h later; mosquitoes, alive or dead, after exposure were stored in RNA later or silica gel, respectively. Mortality rates were interpreted according to WHO recommendations: mortality rate of 98% or above indicates susceptibility, mortality rate between 90 and 97% suggests a possibility for resistance that should be confirmed with further bioassays, and mortality rate less than 90% indicates confirmed resistance [14].

Adult synergist assay with PBO
To evaluate the effectiveness of insecticide detoxification by the mosquitoes, the specific enzyme inhibitor 4% piperonyl butoxide (PBO) was evaluated to address the potential role of P450s in insecticide resistance. Female adults, 3–5 days old, were pre-exposed for one hour to PBO-impregnated papers and then immediately exposed to the selected insecticide. Mortality was scored 24 h later and compared to the results obtained with each insecticide without synergist according to the WHO standards [14].

Knockdown resistance (*kdr*) genotyping
Approximately 30 mosquitoes per city were genotyped for three different *kdr* mutations: V1016I, V410L and F1534C. Genomic DNA of 30 individual mosquitoes per population was extracted using the Livak protocol [24]. Three kdr mutations (F1534C, V1016I and V410L) were chosen for this study because these mutations have been described as involved in the pyrethroids resistance of *Ae. aegypti* mosquito [4, 25]. These mutations have been also reported in Africa [11, 15, 26]. Based on Moyes et al., review, F1534C and V410L are associated to insecticide resistance and V1016I is associated to insecticide

resistance when combined to other *kdr* mutations. Genotyping of the V1016I, V410L and F1534C mutations was performed by real-time melting curve quantitative PCR [27]. Each PCR reaction was performed in a 21.5 µl volume PCR tube containing 2 µl of DNA sample, 10 µl of SYBR® Green (SuperMix), and 1.25 µl of each primer. The thermocycle parameters were: 95 °C for 3 min, followed by 40 cycles of (95 °C for 20 s, 60 °C for 1 min and 72 °C for 30 s) and then a final step of 72 °C for 5 min, 95 °C for 1 min, 55 °C for 30 s and 95 °C for 30 s.

Gene expression
RNA extraction and cDNA synthesis
For this experiment three groups of mosquitoes were used: unexposed (control) to insecticide individuals from G1, exposed G1 individuals that survived resistance assays (resistant), and susceptible (laboratory susceptible strains). For each group three replicates of 10 mosquitoes per species were performed. RNA was extracted using the PicoPure RNA Isolation Kit (Arcturus® Picopure RNA Extraction Kit Life Technologies, California, USA), following the manufacturer's recommendations. Quality and quantity of RNA obtained were assessed using a "NanoDrop Lite" spectrophotometer (Thermo Scientific Inc., Wilmington, USA) and stored at -80 °C.

Extracted RNA was used to synthesize complementary DNA (cDNA) using the Superscript III kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The resulting cDNA was purified using a QIAquick spin column (QIAuick PCR Purification Kit, Qiagen) and diluted 2-fold to accommodate the volumes of the reaction.

Quantitative-reverse transcriptase PCR
Candidate genes for expression analysis were chosen based on previous implications of involvement in metabolic resistance [4, 17]. Standard curve analyses were performed for each primer pair to check the specificity and efficiency of amplifications. Four cytochrome P450 candidate genes were chosen for analysis in *A. aegypti* (*Cyp9M6F88/87*, *Cyp9J28a*, *Cyp9J10,* and *Cyp9J32*) and only one in *A. albopictus* (*Cyp6P12*). The reactions were performed in a volume of 20 µl with 10 µl sybrGreen (Applied Biosystems, Texas, USA), 0.6 µl of each primer (10 µm), 7.8 µl of ddH2O, and 1 µl of CDNA, under the following conditions: 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 10 s. The relative expression level and fold change (FC) of each candidate gene compared to susceptible strains were calculated using the 2-ΔΔCT method, integrating the efficiency of the PCR [17] after normalization with housekeeping genes: Aaeg60sL8, RPF7, RSP7, and tubulin. All primer

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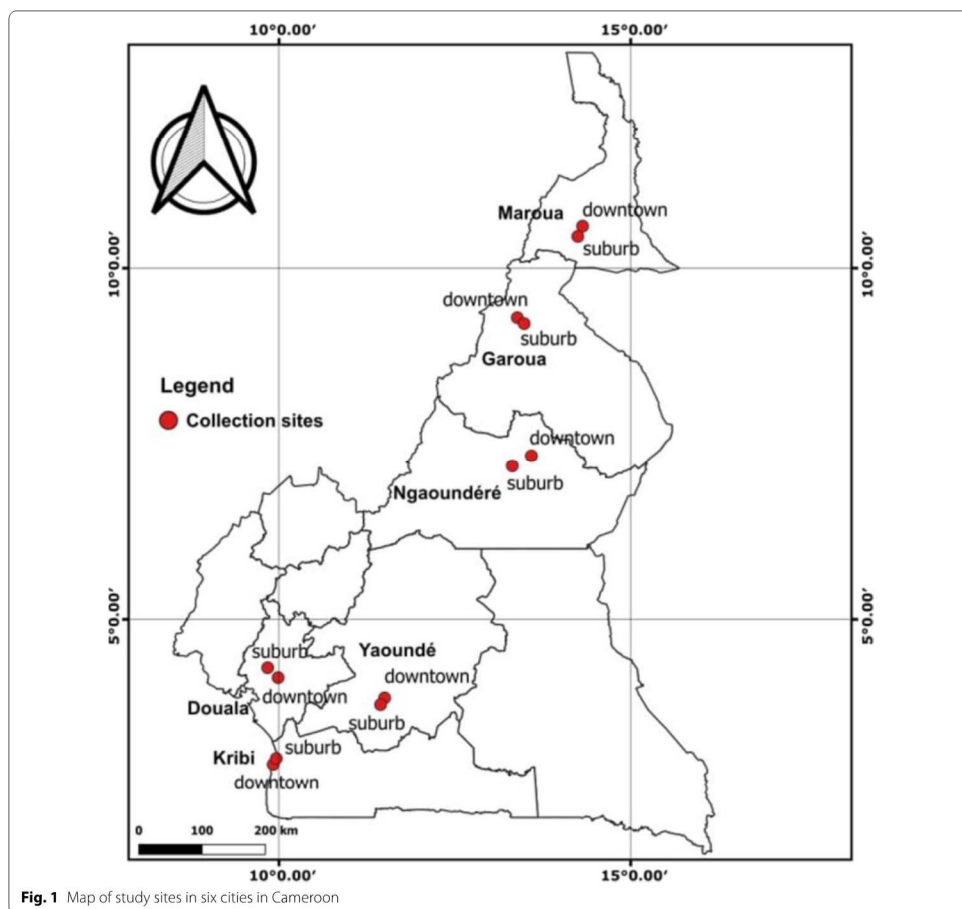
Fig. 1 Map of study sites in six cities in Cameroon

sequences and their origins are shown for *A. albopictus* and *A. aegypti* in Tables 1 and 2, respectively. The Mx Pro software integrated into the Agilent brand TaqMan machine was used (MxPro-Mx3005P v4.10, Stratagene, California, USA).

Data analysis

Comparisons of mortality rates between species and land use category were conducted using generalized linear models (GLM) with a binomial distribution and logit link function using the *lme4* package in R version 3.6.1 (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

The comparison of mortality rate after pre-exposure of mosquitoes to synergist and without pre-exposure to synergist was performed using a Chi-square test. The relative expression level and fold change (FC) of each target gene in field samples relative to the susceptible Benin (*Ae. aegypti*) or VCRU (*Ae. albopictus*) were calculated according to the $2^{-\Delta\Delta^{CT}}$ method incorporating the PCR efficiency after normalization with the housekeeping genes. This analysis was performed using GraphPad Prism 8.0.2 (GraphPad Software, San Diego, California, USA). *P*-value < 0.05 was considered as statistically different.

Results

Insecticide resistance profile for *Aedes aegypti*

In total we tested 10 populations of *Ae. aegypti* with four insecticides (Fig. 2). Susceptibility for three pyrethroid varied among locations. To 0.25% permethrin, high resistance was observed with mortality rates ranging from 24.27% in urban Kribi to 85.89% in urban Ngaoundéré, probable resistance in urban Maroua (mortality rate of 95.43%); susceptibility in suburban Ngaoundéré, urban Garoua, suburban Garoua, and suburban Maroua (mortality rate from 97.82 to 98.80%). Remarkably, even when using 0.75% permethrin, which is triple the dose recommended for *Aedes* control, high levels of resistance were still observed. For example, the mortality rates to the 0.75% permethrin were 5.83% in urban Douala, and 40.48% in suburban Douala. For 0.03% deltamethrin, resistance was found in urban Yaoundé, urban Kribi, urban Ngaoundéré, urban and suburban Douala (mortality rates varying from 23.30% in urban Douala to 88.20% in urban Yaoundé); probable resistance in urban Maroua and susceptibility in suburban Ngaoundéré, urban Garoua, suburban Garoua, and suburban Maroua (mortality rate varying from 98.80% in suburban Maroua to 100% in suburban Ngaoundéré). Six of 10 populations tested were resistant to alphacypermethrin (urban Yaoundé, urban Kribi, urban Ngaoundéré, urban Maroua, and urban and suburban Douala); three others exhibited probable resistant (suburban Ngaoundéré, urban Garoua, and suburban Maroua) and the last one was fully susceptible (suburban Garoua). A moderate level of resistance was reported against bendiocarb with mortality rates varying from 69.31% in urban Yaoundé to 90.26% in suburban Maroua. In addition, probable resistance was detected in six populations with mortality rates ranging from 91.79% in Douala suburban to 95.50% in Ngaoundéré urban. Only the population from urban Maroua was susceptible to bendiocarb (mortality rate of 98.91%).

The best fitting model for predicting insecticide mortality in *Ae. aegypti* was a triple interaction of environment (urban or suburban), city, and insecticide (Additional file 1). On average across all cities and insecticides *A. aegypti* from urban populations had lower mortality to insecticides than those from suburban habitat ($P < 0.01$) (Fig. 3). Across all cities and environments bendiocarb caused significantly higher levels of mortality compared to alphacypermethrin ($P < 0.01$), deltamethrin ($P < 0.01$), and 0.75% permethrin ($P < 0.01$). However, these simple patterns had exceptions; in Yaoundé Bendiocarb had the lowest mortality rates and insecticide mortality was lower in suburban Garoua versus urban Garoua (Fig. 3).

Overall mortality to insecticides showed a strong latitudinal gradient, with higher mortality rates in northern cities (Fig. 3).

Insecticide resistance profile for *Aedes albopictus*

Five *Ae. albopictus* populations were tested (urban Yaoundé, suburban Yaoundé, urban Kribi, suburban Douala, and suburban Kribi). The results are presented in Fig. 4. The analysis showed that resistance or probable resistance was observed to the three pyrethroids used. All populations tested with 0.25% permethrin showed high resistance with mortality rates between 17.35% (urban

Table 1 Primer sequences for the evaluation of the level of expression of metabolic resistance genes by RT-qPCR and their origins in *Aedes albopictus*

Genes	Forward primer	Reverse primer	References
Cyp6P12	CGTGCGCTTTTGGGATTGAG	ATCGTCCGTGCCAAATCCTT	[17]
RSP7	AAGGTCGACACCTTCACGTC	CGCGCGCTCACTTATTAGAT	[17]
qTubulin	CCGCACTCGAGAAGGATTAC	GTGGTTCGGTTTGACTTCGT	[17]

Table 2 Primer sequences for the evaluation of the level of expression of metabolic resistance genes by RT-qPCR and their origins in *Aedes aegypti*

Genes	Forward primer	Reverse primer	References
Cyp9J10	ATCGGTGTTGGTGAAAGTTCTGT	CATGTCGTTGCGCATTATCCC	[46]
Cyp9J28	CCACTGACGTACGATGCGA	GCCGATCAGTGGACGGAGC	[17]
Cyp9M6	TCGGTGCACAATCCAAACAAC	GTCGGGTACGACCAACGAAA	[18]
Cyp9J32	CGGTCCGCTTATGACGAAGAG	TTTGTTCGCTCCGAAGAGTGG	[47]
RPS3	AGCGTGCCAAGTCGATGAA	GTGGCCGTGTCGACGTACT	[18]
Ae60sL8	CTGAAGGGAACCGTCAAGCAA	TCGGCGGCAATGAACAACT	[17]

Fig. 2 Mortality rates of adult *Aedes aegypti* from urban and suburban habitats in six cities in Cameroon 24 h after exposure to insecticides alone or with 1 h preexposure to synergist. Error bars represent standard error of the mean. PBO: Piperonyl butoxide

Yaoundé) and 81.15% (suburban Douala). When the dose of permethrin was increased to 0.75%, some recovery of susceptibility was restored. In urban Yaoundé, the 0.25% permethrin mortality rate was 17.35%, but when 0.75% permethrin was used the mortality rate increased to 91.59%. Two of five populations were found resistant to deltamethrin; urban Yaoundé with mortality rate of 69.47%, and suburban Douala with mortality rate of 84.11%, whereas the three others were shown to exhibit probable resistance (suburban Yaoundé, urban Kribi, and suburban Kribi). To alphacypermethrin, resistance was observed in suburban Douala with mortality rate of 71.21%, urban Yaoundé with mortality rate of 78.63%, and suburban Yaoundé with mortality rate of 89.40%; probable resistance was found in urban Kribi with mortality rate of 92.70%, and suburban Kribi with mortality rate of 95.17%. Moderate level of resistance was reported to bendiocarb with mortality rates varying from 86.75% in suburban Kribi to 98.95% in urban Yaoundé. Environment was not a statistically significant predictor for resistance in *Ae. albopictus.*

In cities where both *Ae. albopictus* and *Ae. aegypti* occur sympatrically (Douala, Kribi, and Yaoundé) *Ae. aegypti* had significantly higher levels of resistance than *Ae. albopictus.* The overall mean mortality to the insecticides standard doses tested were 54.7% and 80.1% for *Ae. aegypti* and for *Ae. albopictus* from sympatric cities $(P < 0.02, \chi^2 = 6.61, df = 1)$.

Tests with synergist PBO

A partial or full recovery of susceptibility to insecticides (permethrin, alphacypermethrin, deltamethrin and bendiocarb) was reported after PBO pre-exposure for both species (Figs. 2, 4).

For *Ae. albopictus* samples, a partial recovery of susceptibility was reported to permethrin from populations in urban Yaoundé (17.35% mortality without PBO and 82.54% after PBO exposure $P < 0.001$), suburban Douala (81.15% mortality without PBO and 99.1% after PBO exposure $P > 0.05$), and urban Kribi (68.08% mortality without PBO and 83.21% after PBO exposure $P > 0.1$). Susceptibility was partially recovered to alphacypermethrin in *Ae. albopictus* populations from urban Yaoundé (78.63% mortality without PBO and 96.36% after PBO exposure $P > 0.05$), suburban Douala (71.21% mortality without PBO and 95.94% after PBO exposure $P > 0.01$), and to bendiocarb in populations from suburban Yaoundé (88.18% mortality without PBO and 98.9% after PBO exposure $P > 0.25$). Full susceptibility recovery was reported in urban Yaoundé (69.47% mortality without PBO and 100% after PBO exposure $P < 0.005$) and to alphacypermethrin in suburban Yaoundé 89.40% mortality without PBO and 100% after PBO exposure $P > 0.25$.

For *Ae. aegypti* samples, a partial recovery of susceptibility was reported to permethrin in populations from urban Yaoundé (78.32% mortality without PBO and
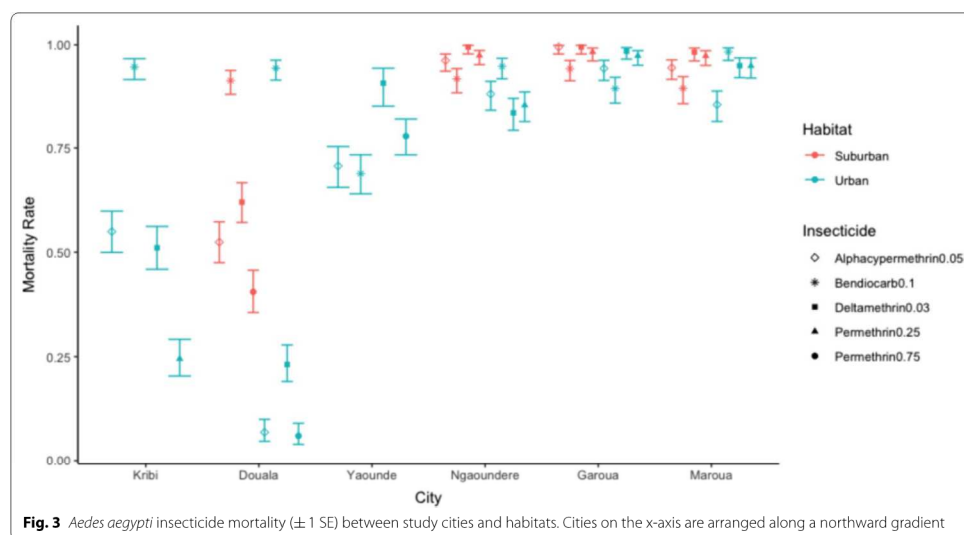
Fig. 3 *Aedes aegypti* insecticide mortality (± 1 SE) between study cities and habitats. Cities on the x-axis are arranged along a northward gradient

92.18% after PBO exposure $P > 0.1$), suburban Douala (40.48% mortality without PBO and 62.13% after PBO exposure $P < 0.01$) and urban Ngaoundéré (85.89% mortality without PBO and 93.25% after PBO exposure $P > 0.5$). Also, susceptibility was partially recovered to alphacypermethrin in *Ae. aegypti* populations from urban Yaoundé (70.71% mortality without PBO and 95.45% after PBO exposure $P > 0.01$), suburban Douala (52.27% mortality without PBO and 54.27% after PBO exposure $P > 0.75$) and urban Ngaoundéré (88.52% mortality without PBO and 98.80% after PBO exposure $P > 0.25$), to deltamethrin in populations from urban Yaoundé (88.20% mortality without PBO and 98.03% after PBO exposure $P > 0.25$), suburban Douala (62.52% mortality without PBO and 68.83% after PBO exposure $P > 0.25$) and urban Ngaoundéré (83.77% mortality without PBO and 98.86% after PBO exposure $P > 0.1$), and to bendiocarb in populations from suburban Douala (91.61% mortality without PBO and 97.79% after PBO exposure $P > 0.5$).

Gene expression
Using qPCR, expression of four cytP450 genes were quantified in *Ae. aegypti* (*Cyp9M6F88/87*, *Cyp9J28a*, *Cyp9J10,* and *Cyp9J32*). Among them, two were significantly overexpressed in field populations compared to a susceptible lab strain (Fig. 5). *Cyp9M6F88/87* was overexpressed in urban Douala sample resistant to permethrin [(fold change (FC) $= 2.54 \pm 0.90$, $P = 0.016$)] and suburban Douala samples resistant to deltamethrin

(FC $= 5.49 \pm 1.64$, $P = 0.003$); and *Cyp9J10* was overexpressed in suburban Douala (FC $= 3.16 \pm 0.40$, $P = 0.013$).

Only one gene (*Cyp6P12*) was assessed in *Ae. albopictus* populations. This gene was significantly more expressed in urban Douala samples resistant to permethrin, compared to a lab susceptible strain (FC $= 5.54 \pm 0.73$, $P = 0.001$) and in urban Yaoundé samples resistant to alphacypermethrin (FC $= 2.48 \pm 0.57$, $P = 0.034$). However, in urban Douala samples resistant to deltamethrin, *Cyp6P12* expression was not significantly different compared to susceptible strain (Fig. 6).

Knockdown resistance (*kdr*) genotyping in *Ae. aegypti*
In this study, three *kdr* mutations were genotyped: V1016I, F1534C and V410L. The results are given in Tables 3, 4 and 5.

For V1016I *kdr* genotyping, 296 samples were examined in total. Among them, 227 (76.69%) were susceptible (1016 V/V), 59 (19.93%) were heterozygote resistant (1016 V/I), and 10 (3.38%) were homozygote resistant (1016I/I). The allele frequencies were 86.66% and 13.34% for alleles V and I respectively.

We examined 296 samples for F1534C genotyping. Among them, 170 (57.43%) were susceptible (1534F/F), 18 (6.08%) were heterozygote resistant (1534F/C), and 108 (36.49.05%) were homozygote resistant (1534C/C). The allele frequencies were 60.47% and 39.53% for alleles
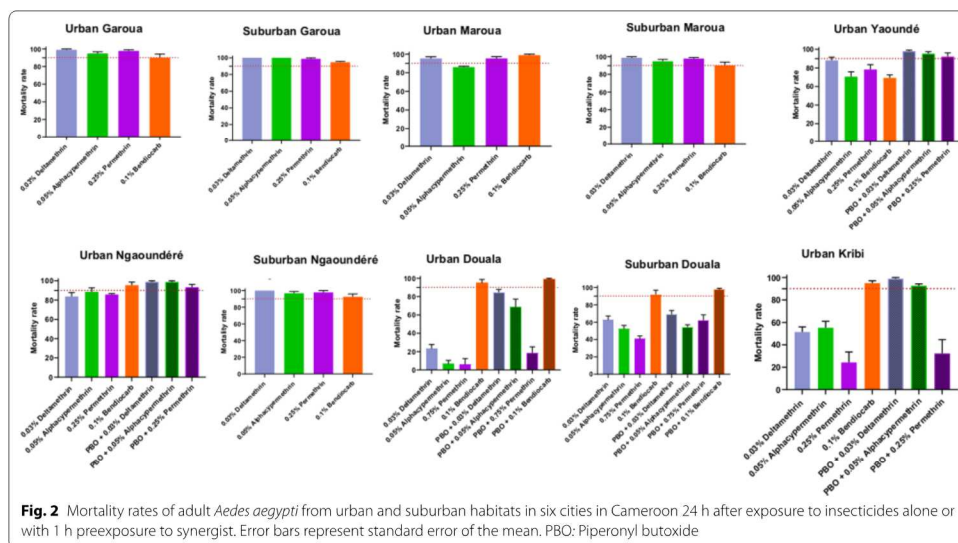
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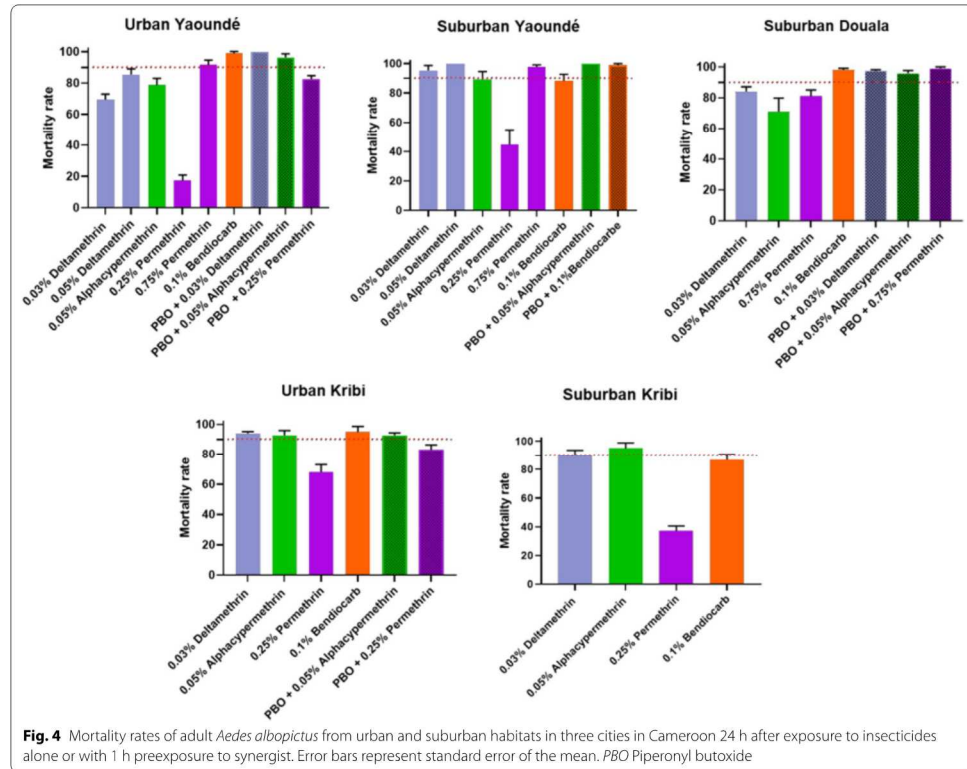
Fig. 4 Mortality rates of adult *Aedes albopictus* from urban and suburban habitats in three cities in Cameroon 24 h after exposure to insecticides alone or with 1 h preexposure to synergist. Error bars represent standard error of the mean. *PBO* Piperonyl butoxide

F and C, respectively. C allele frequency had increased from levels first found in 2017 in 5 of the studied cities (Fig. 7).

We genotyped 299 samples for V410L *kdr* and 212 of samples (70.91%) were susceptible (410 V/V), 59 (19.73%) were heterozygote resistant (410 V/I), and 28 (9.36%) were homozygote resistant (410I/I). The allele frequencies were 80.77% and 19.23% for alleles V and L, respectively.

Discussion

The aim of this study was to evaluate insecticides resistance profiles of *Ae. albopictus* and *Ae. aegypti* collected in several locations in Cameroon and explore the potential mechanisms. Analyses revealed resistance to all four insecticides tested (deltamethrin, alphacypermethrin, permethrin, and bendiocarb) for both *Aedes* species. While the loss of susceptibility to these insecticides in *Aedes* was previously reported in some locations in

Cameroon [10, 28, 29] and outside Africa [30–33], this study expanded the spatial scale of insecticide resistance assessment in Cameroon while simultaneously comparing two species. The cause of resistance to insecticides in both *Ae. aegypti* and *Ae. albopictus* remains unclear as the use of insecticides against *Aedes* is scarce in African countries [10, 15, 30]. Nonetheless alleles, such as F1534C, that confer insecticide resistance continue to increase in frequency over time indicating selective pressure towards greater insecticide resistance. Given that the source of selective pressure for resistance is unknown, a return to susceptibility seems improbable [15] and could have operational consequences. The reduced susceptibility to the pyrethroids tested may pose a serious threat to future vector control programs, because pyrethroids are recommended for the control of adult *Aedes* mosquitoes, especially in case of disease outbreaks [34], and are commonly used in insecticide treated nets and uniforms.

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Fig. 5 Expression of the *CYP9M6F88/87, CYP9J28a, CYP9J10, CYP9J32* genes in samples of *Aedes aegypti* from urban Yaoundé and urban and suburban Douala

Fig. 6 Expression of the *CYP6P12* gene in samples of *Aedes albopictus* from urban Yaoundé, suburban Kribi, and urban Douala
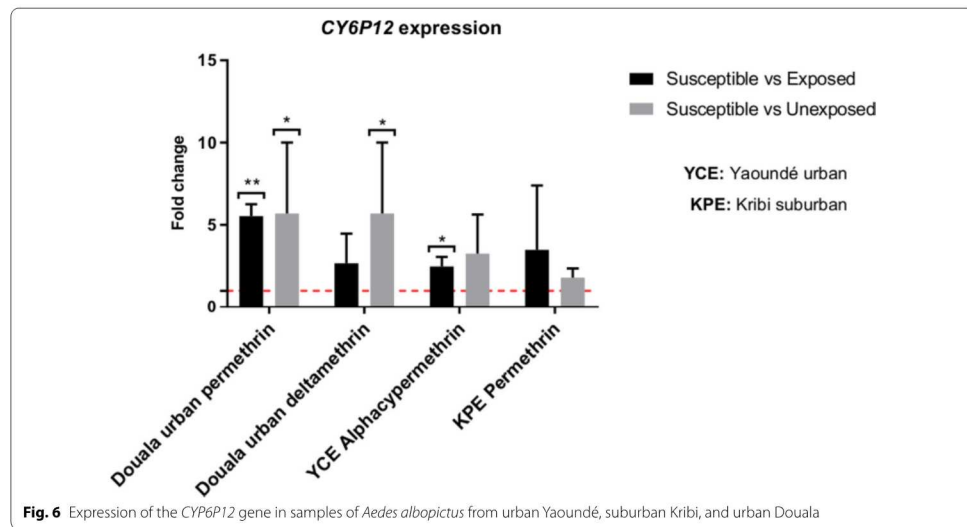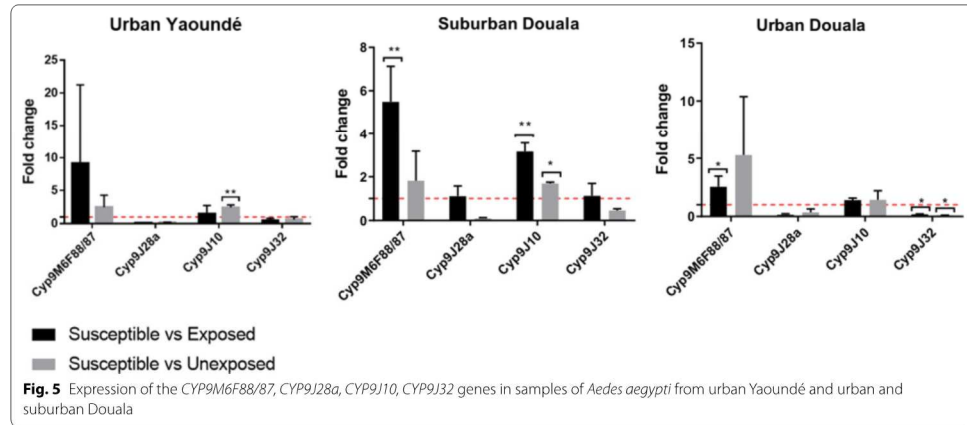
Aedes aegypti populations from the northern part of Cameroon were more susceptible to pyrethroids than those from the southern part. This suggests that resistance to this insecticide family has not yet spread throughout the entire country and these insecticides are still effective in controlling *Aedes* in some locations of Cameroon. Previous studies on *Anopheles* in Cameroon showed the same latitudinal gradient to resistance distribution [35]. Climate is a potential contributing mechanism; while the relationship between insecticide resistance and climate for *Aedes* mosquitoes is largely unexplored, solar radiation and humidity were the highest-ranked predictor variable for resistance levels in Cameroonian *Anopheles* [35]. As with our study, *Anopheles* mosquitoes from wet tropical areas generally had higher levels of resistance than those from dry arid climates.

Table 3 1016 genotype numbers and the allelic frequency of the Isoleucine (I) mutation of *Aedes aegypti*

Location	V1016 genotypes				Allelic frequencies	
	VV	VI	II	VV + VI + II	V	I
Urban Douala	21	6	0	27	0.89	0.1
Suburban Douala	22	8	0	30	0.87	0.13
Urban Yaoundé	27	2	1	30	0.93	0.07
Urban Maroua	13	14	2	29	0.69	0.31
Suburban Maroua	30	0	0	30	1	0
Urban Garoua	22	8	0	30	0.87	0.13
Suburban Garoua	27	2	1	30	0.93	0.07
Urban Ngaoundéré	21	8	1	30	0.83	0.17
Suburban Ngaoundéré	30	0	0	30	1	0
Urban Kribi	14	11	5	30	0.65	0.35
Total	227	59	10	296	0.87	0.13

V: Valine; I: isoleucine; VV: absence of the V1016I mutation; VI: presence of the V1016I mutation with 2 alleles: one resistant, allele I and another susceptible V allele; II: presence of the V1016I mutation with the 2 resistant alleles

Table 4 1534 genotype numbers and the allelic frequency of the Cysteine (C) mutation of *Aedes aegypti*

Location	F1534 genotypes				Allelic frequencies	
	FF	FC	CC	FF + FC + CC	F	C
Urban Douala	0	3	26	29	0.05	0.95
Suburban Douala	1	5	24	30	0.12	0.88
Urban Yaoundé	27	2	1	30	0.93	0.07
Urban Maroua	8	1	19	28	0.30	0.70
Suburban Maroua	30	0	0	30	1	0
Urban Garoua	30	0	0	30	1	0
Suburban Garoua	26	2	1	29	0.93	0.07
Urban Ngaoundéré	18	5	7	30	0.68	0.32
Suburban Ngaoundéré	30	0	0	30	1	0
Urban Kribi	0	0	30	30	0	1
Total	170	18	108	296	0.60	0.40

F: phenylalanine; C: cysteine; F/F: absence of the F1534C mutation; F/C: presence of the F1534C mutation with 2 alleles: one resistant, allele C and another susceptible F allele; C/C: presence of the F1534C mutation with the 2 resistant alleles

Pyrethroid resistance was more frequent in urban settings, which mirrors patterns in *Anopheles* mosquitoes in Cameroon [36]. This may be the result of a more intensive use of insecticide treated nets (ITN) and household insecticide (e.g., mosquito coils) usage in urban settings. However, both *Ae. aegypti* and *Ae. albopictus* feed primarily outdoors and during daylight in Cameroon and are thus unlikely to encounter ITNs [37, 38]. Land use could also affect resistance by determining the availability of larval habitat types since *Ae. aegypti* insecticide resistance levels can vary between larvae that developed in tires versus water containers [15].

With pre-exposure to PBO, partial or full recovery of susceptibility to all insecticides tested in *Ae. aegypti* and *Ae. albopictus* was observed. Similar results have been seen in several other African countries [10, 15, 28, 29, 33], as well as outside Africa [16, 17]. These findings suggested the important role played by P450 genes in the resistance to pyrethroids (deltamethrin, alphacypermethrin, and permethrin) and carbamates (bendiocarb). These observations were confirmed by the overexpression of genes such as *Cyp9M6F88/87* and *Cyp9J10* in some *Ae. aegypti* populations and *Cyp6P12* in *Ae. albopictus* populations in Cameroon. The implication of these genes in metabolic resistance were previously demonstrated [16–19, 39].

Three possible *kdr* mutations were genotyped in this study: F1534C, V1016I, and V410L, which are involved in pyrethroids resistance in *Aedes* mosquito [11, 40–42]. These three *kdr* mutations have been previously

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Table 5 410 genotype numbers and the allelic frequency of the Leucine (L) mutation of *Aedes aegypti*

Location	V410 genotypes				Allelic frequencies	
	VV	VL	LL	VV + VL + LL	V	L
Urban Douala	22	7	1	30	0.85	0.15
Suburban Douala	23	7	0	30	0.88	0.12
Urban Yaoundé	27	2	1	30	0.93	0.07
Urban Maroua	14	15	1	30	0.72	0.28
Suburban Maroua	30	0	0	30	1	0
Urban Garoua	22	8	0	30	0.87	0.13
Suburban Garoua	26	2	1	29	0.93	0.07
Urban Ngaoundéré	1	9	20	30	0.18	0.82
Suburban Ngaoundéré	30	0	0	30	1	0
Urban Kribi	17	9	4	30	0.72	0.28
Total	212	59	28	299	0.81	0.19

V: valine; L: leucine; VV: absence of the V410L mutation; VL: presence of the V410L mutation with 2 alleles: one resistant, allele L and another susceptible V allele; LL: presence of the V410L mutation with the 2 resistant alleles
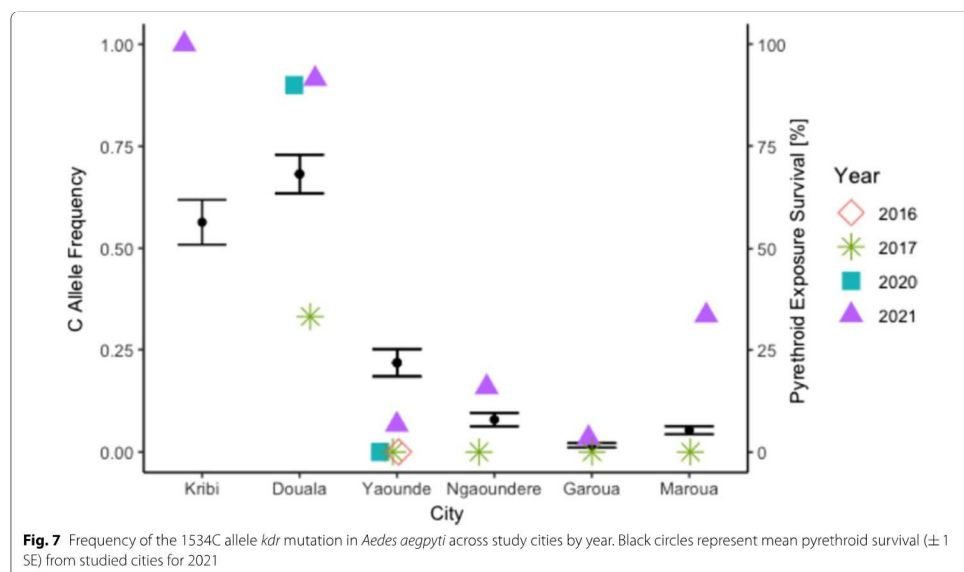
Fig. 7 Frequency of the 1534C allele *kdr* mutation in *Aedes aegpyti* across study cities by year. Black circles represent mean pyrethroid survival (± 1 SE) from studied cities for 2021

reported in Africa [15, 42–44], but only F1534C and V1016I were found previously in Cameroon [9, 10]. This study revealed for the first time the presence of a V410L mutation in *Ae. aegypti* in Cameroon. This mutation was first reported in 2017 in Brazil [7], and its first evidence in Africa was in 2020 in Angola [11]. V410L, alone confers low levels of resistance to insecticides, but when it co-occurs with V1016I or F1534C it yields higher levels

of resistance [7, 11]. The frequency of V410L was moderate compared to frequencies in Angola, supporting the hypothesis of a novel introduction of this mutation. The F1534C and V1016I *kdr* mutations are common in *Ae. aegypti* and have a worldwide distribution [4]. V1016I and V410L have a moderate frequency, while F1534C has a high frequency and has increased in *Ae. aegypti* populations across Cameroon since the mutation was first

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observed there in 2017. This result supports the observation made previously in Cameroon suggesting an introduction and a gradual spread of the F1534C mutation in the country [10]. Broadly, the detection of *kdr* mutations are more frequent in Africa than was observed in the past [45]. Faced with their involvement in pyrethroid resistance, these three *kdr* mutations could have an impact on vector control measures.

The main limitation of the study is that we did not assess whether there is an association between the phenotypic resistance observed and the *kdr* mutations detected.

Conclusions

Our study revealed the loss of susceptibility to four insecticides: deltamethrin, alphacypermethrin, permethrin, and bendiocarb, for both *Ae. aegypti* and *Ae. albopictus*. These insecticides are most likely to be used for mosquito control in outbreak responses and for reducing disease risk to deployed military personnel, and this study suggests these mitigation strategies will have limited efficacy. The level of loss of susceptibility varied according to the city, land use class, and species. Our results showed that several mechanisms are involved in resistance, and this can impact the strategies of *Aedes* control in Cameroon including the first detection of the V410L mutation in Cameroon. Further investigations including testing novel insecticides are needed to help to put in place effective strategies to control arbovirus vectors in Cameroon.

Abbreviations

DNA: Deoxyribonucleic acid; kdr: Knockdown resistance; RNA: Ribonucleic acid; PBO: Piperonyl butoxide; PCR: Polymerase chain reaction; *VGSC*: Voltage-gated sodium channel; WHO: World Health Organization.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40249-022-01013-8.

> **Additional file 1.** Logistic regression of *Aedes aegypti* mortality to insecticides across cities and habitat types. Suburban habitat, city of Douala, and Bendiocarb were reference levels.

Availability of data and materials

All the relevant data generated during this study are included in the manuscript.

Declarations

Ethics approval and consent to participate

An oral consent form was obtained from the head or representative of each household or garage owner prior to collect the mosquito larvae.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Chapter 3: Comparing relative transmission potential to population seroprevalence for seven arboviruses: yellow fever, chikunguna, zika, and four dengue serotypes

Abstract

Dengue (DENV), chikungunya (CHIKV), yellow fever (YFV), and Zika virus (ZIKV) are mosquito-borne viruses which account for millions of human infections annually, primarily in tropical regions. Despite the presence of multiple arboviruses in many locations, little comparative work has been done to examine relative transmission intensity through seroprevalence, and to what extent this is determined by differences in host and vector in competence between viruses. We calculated relative seroprevalence estimates for DENV (serotypes 1-4), CHIKV, YFV, and ZIKV from 204 seroprevalence studies with a total of 95,000 individuals on five continents. We then calculated the relative epidemic potential, R_0, for the same seven arboviruses for both *A. aegypti* and *A. albopictus* using prior clinical and laboratory studies on vector and host competence. We hypothesized relative seroprevalence of a virus would be positively correlated to its R_0 estimates. Globally, CHIKV and YFV had the highest relative seroprevalence, followed by ZIKV and DENV-2, DENV-3, DENV-1, and DENV-4, whereas in Asia YFV seroprevalence was zero and in the Americas ZIKV had the highest seroprevalence and CHIKV was the third lowest. Relative R_0 values were highest for CHIKV followed by the four DENV and ZIKV, with YFV being much lower. Relative R_0 values were higher for *A. aegypti* than *A. albopictus*. Relative R_0

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for *A. aegypti* was significantly correlated with higher seroprevalence for the seven arboviruses in Asia, but not in other continents or globally. These results demonstrate substantial variation in seroprevalence and relative R_0 among these arboviruses, but also regional variation in relative seroprevalence differences. This suggests that predicting relative exposure of different pathogens may require more detailed local spatio-temporal estimates of host and vector competence.

Introduction

Viruses transmitted by arthropods (arboviruses) are of increasing global concern and account for millions of annual cases of human illness and tens of thousands of fatalities (Kilpatrick & Randolph 2012, Puntasecca et al. 2021, Yang et al. 2021). Increased global trade and travel have led to regular introductions of novel pathogens including many arboviruses such as West Nile, Zika, and Chikungunya (Dufft et al. 2009, Kilpatrick & Randolph 2012; Possas et al. 2017, WHO 2020a). The total fraction of the population infected over the course of an epidemic is determined, in part, by the reproductive number of the pathogen, R_0, which also influences long term steady state seroprevalence (Dye 1992). Despite the presence of multiple arboviruses in human populations, little comparative work has been done to determine relative transmission intensity through seroprevalence, and to what extent this is determined by differences in host and vector in competence between viruses.

Arboviruses spread by *Aedes* mosquitoes, including CHIKV, DENV, yellow fever (YFV), and Zika virus (ZIKV) account for the majority of human arbovirus

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infections (World Health Organization 2020). Most transmission of these arboviruses occurs in urban areas by two anthropophillic vectors, *A. aegypti* and *A. albopictus* (Weaver & Reisen 2010). The urban cycles of these viruses share common vectors and a single host, humans. As a result, in most urban areas, relative transmission intensity will depend only on differences in host and vector competence among the viruses which are key components of R_0 (Dye 1992). Host competence depends on both the levels of viremia (the concentration of virus in blood) reached within human hosts and the duration of infectious viremia. Vector competence, the probability of a mosquito transmitting virus after feeding on an infected host, depends on host viremia, temperature, and time since feeding, and often differs between vector species and populations (Azar & Weaver 2019, Souza-Neto et al. 2019).

Our goal was to estimate the relative seroprevalence of these seven arboviruses across human populations and compare these to estimates of relative R_0 based on host and vector competence. Antibodies against these viruses persist for up to 60 years following exposure (Imrie et al. 2007; Wang & Sekaran 2010), and thus provide an estimate of previous exposure of a population (Imrie et al. 2007; Wang & Sekaran 2010). We collected a large number of seroprevalence estimates to try to average across sources of variation, including movement of people among locations and episodic transmission. We then estimated relative R_0 across all seven arboviruses using measures of human viremia and mosquito vector competence from previous studies. Numerous prior studies have estimated R_0 for individual viruses or pairs of arboviruses in one or two vectors (Chitnis et al 2006, Gardner & Ryman 2010,

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Lambrecths et al. 2010, Weaver et al. 2015, Manore et al. 2014, Vasilakis & Weaver 2017, Mordecai et al. 2017, Liu et al. 2020), but none of these studies has estimated relative R_0 for all seven of the arboviruses transmitted primarily by *A. aegypti* and *A. albopictus*. We compared relative seroprevalence to relative R_0 for the seven arboviruses for both *A. aegypti* and *A. albopictus* and hypothesized that these would be positively correlated.

Methods

Relative transmission potential, R_0

We compiled data on host and vector competence for CHIKV, YFV, ZIKV, and the four serotypes of DENV for both *A. aegypti* and *A. albopictus* (Table S1. Human viremia data were reported relative to time since symptom onset or resolution and measured in either PFU/mL or viral copies/mL. DENV viremia was converted from viral copies/mL to PFU/mL using the equation Log_{10} PFU/mL = [0.974 * Log10 viral copies/mL] - 2.807 (Fernandes-Monteiro et al. 2015). We analyzed six days of viremia for each virus after which infectiousness of most viruses decreased to zero or near zero (see below). We fit a linear model to compare viremia between viruses for each of the six days in R and performed a post-hoc comparison of viremia between viruses using the emmeans package (Lenth 2022).

For vector competence, we collected data on the fraction of *A. aegypti* and *A. albopictus* mosquitoes that transmitted virus after feeding on infected blood with known viremia. The majority of vector competence studies provided infectious dose

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blood-meal viremia in PFU/mL. For studies which measured viremia in Tissue Culture Infectious Dose (TCID50) we converted those data to PFU/mL using the equation $\text{Log}_{10}\,\text{PFU mL}^{-1} = -0.567306 + 0.987227\,\text{Log}_{10}\,\text{CID50 mL}^{-1}$ (Kilpatrick et al. 2007) and for studies measuring viremia in Mouse Lethal Dose (MLD50) we used $1.395\,\text{Log}_{10}\,\text{PFU mL}^{-}/\text{MLD50}$ (Freire et al. 2007).

We fit models to estimate the fraction of mosquitoes transmitting virus as a function of time since feeding and host viremia. We fit empirical data on the fraction transmitting on a given day to the cumulative distribution of a gamma distribution representing the extrinsic incubation period, in days, using a Bayesian framework via the R package *rethinking* (Ferguson et al. 2016, McElreath 2016). We fit all data for three viruses (CHIKV, YFV, and ZIKV) and both mosquitoes simultaneously with an additive model of vector species and virus to leverage information for virus-vector pairs with limited information. We used vague priors for all parameters (gaussian distributions with mean = 0, SD = 20); alternative priors produced nearly identical posterior estimates. We performed similar analyses for the four serotypes of DENV. We fit separate models for DENV because data for all four viruses for both viremia and vector competence came from a single location and set of studies (Nguyen et al. 2013, Whitehorn et al. 2015) and the units that viremia were measured in differed between the DENV studies (copies/ml) and the other three viruses (PFU/ml).

We calculated epidemic potential by estimating the infectiousness of human viremias for each virus on each day using the vector competence models (estimated 14 days after feeding) and then summing across six days of viremia for each virus.

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We estimated uncertainty in relative R_0 estimates using both variation in daily viremia among people and the uncertainty in the vector-competence-viremia relationships.

Seroprevalence estimates. We collected seroprevalence data from the literature by searching Google Scholar and Web of Science. Our search terms consisted of a pathogen (CHIKV, DENV 1-4, YFV, and ZIKV) or the term "arbovirus", seroprevalence or serosurvey or antibodies, and a location including countries and continents. We included articles that simultaneously measured antibodies for at least two of the seven arboviruses in the same population so that we could estimate the relative differences in seroprevalence among two or more viruses. We excluded studies done in response to outbreaks of specific viruses and studies of DENV seroprevalence which did not specify serotype. We categorized seroprevalence data by continent for Asia, Africa, and the Americas.

Calculating comparative seroprevalence. We compared seroprevalence among viruses using a generalized linear model with a binomial distribution with virus as a fixed effect and study ID as a random effect using the lme4 package in R version 4.2.1 (Bates et al. 2015). We fit models both to all serosurvey data and to data for each continent. For Asia, where all YFV studies had a seroprevalence of zero, we fit a similar model using both study ID and virus as fixed effects using the *brglm2* package in R to better estimate the YFV coefficient (Kosmidis 2017). We then used the fitted

coefficients for each arbovirus as the response variable and estimates of relative R_0 for either *A. aegypti* or *A. albopictus* as the predictor variable using a linear model. We performed these analysis for both global and continental seroprevalence data with a single estimate for relative R_0 for each virus because we had insufficient host and vector competence data to estimate continent-specific relative R_0 values.

Results

Viremia. Viremia among human patients differed significantly between arboviruses, with CHIKV having significantly higher viremia than any other virus. None of the other viruses differed significantly from one another in viremia (Figure 1, Table S2).

Vector Competence. Vector competence varied significantly among viruses and between mosquito species (Figure 2; Figures S1 and S2, Table S3). Vector competence was higher for *A. aegypti* for all seven arboviruses, with the exception of DENV-1 and DENV-4 at titers above 8 PFU/mL (Figure 2).

Relative R_0. Daily human infectiousness to mosquitoes and thus relative R_0 differed between arboviruses (Figure 3). R_0 was greatest for CHIKV, followed by DENV-2, DENV-1, DENV-3, DENV-4, ZIKV, and YFV with *A. aegypti* as the vector. For *A. albopictus* the ranked order of R_0 from greatest to least was CHIKV, DENV-1, DENV-3, DENV-4, ZIKV, DENV-2, and YFV.

Seroprevalence for seven arboviruses. We reviewed 611 articles and identified 80 which contained 202 unique serosurveys that matched our criteria (Supplemental References). Serosurvey data represented 95,000 individual blood samples from 46 countries (Figure S3). In the full dataset, relative seroprevalence differed significantly among arboviruses, with CHIKV and YFV being highest, followed by ZIKV and DENV-2, DENV-3, DENV-1, and DENV-4 (Figure 4, Table 1, Figure 5A). However, there were substantial differences among the three continents (Tables S4-S6). Patterns in Africa were somewhat similar to the global dataset, in part because Africa made up a substantial fraction of all studies (Figure S3), but DENV-4 and DENV-1 switched places in relative seroprevalence, and ZIKV was relatively lower (Figure 5 A,B). In the Americas, ZIKV and DENV-2 seroprevalence was the highest and DENV-4 was the lowest. Finally, in Asia, YFV was absent and all YFV seroprevalence estimates were 0%, whereas CHIKV and DENV-3 had the highest seroprevalence. The differences in seroprevalence among arboviruses were much larger in the Americas and Asia than in global and especially African datasets (Figure 6, Tables S4-S6). In areas in the Americas with average transmission, ZIKV and CHIKV had very high estimated seroprevalence (>80%), whereas the four dengue viruses were much lower (<50%) (Figure 6C). In Asia, CHIKV, DENV-3 and DENV-2 had the highest estimated seroprevalence whereas YFV seroprevalence was zero in all studies (Figure 6D).

Comparison of relative R_0 and relative seroprevalence. Relative seroprevalence was significantly positively correlated with relative R_0 for *A. aegypti* in Asia, but no other correlations were significant with either species as the vector (Figure 5; Figure S4). CHIKV had very high relative R_0 and seroprevalence in all regions, and DENV-4 had a low seroprevalence in all regions and a moderately low relative R_0. However, ZIKV had the 2nd lowest relative R_0 and the highest seroprevalence in the Americas and was moderately high in Asia and the global dataset. The four serotypes of DENV also showed spatial variation in relative seroprevalence and differences among these four viruses was variable and not correlated with relative R_0 in most regions (Figure 5).

Discussion

We found large differences among arboviruses in seroprevalence, but differences varied substantially among continents, and differences in seroprevalence were only correlated with relative R_0 for one vector, *A. aegypti*, in one of three regions and not in the global analysis. We also found large differences in relative R_0 among viruses due to both significant differences in viremia as well as vector competence. The lack of correlation between relative R_0 and seroprevalence for the global dataset and for Africa was surprising, because we had a large dataset of seroprevalence studies and a relatively large dataset to estimate vector competence. However, the analysis only included seven arboviruses and two viruses with low relative R_0 values played a key role in the lack of a correlation with relative

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seroprevalence: YFV and ZIKV. YFV had the lowest estimated relative R_0, by far, but had the second highest relative seroprevalence in Africa and in the global dataset. ZIKV had the second lowest relative R_0, but had the highest seroprevalence in the Americas, and intermediate seroprevalence in Asia and globally. Clearly the relative R_0 values didn't match the observed relative seroprevalence for these viruses.

The low estimate of relative R_0 for YFV was especially puzzling. We had a robust dataset for calculating YFV vector competence, but these data suggested that it was significantly lower than any other virus for both vectors. Human viremia data were limited for YFV, but no more so than for CHIKV or ZIKV, all of which had no more than 11 viremia values for a single day post symptom onset. In any case, YFV had the lowest viremia of the seven viruses on 3 of the 6 days and overall were in the lowest group with DENV-1, DENV-2 which had much higher relative vector competence. The low estimated relative R_0 of YFV is surprising in light of its very high seroprevalence in Africa. Human YFV infections were detected in 46 countries between 1970-2016 (Shearer 2018), and in 10 African countries and 2 South American countries in 2022 (WHO 2022, PAHO 2022). Given the recent and ongoing outbreaks of YFV in Africa (WHO 2022) and potential for expansion, more research is needed on human viremia data for YFV and to understand the variation in YFV vector competence among mosquito populations (Figure 2).

The mismatch between relative seroprevalence and relative R_0 may arise from spatial or temporal variation among viruses, mosquitoes, or human populations and their contributions to relative epidemic potential. For example, there are substantial

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differences between the Asian and Reunion strains of CHIKV in vector competence

(Tsetsarkin et al. 2007). Similarly, there are clear spatial and temporal differences in

vector competence among mosquito populations (Gubler et al. 1979, Bosio et al.

1998, Bosio et al. 2000, Goncalves et al. 2014). Innate differences in viremia among

human populations may also contribute to different epidemic potential in different

populations, but they have not been studied. However, evidence suggest that host

competence is mediated by prior exposure to related viruses. For example, prior

exposure to DENV may lead to partial protection against YFV, and been posited as

one of the reasons for YFVs absence from Asia (Kuno 2020), and patients with

secondary infections with DENV clear the virus more quickly than primary infections

(Ben-Shachar et al 2016). Lastly, the lack of correlation between relative R_0 and

seroprevalence could be due to factors influencing seroprevalence, including episodic

transmission, human migration, or differential waning of antibodies. The variation in

relative seroprevalence rankings among studies (Figure 4) indicate that there is

substantial uncertainty in which virus will lead to the highest exposure a given

population at a point in time.

Despite these uncertainties, our results provide predictions about the relative

likelihood of pathogen establishment among arboviruses. If the pathogen with both

the highest seroprevalence and highest relative R_0, CHIKV, is unable to become

established in a given location (e.g. the United States), then our results suggest it is

less likely that the other arboviruses in this study would be able to become established

in the same population. There have been no locally acquired cases of CHIKV in the

US since 2015, despite numerous travel-associated cases (CDC 2022), suggesting that ZIKV, YFV, and the four serotypes of DENV are also unlikely to become established unless conditions (e.g. climate, larval habitat, poverty) change.

There appear to be substantial differences among the seven arboviruses we examined based both on relative seroprevalence and estimates of relative R_0. However, the lack of a correlation between relative seroprevalence and relative R_0 for most regions suggests that finer scale data on viruses, vectors and hosts may be needed to predict the relative transmission potential of different viruses in a given population. Measurements of vector competence and viremia for all seven arboviruses in replicated population of mosquitoes and humans would help illuminate the differences in relative R_0, and may uncover cryptic coevolution between viruses, vectors and hosts.

Tables and Figures

Table 3.1. Analysis of global seroprevalence data using a generalized linear model with a binomial distribution and a logit link with virus type as a fixed effect and study ID as a random effect. CHIKV was the reference level.

	Estimate	Std. Error	z value	P-value
Intercept (CHIKV)	-1.27	0.15	-8.61	< 0.001
DEN-1	-1.33	0.034	-39.41	< 0.001
DEN-2	-0.64	0.034	-18.67	< 0.001
DEN-3	-0.82	0.038	-21.79	< 0.001
DEN-4	-2.21	0.041	-53.29	< 0.001
YFV	-0.047	0.022	-2.16	0.030
ZIKV	-0.65	0.022	-29.51	< 0.001
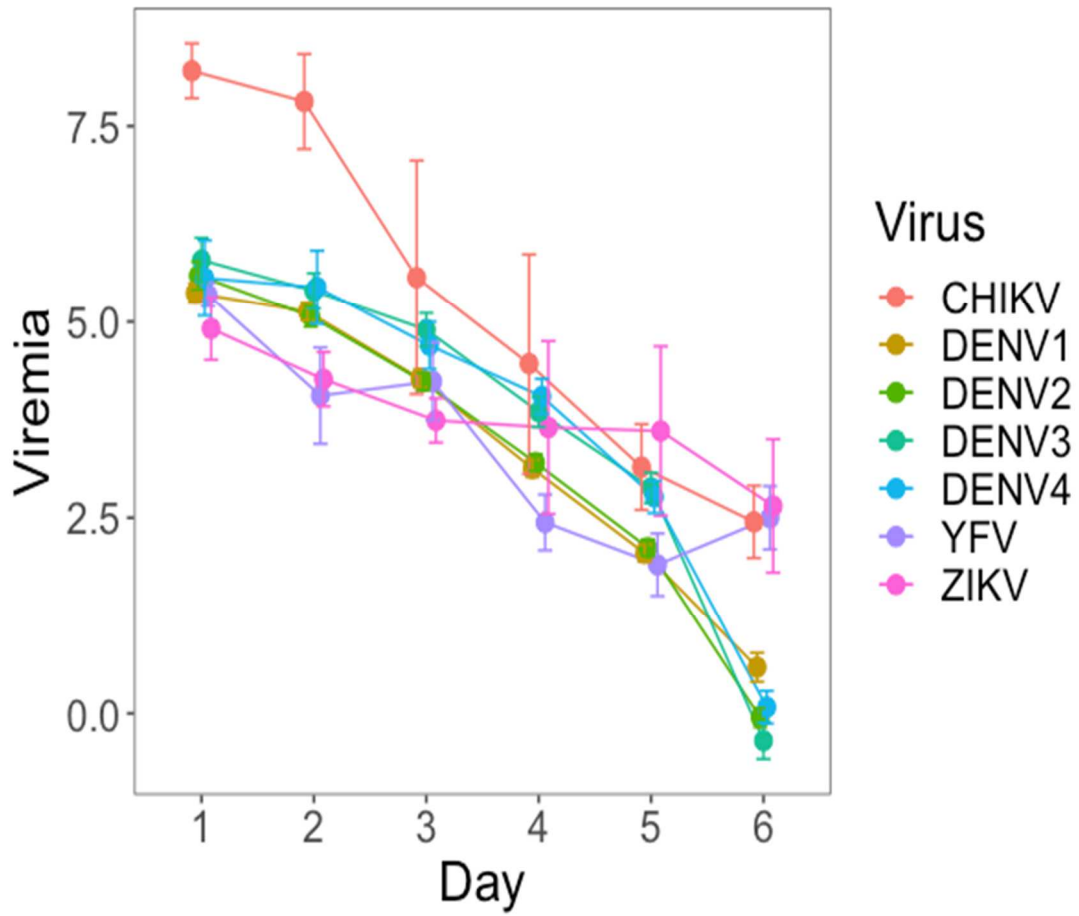
Figure 3.1. Human viremia (PFU/mL) by date of symptom onset.
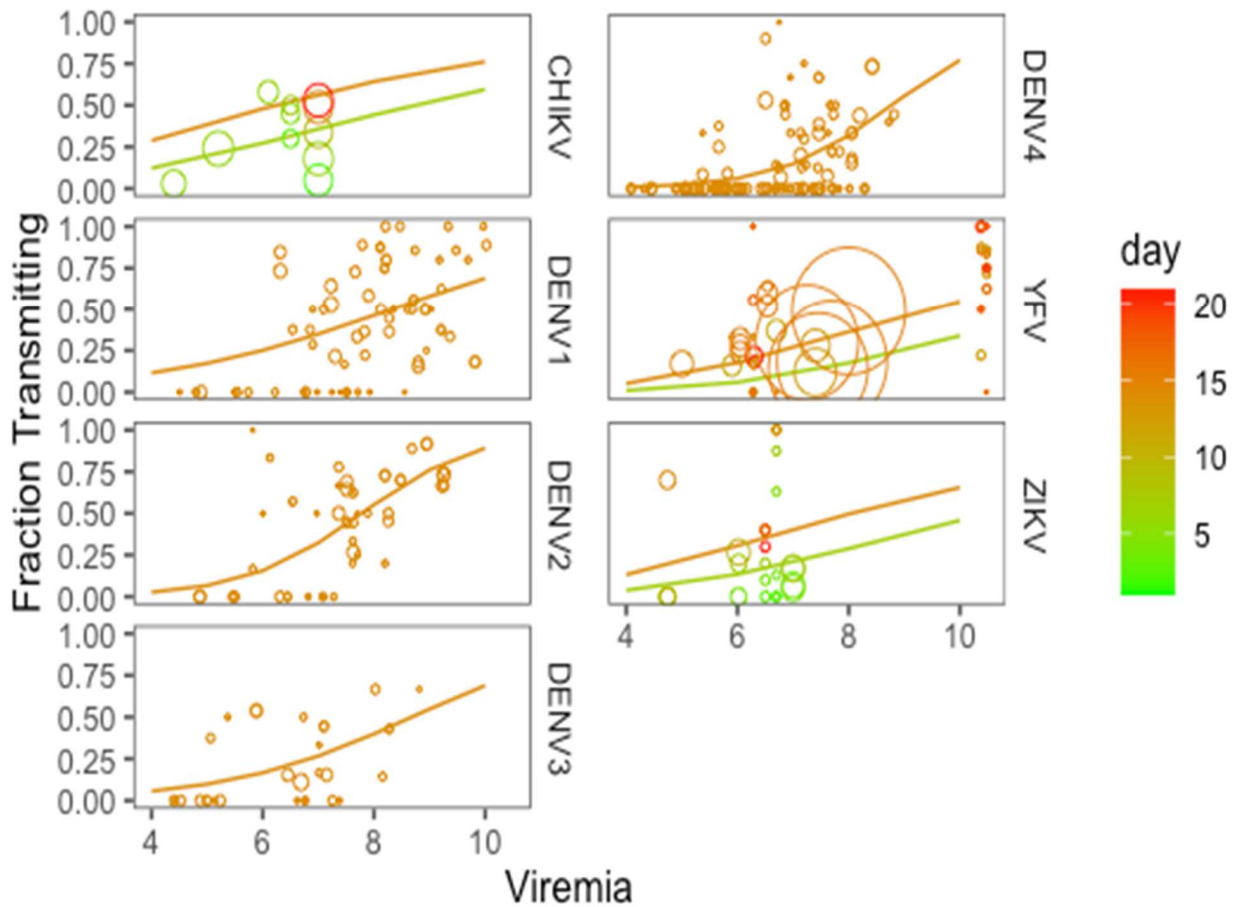
Figure 3.2. Predicted fraction of *A. aegypti* transmitting virus by blood meal viremia. Circles represent vector competence study data with circle size corresponding to *A. aegypti* sample size. Data for CHIKV, YFV, and ZIKV are plotted for days 7 and 14 post-feeding. DENV1-4 was plotted for day 14 since source study data only measured vector competence on day 14.
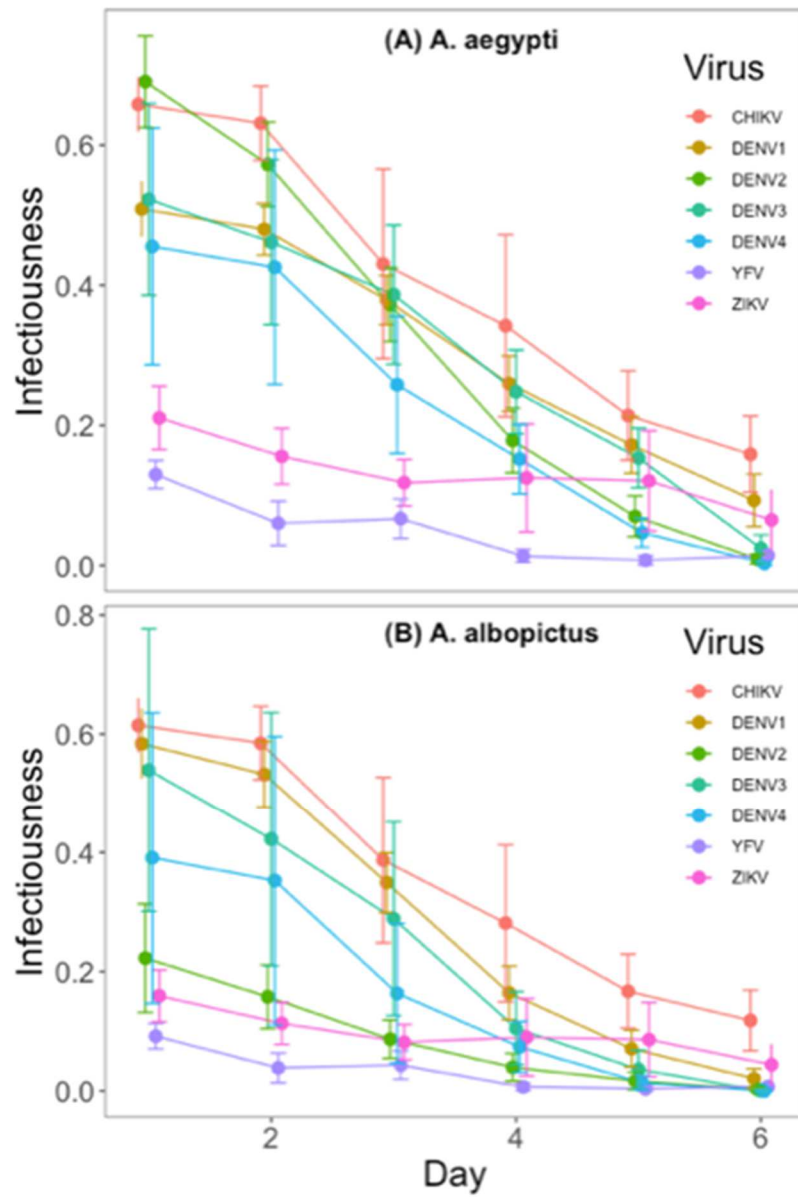
Figure 3.3. Daily estimates of human infectiousness (±1 SE) for seven arboviruses for *A. aegypt*i (top) and *A. albopictus* (bottom).
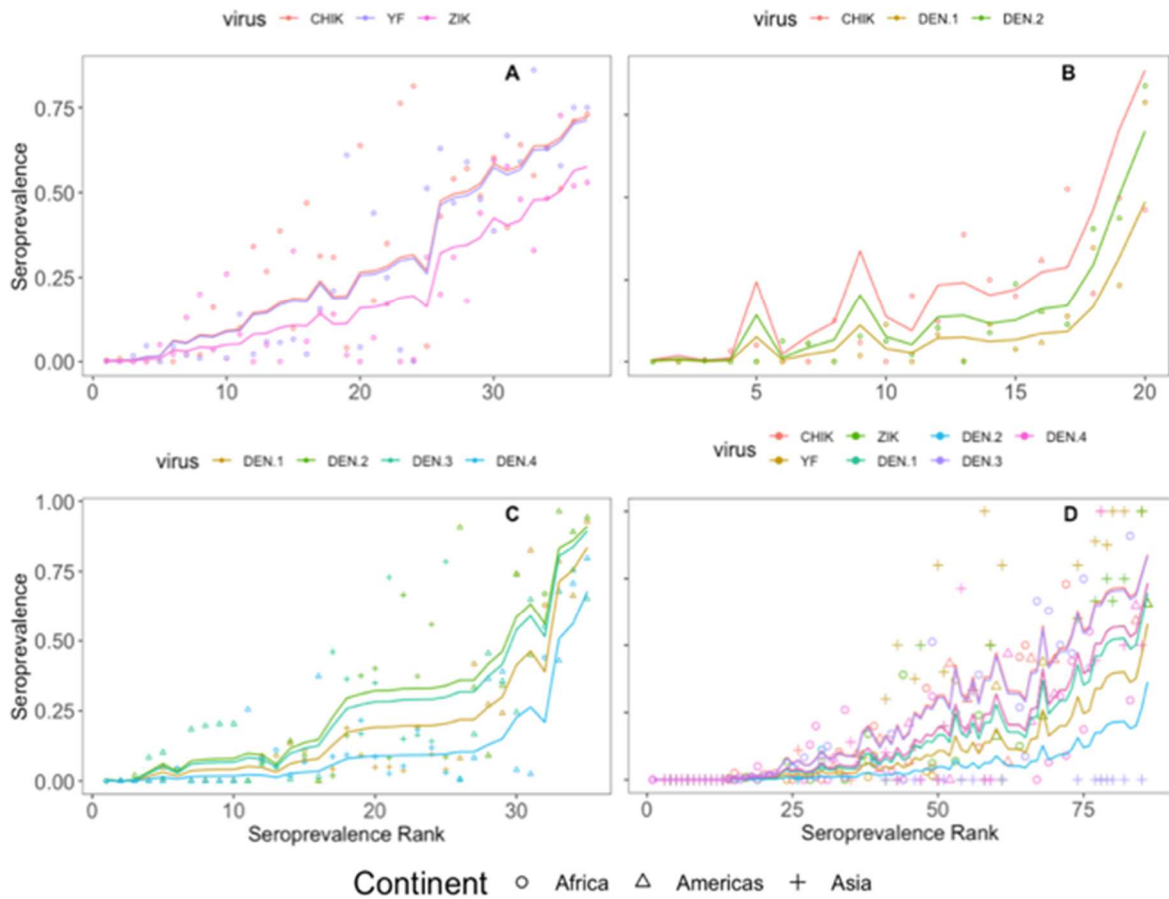
Figure 3.4. Relative seroprevalence for studies simultaneously comparing CHIKV, YFV, and ZIKV (A), CHIKV and DENV1-2 (B) ,DENV1-4 (C), and all remaining studies (D) with studies ranked by average seroprevalence along the x-axis. Lines represent predicted seroprevalence values based on fitted model with virus type and study ID as predictor variables.
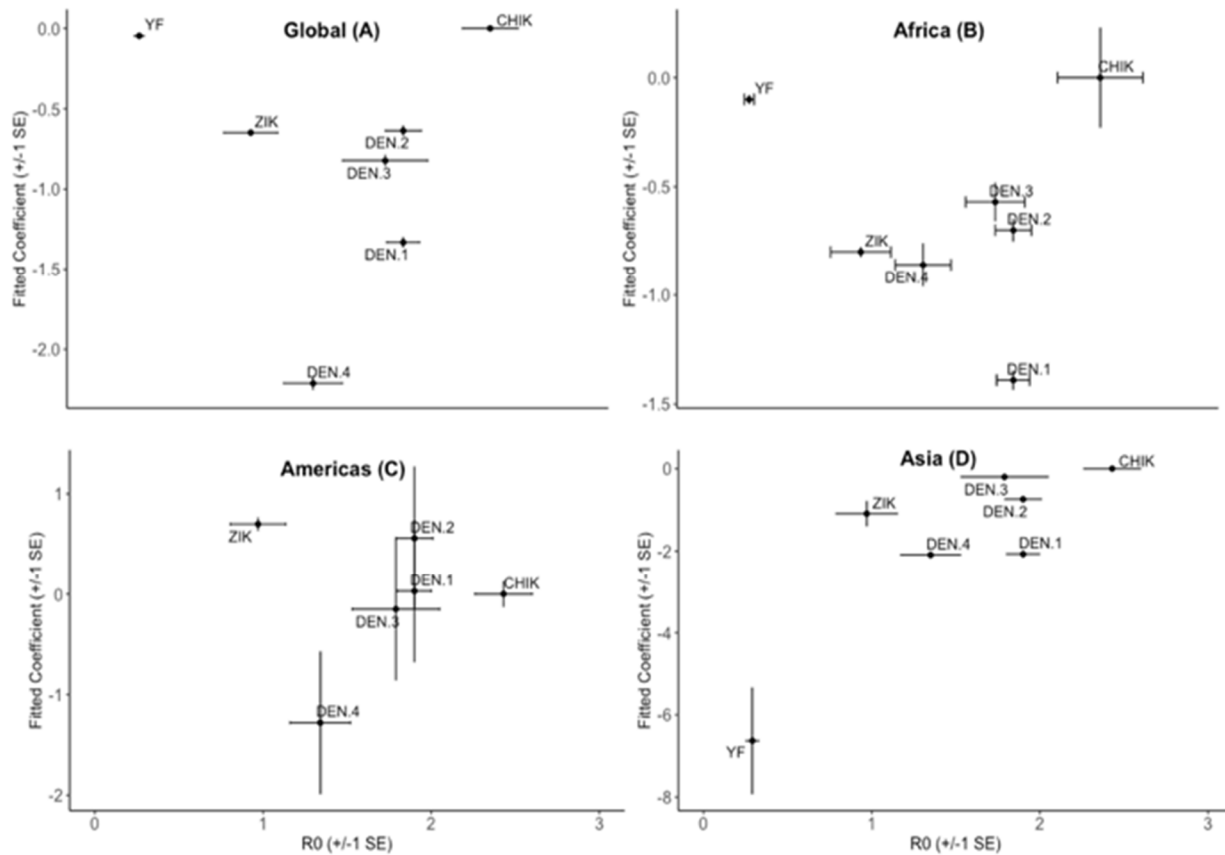
Figure 3.5. Fitted coefficients of relative seroprevalence plotted against relative R_0 estimates for *A. aegypti* for all data (A), Africa (B), the Americas (C), and Asia (D). Seroprevalence coefficients were significantly positively correlated with relative R_0 in Asia (Panel D; Seroprevalence Fitted Coefficients = -5.82 + 2.62 (± 0.81)* R_0, P = 0.023), but not for the global dataset (Panel A; Seroprevalence Fitted Coefficients = -0.72 - 0.062 (± 0.48)* R_0, P = 0.90), in Africa (Panel B; Seroprevalence Fitted Coefficients = -0.51 - 0.080 (± 0.29)* R_0, P = 0.80) or the Americas (Panel C; Seroprevalence Fitted Coefficients = -0.10 + 0.045 (± 0.69)* R_0, P = 0.95). YFV was not included in the Americas due to a lack of serological data.

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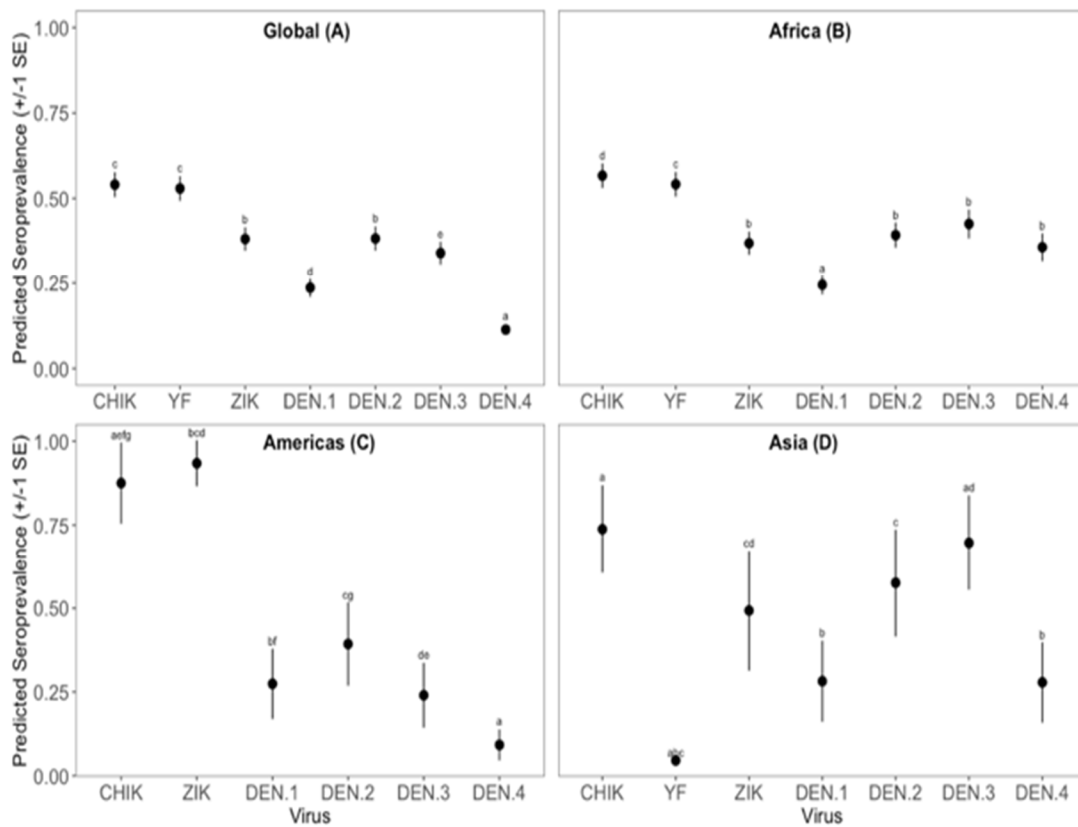
Figure 3.6. Estimated seroprevalence for each arbovirus for all data (A), Africa (B), the Americas (C), and Asia (D) for an intermediate value of average seroprevalence. *S*tatistically significant differences between viruses are indicated with compact letter display. YFV was not included in the Americas due to a lack of serological data.

Conclusion:

The unifying goal of this research is an improved understanding of the ecological factors that affect human disease risk whether through how land use influences mosquito abundance, the efficacy of insecticides used to control mosquito populations, and how variations in host and vector transmission capabilities of viruses determines the scope of epidemics. Better understanding the ecological determinants of human disease risk can then be used to devise better control strategies which reduce human disease burden. The ongoing urbanization of our planet is one of the most drastic changes effecting the natural world and as such a pressing area of study in ecology. Urbanization, broadly defined as the process by which land is covered by impervious surfaces by humans, is among the most drastic and consequential form of human land use and plays a pivotal role in shaping the biotic and abiotic conditions faced by many organisms (Gubler et al. 2011). This research provides new insights into species abundance in response to urbanization and thus how the process shapes disease risk across a grand temporal and spatial scale from two globally important mosquito species. While urbanization was strongly correlated with mosquito abundance and insecticide resistance, the precise mechanisms of this relationship remains largely unresolved.

Urbanization positively increases the abundance of one major global vector at a constant rate despite vastly different climates and ecotypes, ranging from the arid Sahel to lush Equatorial Forests. While simultaneously, urbanization decreases, has no effect, and increases the abundance of another species even in cities sharing near

identical climates. These results show that while one species' abundance in response to urbanization can be a linear relationship, other species can have completely divergent relationships on a city-by-city basis.

The correlates of urbanization one would expect to determine mosquito abundance, such as host abundance, larval habitat availability, and number of human structures, were indeed significantly correlated with abundance, but none were better predictors than the broader vague categorization of our urbanization index. Other factors important to mosquito abundance which we expected to be mediated by urbanization, temperature and humidity, were not significantly correlated with urbanization.

Furthermore, urbanization shapes disease risk by decreasing the efficacy of insecticides, the primary public health tool for controlling arbovirus disease outbreaks. The mechanisms of the positive relationship between insecticide resistance (i.e. increased survival to insecticide exposure) are another large gap in our understanding. Generally urbanization takes place at the expense of agricultural land where the vast majority of insecticide use occurs. In the absence of public health vector control programs (as is the case in Cameroon) what mechanisms then would potentially lead to greater resistance with increased urbanization? Does this phenomenon hold true for other vectors, or arthropods in general, as urbanization increases? Further elucidating the mechanisms of urbanization's effects on abundance and insecticide resistance is clearly an area in need of more research.

Considering our results in the context of climate change, another area of urgent focus in the field of ecology, the potential effects of climate change on disease risk are unclear as a result of this research. Increased temperatures led to lower abundance of *A. aegypti* across all habitats and increased humidity was significantly correlated with greater *A. albopictus* abundance. Increased rainfall was correlated with greater abundance of both species as well. Furthermore, inseciticde resistance decreased along a south to north gradient as the climate became hotter and drier. As such, as hotter drier climates arise this could likely reduce disease risk by limiting vector populations.

Our estimate of arbovirus transmission potential (relative R_0) provide further hope that increased burden and spread of vector borne disease to all corners of the world is not inexorable. If the pathogen with both the highest seroprevalence and highest relative R_0, CHIKV, is unable to become established in a given location (e.g. the United States), then our results suggest it is less likely that the other arboviruses in this study would be able to become established in the same population. These calculations provide a plausible explanation for why the ranges of our studied arboviruses are considerably smaller than their vector populations.

Comparing these calculations of relative R_0 to human seroprevalence as a measure of transmission intensity shows a disconnect between calculated predictions of pathogen fitness and the actual transmission intensity of these pathogens in field settings. Our results show that a pathogen, such as yellow fever, may have relatively poor host and vector competence in some populations and thus have lower predicted

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fitness, but simultaneously have higher relative levels of host antibodies in field observations. Essentially, pathogens which by our measure should have the lowest fitness and thus lower infection rates somehow show among the highest level of fitness in infecting humans. The potential explanations for this mismatch are a target rich area for future research. Our novel methodology of comparing calculations of relative transmission potential from laboratory derived data on host and vector competences with seroprevalence (or some other measure of prior infection) can be applied to other host-pathogen systems to test if calculations of pathogen fitness match field observations.

In our study system, the mismatch between relative seroprevalence and relative R_0 may arise from spatial or temporal variation among viruses, mosquitoes, or human populations and their contributions to relative epidemic potential. In both *A. aegypti* and *A. albopictus*: vector competence can vary enormously within the same mosquito populations over relatively short spatial and time scales (<1 year) (Gubler et al. 1979, Kilpatrick et al. 2010, Goncalvez et al. 2014). Host competence and seroprevalence can also be mediated by prior exposure to other arboviruses, possibly different rates of waning in antibodies, or perhaps by variations between human population in viremia. The extent to which any of these variations determine transmission intensity within a population is largely unexplored. Further studies on the factors underpinning host and vector competence are needed to understand if our estimates of host and vector competence can be extrapolated beyond any one particular place and time.

The ranges of both *A. aegypti* and *A. albopictus* are expanding and our increasingly interconnected world allows for pathogens to spread quickly (Shragai et al. 2017). Understanding and reducing disease risk relies on accurate assessments of host and vector competence, knowledge of the environmental factors determining vector abundance, and availability of effective means of controlling vector populations. Unlike most fields of ecology, we seek to actively disrupt and destroy our study organisms of disease-spreading vectors and hope the knowledge gained from this research will be useful in doing so.
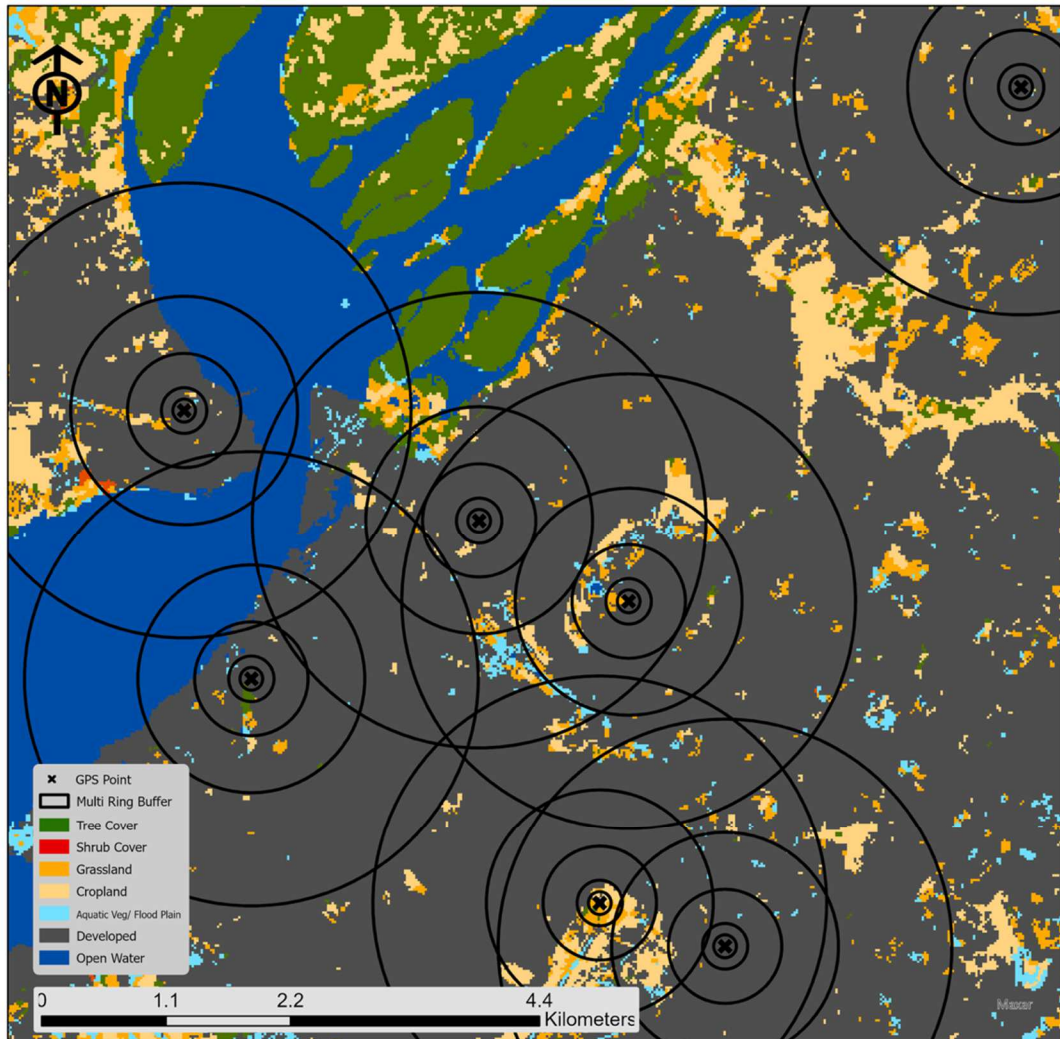
Figure S1: Example of land use data buffers (100m-2km) generated for collection sites in the city of Kribi.
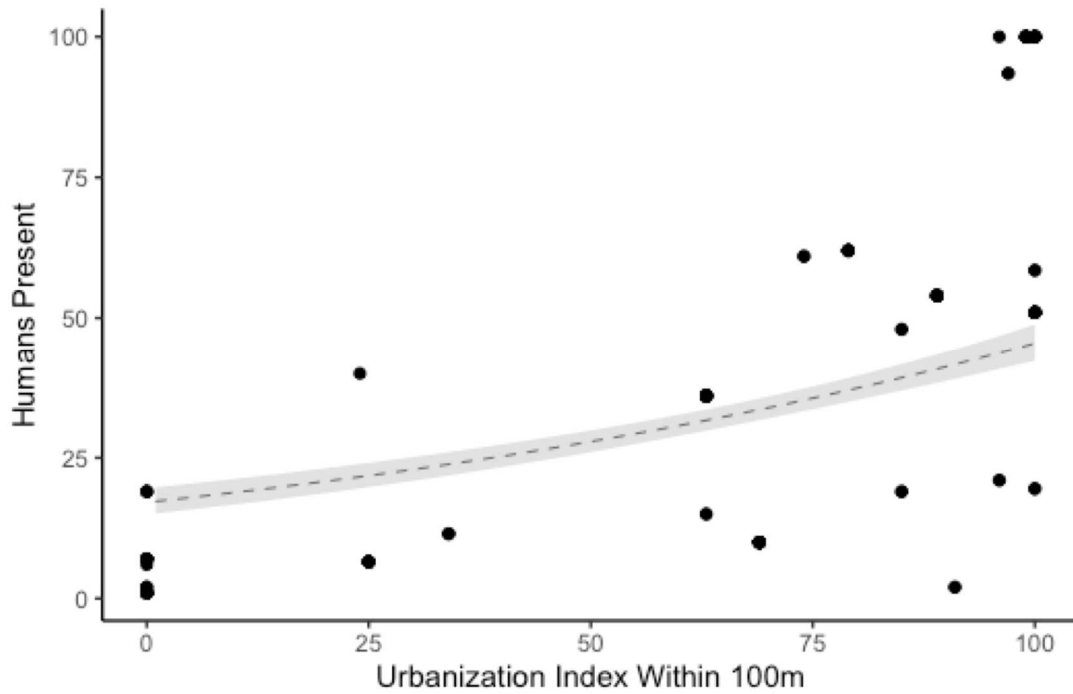
Figure S2. Available human hosts observed at collection sites in Douala, Kribi, and Yaounde. Lines and ribbons show fitted models with urbanization index as predictor and 95% CI, respectively.
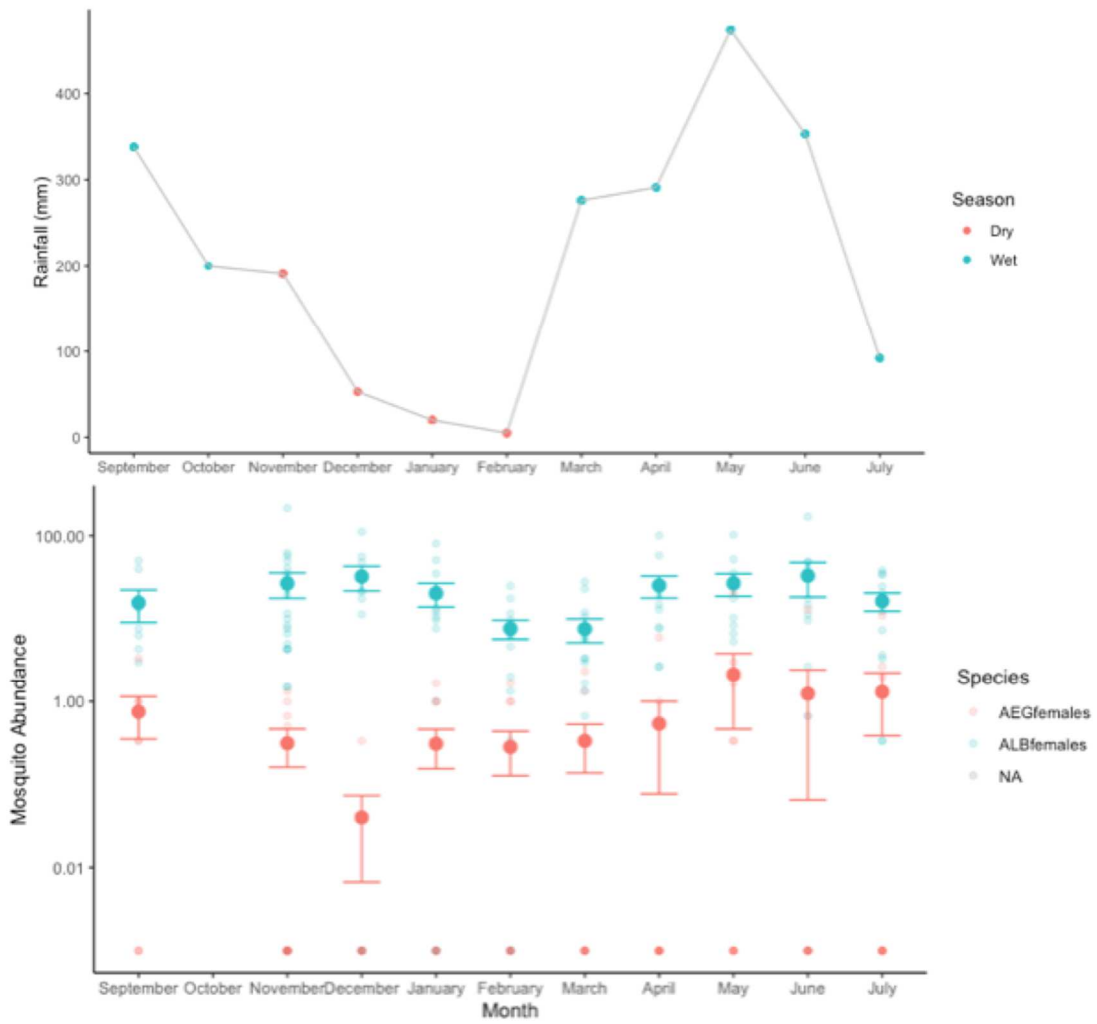
Figure S3. Monthly rainfall and mosquito abundance for the city of Yaounde.

Dark circles represent the mean catch rate per hour across all sites (+/- 1SE).
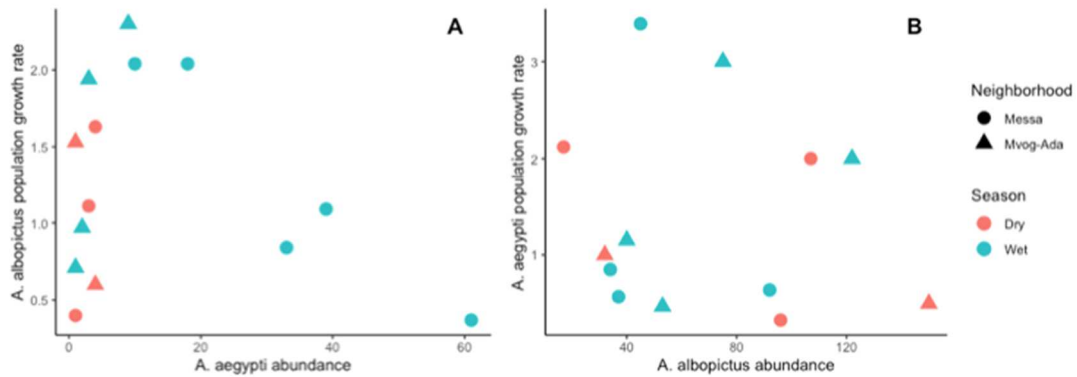
Figure S4. Monthly population growth rate of *A. albopictus* plotted against abundance of *A. aegypti* in the preceding month (A) and vice versa (B) . The relationship was not significant for an effect of *A. aegypti* abundance on *A. albopictus* population growth rates the following month (Log(ALBPopGrowth)=0.22-0.011 (± 0.0089)*AEG, P = 0.27) nor for an effect of *A. albopictus* abundance on the following month *A. aegypti* population growth rates (Log(AEGPopGrowth)=0.14-0.0026 (± 0.0059)*ALB, P = 0.66).

Table S1. Previous studies on *Ae. aegypti* abundance and urbanization.

Study	Location	Urbanization Metric	Urbanization Correlation w/*Ae. aegypti* Abundance
Braks et al. 2003	Rio de Janeiro & Nova Iguacu, Brazil; Boca Raton & West Palm Beach, FL, USA	Qualitative (Urban, suburban, rural)	Positive
Carbajo et al. 2006	Buenos Aires, Argentina	Quantitative GIS Land Cover Analysis (100m and 300m)	Positive
Rey et al. 2006	Manatee, Miami-Dade, & Palm Beach Co., FL, USA	Quantitative GIS Land Cover Analysis (100m)	Positive
Tsuda et al. 2006	Chiangmai Province, Thailand	Qualitative (Urban, Transition, Rural)	Positive
Honorio et al. 2009	Rio de Janeiro, Brazil	Qualitative (Urban, Low Vegetation, Medium Vegetation, High Vegetation)	Positive
Higa et al. 2010	Vietnam	Qualitative (Urban, Transition, Rural)	Mixed (regional variation)
Fatima et al. 2016	Pakistan	Quantitative GIS Land Cover Analysis (30m)	Positive
Zahouli et al. 2016	Cote D'Ivoire	Qualitative (Urban, Suburban, Rural)	Positive
Ndenga et al. 2017	Kenya	Qualitative (Urban, rural)	Positive
Overgaard et al. 2017	Colombia	Qualitative (Urban, rural)	Positive
Dalpadado et al. 2018	Gampaha District, Sri Lanka	Qualitative (Urban, Suburban, Rural)	Positive
Estallo et al. 2018	Cordoba, Argentina	Quantitative GIS Land Cover Analysis (10m)	Positive
Talaga et al. 2020	Kourou, French Guiana	Quantitative GIS Land Cover Analysis (70m)	Moderately Urbanized site had highest density

Table S2. Previous studies on *Ae. albopictus* abundance and urbanization. Study number indicates study location and result in Figure 8.

#	Study	Location	Urbanization Metric	Urbanization Correlation w/*Ae. albopictus* Abundance
1	**Hornby et al. 1994**	Lee County, FL, USA	Qualitative (Urban, Suburban)	Negative
2	**Barker et al. 2003**	Virginia, USA	Qualitative (Forest, Yard Bordering Forest, Yard)	Positive (Unforested sites had highest abundance)
3	**Braks et al. 2003**	Rio de Janeiro & Nova Iguacu, Brazil; Boca Raton & West Palm Beach, FL, USA	Qualitative (Urban, suburban, rural)	Negative
4	**Rey et al. 2006**	Manatee, Miami-Dade, & Palm Beach Co., FL, USA	Quantitative GIS Land Cover Analysis (100m)	Negative
5	**Tsuda et al. 2006**	Chiangmai Province, Thailand	Qualitative (Urban, Transition, Rural)	Negative
6	**Honorio et al. 2009**	Rio de Janeiro, Brazil	Qualitative (Urban, Low Vegetation, Medium Vegetation, High Vegetation)	Negative
7	**Higa et al. 2010**	Vietnam	Qualitative (Urban,	None

			Transition, Rural)	
8	**Bagny et al. 2012**	Mayotte, France	Quantitative GIS Land Cover Analysis (25m)	Positive
9	**Li et al. 2014**	Guangzho, China	Qualitative (Urban, suburban, rural)	Positive
10	**Samson et al. 2015**	Cap-Haitien, Haiti	Quantitative GIS Land Cover Analysis (6.5m)	Positive
11	**Baldacchino et al. 2017**	Belluno & Trento, Italy	Quantitative GIS Land Cover Analysis (250m)	Positive
12	**Dalpadado et al. 2018**	Gampaha District, Sri Lanka	Qualitative (Urban, Suburban, Rural)	Negative
13	**McClure et al. 2018**	Big Island, Hawaii, USA	Quantitative GIS Land Cover Analysis	Positive
14	**Arduino et al. 2020**	Sao Paulo, Brazil	Qualitative (Urban, Forest, Grass-Shrubs)	Positive
15	**Westby et al. 2021**	St. Louis, Missouri, USA	Qualitative (Urban, Suburban, Rural)	Positive (Urban & Suburban equal, but both higher than rural)

Table S3: Sequence of primers used for viral detection.

Primers	Sequence (5'-3')	References
ZIKV-forward	nt9271-AARTACACATACCARAACAAAgTggT9297	(Lanciotti *et al.*, 2008)
ZIKV-reverse	nt9352-TCCRCTCCCYCTYTggTCTTg-9373	
ZIKV-probe	nt9304-FAM-CTYAgACCAgCTgAAR-BBQ-9320	
CHIKV-forward	AAGCTYCGCGTCCTTTACCAAG	(Pastorino *et al.*, 2005)
CHIKV-reverse	CCAAATTGTCCYGGTCTTCCT	
CHIKV-probe	FAM-CCAATGTCYTCMGCCTGGACACCTTT-TAMRA	
DENV-forward	AGGACYAGAGGTTAGAGGAGA	(Leparc-Goffart *et al.*, 2009)
DENV-reverse	CGYTCTGTTGCCTGGAWTGAT	
DENV-probe	FAM-ACAGCATATTGACGCTGGGARAGACC-TAMRA	

Table S4: Average hourly mosquito collection rate by city and species.

City	*A. aegypti* females/hour	**SD of** *A. aegypti* females/hour	*A. albopictus* females/hour	**SD of** *A. albopictus* females/hour
Douala	18.2	29.7	8.9	10.3
Garoua	2.3	3.1	0.0	0.0

Kribi	4.4	11.1	13.2	11.3
Maroua	1.4	2.1	0.0	0.0
Ngaoundere	1.2	1.3	0.0	0.0
Yaounde	0.7	2.4	21.7	30.6

Table S5: Hourly collection rate of mosquitoes from each collection site. Urbanization indices are included for each location.

Site	City	UI 100M	UI 200M	UI 500M	UI 1KM	UI 2KM	*A. aegypti* females/hr	*A. albopictus* females/hr
Akwa	Douala	96	90	90	85	81	5.3	9.7
Bepanda	Douala	85	85	87	88	92	7.2	0.0
Bonaberi	Douala	97	93	91	76	64	21.5	17.0
Brazzaville	Douala	100	100	98	92	89	10.0	0.0
Deido	Douala	100	100	98	91	78	67.0	2.3
Kotto	Douala	100	99	98	93	79	2.7	19.3
Logbessou	Douala	75	86	88	59	58	15.7	10.5
New Bell	Douala	19	30	70	80	88	23.7	0.7
Ngodji	Douala	0	0	0	0	5	0.0	5.3
Yassa	Douala	24	25	23	25	22	3.0	29.3
Bockle-Siha	Garoua	0	0	0	1	1	0.0	0.0
Camp-Chinois	Garoua	97	97	74	63	42	1.3	0.0
Foulbere	Garoua	100	98	98	83	54	6.5	0.0
Lainde	Garoua	56	51	40	50	36	3.5	0.0
Nassarao	Garoua	67	54	43	21	14	1.7	0.0
Poumpoumre	Garoua	35	32	56	61	50	5.2	0.0
Rocade	Garoua	43	17	7	16	22	3.3	0.0

Roumde Adjia	Garoua	100	96	63	58	61	0.0	0.0
Roumde-Adja	Garoua	100	96	63	58	61	0.0	0.0
Sanguere-Paul	Garoua	0	0	0	2	1	0.0	0.0
Yelwa	Garoua	100	98	85	68	54	0.8	0.0
Zoological Garden	Garoua	53	43	63	70	57	2.7	0.0
Bebwambé	Kribi	0	5	13	17	20	0.6	11.6
Bikondo	Kribi	0	0	0	1	2	0.0	2.0
Dombe	Kribi	85	77	68	55	32	1.0	11.7
Kingué 1	Kribi	1	14	29	37	32	0.0	0.0
Kingué 2	Kribi	96	89	75	69	47	43.7	2.0
Londji	Kribi	0	0	3	21	25	1.9	21.5
Mboamanga	Kribi	91	83	60	52	42	17.0	22.5
Mokolo	Kribi	63	58	61	56	43	1.0	13.3
Ngoyé	Kribi	74	63	57	58	51	1.2	10.5
Polongwe	Kribi	34	12	2	2	6	1.1	13.9
Zaire	Kribi	100	94	73	57	35	2.2	10.7
Comice	Maroua	88	93	76	55	43	0.7	0.0
Djarengol	Maroua	64	56	49	51	43	3.0	0.0
Domayo	Maroua	74	55	58	71	52	0.5	0.0
Dougoi	Maroua	93	88	79	79	62	1.6	0.0
Florina	Maroua	0	0	2	1	8	0.5	0.0
Louggol	Maroua	0	0	0	0	0	0.2	0.0
Makabaye	Maroua	41	34	19	12	13	0.0	0.0
Salak	Maroua	16	22	13	10	7	4.3	0.0
Zaika	Maroua	1	0	0	1	8	0.3	0.0
Bamnyanga	Ngaoundere	46	31	24	14	18	1.3	0.0

Beka Hosere	Ngaoun dere	0	0	2	3	12	1.0	0.0
Burkin a	Ngaoun dere	100	98	82	54	31	0.3	0.0
Djakbo l	Ngaoun dere	60	62	72	61	59	1.2	0.0
Gada Maban ga	Ngaoun dere	0	0	1	2	12	0.8	0.0
Joli Soir	Ngaoun dere	100	100	98	87	65	0.8	0.0
Mabor no	Ngaoun dere	50	32	14	10	6	0.3	0.0
Madag ascar	Ngaoun dere	96	98	93	79	54	0.7	0.0
Mboun djere	Ngaoun dere	100	100	88	79	65	4.3	0.0
Sabong ari	Ngaoun dere	93	83	57	53	41	2.0	0.0
Afanoy oa I	Yaound e	0	0	0	1	1	0.0	5.1
Afanoy oa II	Yaound e	25	10	2	1	1	0.3	8.9
Essos	Yaound e	100	100	98	96	91	0.3	28.2
Mendo ng	Yaound e	89	86	85	84	68	0.0	24.8
Messa	Yaound e	79	66	81	86	86	5.5	16.3
Mvog-Ada	Yaound e	100	100	93	95	86	1.1	24.8
Nkolbis son	Yaound e	69	62	43	38	48	0.1	18.2
Omnis port	Yaound e	99	89	85	93	92	0.1	66.4
Tsinga	Yaound e	63	72	58	54	43	0.2	16.1
Zamass i	Yaound e	0	4	1	0	0	0.1	0.9

Table S6: Analysis of *A. aegypti* abundance with urbanization index (UI), temperature, humidity, and prior month's rain in six cities as predictors using a generalized linear model with negative binomial distribution and a log link. The city of Douala was the reference level.

Predictor	Estimate	SE	Z-Value	P
(Intercept)	6.075	2.80	2.17	0.030
UI_1KM	0.026	0.0044	5.80	≤0.001
Monthly_Rain (mm)	0.0027	0.0011	2.52	0.012
City of Garoua	-0.39	0.75	-0.52	0.61
City of Kribi	-0.98	0.71	-1.39	0.17
City of Maroua	-1.49	0.73	-2.03	0.042
City of Ngaoundere	-2.40	0.82	-2.92	0.0035
City of Yaounde	-3.91	0.64	-6.12	≤0.001
Temperature	-0.18	0.070	-2.62	0.0088
Humidity	-0.0068	0.011	-0.60	0.55

Table S7: Analysis of *A. albopictus* abundance with urbanization index (UI) interacting with city and temperature, humidity, and prior month's rain in three cities as predictors with a generalized linear model with a negative binomial distribution and a log link. The city of Douala was the reference level.

Predictor	Estimate	SE	Z- value	P
(Intercept)	-0.314	2.45	-0.13	0.90
UI_2KM	-0.023	0.012	-1.96	0.049
City of Kribi	-1.076	0.96	-1.12	0.26
City of Yaounde	-1.924	0.86	-2.23	0.026
Monthly_Rain (mm)	0.0013	0.00067	2.0080	0.045
Temperature	0.072	0.057	1.25	0.21
Humidity	0.021	0.010	2.015	0.044
UI_2KM:Kribi	0.021	0.019	1.10	0.27
UI_2KM:Yaounde	0.047	0.012	3.93	≤0.001

Table S8. Analysis of temperature in response to urbanization index (UI) at 100m interacting with city as predictors with a generalized linear model with a negative binomial distribution and a log link. The city of Kribi was the reference level.

Predictor	Estimate	SE	Z- value	P
(Intercept)	3.33	0.059	56.41	≤0.001
UI_100M	-0.00026	0.0010	-0.27	0.79
City of Douala	0.045	0.14	0.33	0.74
City of Yaounde	-0.094	0.070	-1.33	0.18
City of Ngaoundere	-0.11	0.11	-1.06	0.29
City of Garoua	0.15	0.095	1.55	0.12
City of Maroua	0.010	0.089	0.12	0.91
UI_100M:Douala	0.000062	0.0018	0.034	0.97
UI_100M:Yaounde	0.00050	0.0011	0.45	0.65
UI_100M:Ngaoundere	0.000046	0.0015	0.030	0.98
UI_100M:Garoua	-0.00024	0.0014	-0.17	0.87
UI_100M:Maroua	0.00011	0.0015	0.074	0.94

Table S9. Analysis of humidity in response to urbanization index (UI) at 100m interacting with city as predictors with a generalized linear model with a negative binomial distribution and a log link. The city of Kribi was the reference level.

Predictor	Estimate	SE	Z- value	P
(Intercept)	4.41	0.076	58.00	≤0.001
UI_100M	-0.00034	0.0013	-0.27	0.79
City of Douala	-0.089	0.18	-0.49	0.62
City of Yaounde	-0.052	0.090	-0.58	0.56
City of Ngaoundere	-0.16	0.13	-1.16	0.25
City of Garoua	-0.25	0.13	-1.92	0.055
City of Maroua	-0.23	0.12	-1.96	0.050
UI_100M:Douala	0.0011	0.0024	0.48	0.63
UI_100M:Yaounde	0.000024	0.0014	0.017	0.99
UI_100M:Ngaoundere	-0.00077	0.0019	-0.40	0.69
UI_100M:Garoua	0.00097	0.0020	0.50	0.62
UI_100M:Maroua	0.0017	0.0019	0.886	0.38

Table S10: Compiled Minimum Infection Rates (MIR) for studies which detected Zika virus in field caught specimens of *A. aegypti* and *A. albopictus*.

Study	Country/ Location	Species	Zika+ Pools	Total Pools	Specimens Tested	Max Pool Size	MIR
Marchette 1969	Malaysia	*A. aegypti*	1	58	1277	80	0.8
Grard 2014	Gabon	*A. albopictus*	2	91	2130	25	0.9
Ferreira-de-Brito 2016	Brazil	*A. aegypti*	3	198	550	10	5.5
Guerbois 2016	Mexico	*A. aegypti*	15	55	279	NA	53.8
Ho 2017	Singapore	*A. aegypti* and *A. albopictus* **	9	517	1375	5	6.5
Cevallos 2018	Ecuador	*A. aegypti*	2	14	193	10	10.4
Correa-Morales 2019	Mexico	*A. aegypti*	260	3120	14,145	Data Unavailable	18.4
Correa-Morales 2019	Mexico	*A. albopictus*	7	52	78	Data Unavailable	89.7
Singh 2019	India	*A. aegypti*	3	55	203	10	14.8
Ali 2020	Malaysia	*A. albopictus*	6	NA	186	25	32.3
Calle-Tobon 2020	Colombia	*A. aegypti*	98	Data Unavailable	6585	10	14.9
Campos 2020	Cape Verde	*A. aegypti*	2	Not Pooled	816	1	2.5
Kosoltanapawit 2020	Thailand	*A. aegypti*	2	Not Pooled	130	1	15.4
Phumee 2020*	Thailand	*A. aegypti*	Data Unavailable	Data Unavailable	Data Unavailable	10	22.4
Parra 2022	Sao Paulo, Brazil	*A. aegypti*	55	607	1026	10	53.6
Parra 2022	Sao Paulo, Brazil	*A. albopictus*	1	11	12	10	83.3

This Study	Cameroon	*A. albopictus*	1	289	7490	30	0.1
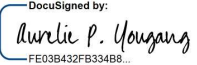
• Study provided an MIR without number of specimens or pools tested.

* Study data did not allow for differentiation of *A. aegypti* and *A. albopictus* so results were combined.

Appendix 2

The text of this dissertation includes a reprint of the following previously published material: Montgomery, M., Harwood, J.F., Yougang, A.P. *et al.* Spatial distribution of insecticide resistant populations of *Aedes aegypti* and *Ae. albopictus* and first detection of V410L mutation in *Ae. aegypti* from Cameroon. *Infect Dis Poverty* **11**, 90 (2022). The co-authors listed in this publication have approved of its inclusion.

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Appendix 3

Table S1. Human viremia data. CHIKV (Appassakij et al 2013, Riswari et al. 2015), DENV1-4 (Nguyen et al. 2013), YFV (Nassar et al. 1995, Monath et al. 2012), ZIKV (Mansuy et al. 2017).

Day	Viremia	SE	SD	N	Virus
1	5.35	0.15	0.21	2	YFV
2	4.06	0.61	1.84	9	YFV
3	4.24	0.50	1.41	8	YFV
4	2.44	0.36	0.80	5	YFV
5	1.90	0.40	0.57	2	YFV
6	2.50	0.40*	0.96	1	YFV
1	8.20	0.35	NA	2	CHIKV
2	7.81	0.61	NA	11	CHIKV
3	5.57	1.49	NA	6	CHIKV
4	4.46	1.40	NA	8	CHIKV
5	3.15	0.55	NA	3	CHIKV
6	2.45	0.46	NA	5	CHIKV
7	2.63	NA	NA	1	CHIKV
11	1.08	NA	NA	1	CHIKV
13	2.98	NA	NA	1	CHIKV
17	1.86	NA	NA	1	CHIKV
-2	5.80	0.63	1.26	1	ZIKV
0	4.53	0.57	1.13	4	ZIKV
1	4.91	0.40	0.89	5	ZIKV
2	4.26	0.35	1.09	10	ZIKV
3	3.74	0.28	0.69	6	ZIKV
4	3.65	1.10	1.91	3	ZIKV
5	3.61	1.08	1.87	3	ZIKV
7	1.70	NA	NA	1	ZIKV

10	1.85	NA	NA	1	ZIKV
1	8.56	NA	NA	1	DENV3
2	7.81	0.30	1.25	18	DENV1
2	8.01	0.25	1.13	20	DENV2
2	8.74	0.25	0.71	8	DENV3
2	6.86	0.74	1.28	3	DENV4
3	8.17	0.15	1.24	65	DENV1
3	7.59	0.14	1.24	74	DENV2
3	7.99	0.26	1.04	16	DENV3
3	6.75	0.46	1.66	13	DENV4
4	7.79	0.17	1.46	78	DENV1
4	6.85	0.16	1.41	83	DENV2
4	7.52	0.33	1.65	25	DENV3
4	6.06	0.42	1.96	22	DENV4
5	6.67	0.18	1.57	78	DENV1
5	5.44	0.16	1.41	83	DENV2
5	6.33	0.31	1.53	25	DENV3
5	4.98	0.40	1.87	22	DENV4
6	5.44	0.14	1.19	77	DENV1

6	3.95	0.14	1.24	82	DENV2
6	4.77	0.36	1.75	24	DENV3
6	3.47	0.24	1.12	21	DENV4
7	4.50	0.11	0.87	60	DENV1
7	3.01	0.14	1.13	61	DENV2
7	3.62	0.34	1.39	17	DENV3
7	2.86	0.00	0.00	19	DENV4
8	3.77	0.31	0.96	10	DENV1
8	2.59	0.40	1.14	8	DENV2
8	2.93	0.37	0.99	7	DENV3
8	2.86	NA	NA	9	DENV4

*Average SE for prior 5 days of YFV.

Table S2. Analysis of viremia with day and virus as predictors. Day 1 and CHIKV were the reference levels.

	Estimate	Std. Error	t-Value	P-Value
(Intercept)	7.2730	0.4176	17.416	< 2e-16
VirusDENV1	-1.8500	0.4511	-4.101	0.000289
VirusDENV2	-1.9128	0.4511	-4.241	0.000196
VirusDENV3	-1.5284	0.4511	-3.388	0.001983
VirusDENV4	-1.5085	0.4511	-3.344	0.002227
VirusYFV	-1.8596	0.4511	-4.123	0.000272
VirusZIKV	-1.4701	0.4511	-3.259	0.002779

	Estimate	Std. Error	t-Value	P-Value
Day2	-0.5155	0.4176	-1.235	0.226592
Day3	-1.3048	0.4176	-3.124	0.003931
Day4	-2.2862	0.4176	-5.474	6.1E-06
Day5	-3.1883	0.4176	-7.635	1.63E-08
Day6	-4.7024	0.4176	11.260	2.69E-12

Table S3. Results from a Bayesian model fit to vector competence data. Coefficients were fit for the mean extrinsic incubation period (EIP) in days, including virus, mosquito species, and viremia, with A. aegypti and ZIKV as the reference level. The model also included two additional study ID intercept parameters (ID1 for *A. albopictus* transmitting CHIV, and ID2 for *A. aegypti* transmitting ZIKV) to account for substantial differences among these two individual studies and all others in the fraction transmitting.

	Mean EIP	SE	95% CI
Intercept (*A. aegypti*, Zika virus)	2.64	0.12	2.41 - 2.87
A. albopictus	0.11	0.052	0.0080 - 0.21
CHIKV	-0.33	0.066	-0.46 - -0.21
YFV	0.26	0.055	0.15 - 0.37
Viremia (PFU/ml)	0.18	0.021	0.14 - 0.23

	Mean EIP	SE	95% CI
ID1 (*A. albopictus* CHIKV)	-2.24	0.28	-2.78 - -1.70
ID2 (*A. aegypti* ZIKA)	-0.89	0.16	-1.20 - -0.58

Table S4. Analysis of seroprevalence data for Africa using a generalized linear model with a binomial distribution and a logit link with virus type as a fixed effect and study ID as a random effect. CHIKV was the reference level.

	Estimate	Std. Error	z value	P-value
Intercept (CHIKV)	-1.47	0.20	-7.24	< 0.001
DEN-1	-1.40	0.046	30.68	< 0.001
DEN-2	-0.71	0.053	13.39	< 0.001
DEN-3	-0.58	0.10	-6.08	< 0.001
DEN-4	-0.86	0.10	-8.33	< 0.001
YFV	-0.10	0.022	-4.60	< 0.001
ZIKV	-0.81	0.024	33.87	< 0.001

Table S5. Analysis of seroprevalence data for the Americas using a generalized linear model with a binomial distribution and a logit link with virus type as a fixed effect and study ID as a random effect. CHIKV was the reference level..

	Estimate	Std. Error	z value	P-value
Intercept (CHIKV)	-1.76	0.61	-2.90	0.0038
DEN-1	0.031	0.71	0.043	0.97

	Estimate	Std. Error	z value	P-value
DEN-2	0.57	0.71	0.80	0.42
DEN-3	-0.15	0.71	-0.21	0.83
DEN-4	-1.28	0.71	-1.81	0.07
ZIKV	0.70	0.068	10.44	< 0.001

Table S6. Analysis of seroprevalence data for Asia using a generalized linear model with a binomial distribution and a logit link with virus type as a fixed effect and study ID as a random effect. CHIKV was the reference level.

	Estimate	Std. Error	z value	P-value
Intercept (CHIKV)	-1.13	0.05	-22.12	< 0.001
DEN-1	-2.072	0.08	-24.74	< 0.001
DEN-2	-0.74	0.08	-9.68	< 0.001
DEN-3	-0.20	0.08	-2.44	0.014
DEN-4	-2.093	0.09	-23.18	< 0.001
YFV	-6.64	1.31	-5.078	< 0.001
ZIKV	-1.087	0.31	-3.55	< 0.001
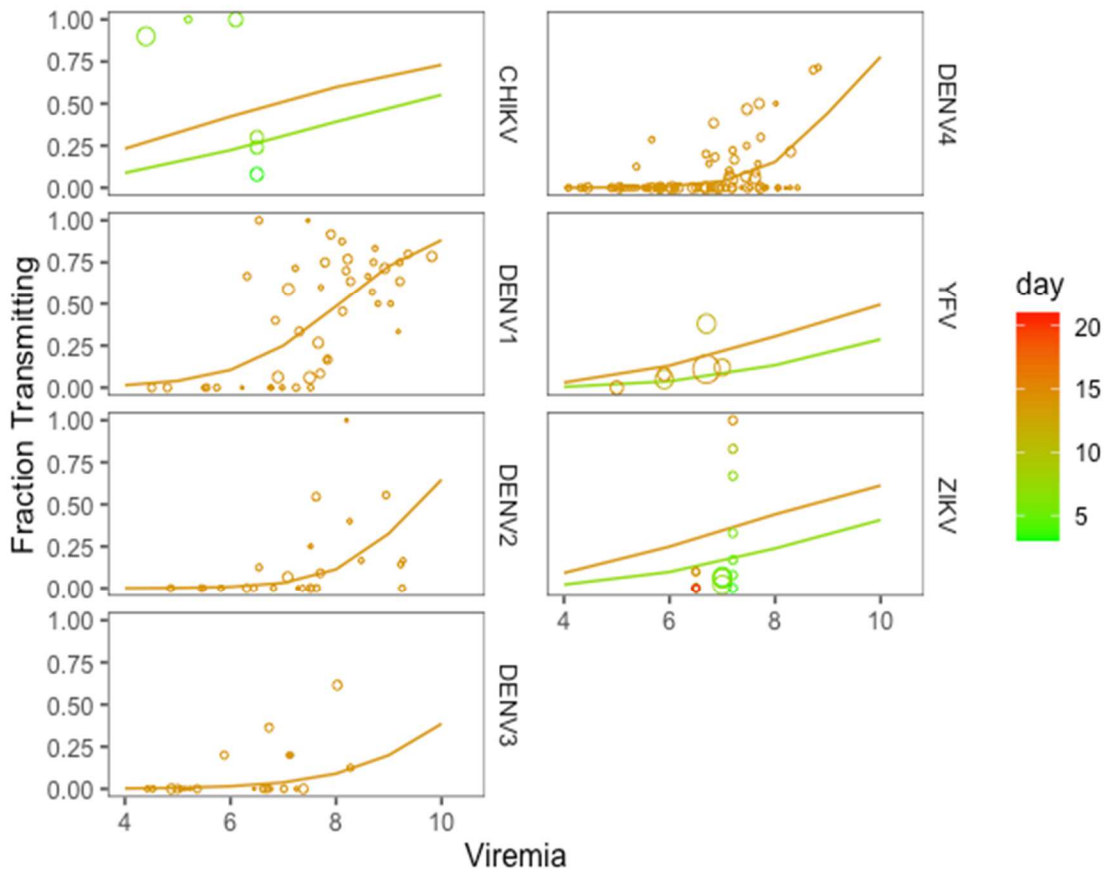
Figure S1. Predicted fraction of *A. albopictus* transmitting virus by blood meal viremia. Circles represent vector competence study data with circle size corresponding to *A. aegypti* sample size. Data for CHIKV, YFV, and ZIKV are plotted for days 7 and 14 post-feeding. DENV1-4 was plotted for day 14 since source study data only measured vector competence on day 14.
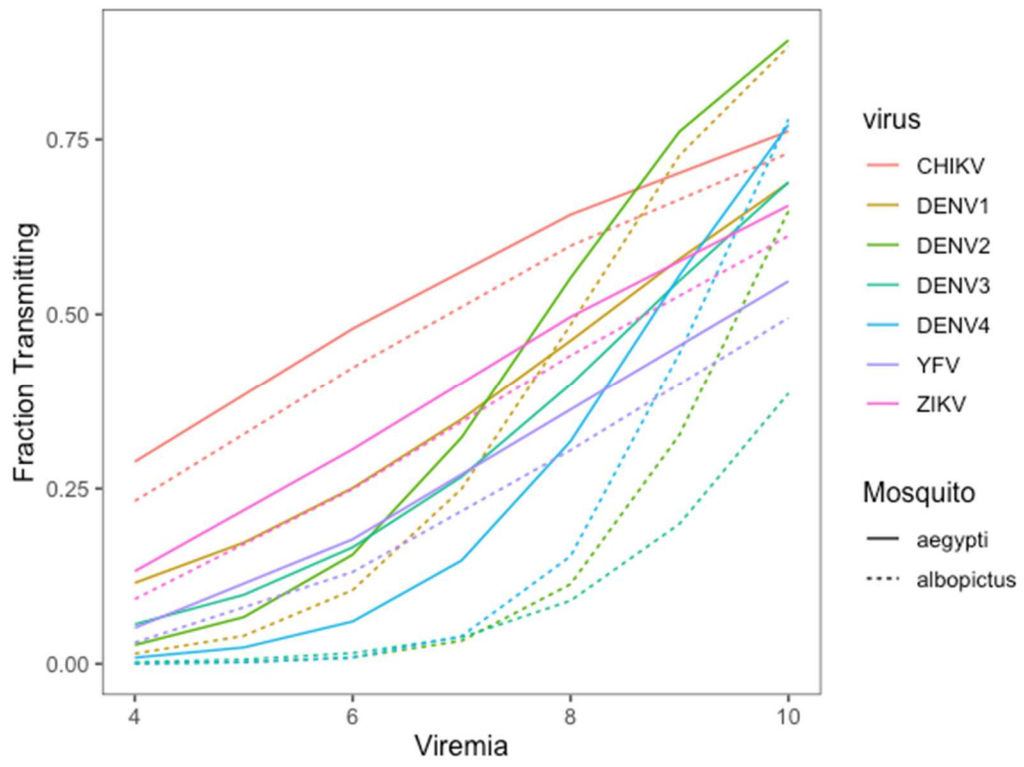
Figure S2. Predicted fraction of mosquitoes transmitting virus by blood meal viremia on day 14.

Figure S3. Number of serosurveys and total sample size by country.
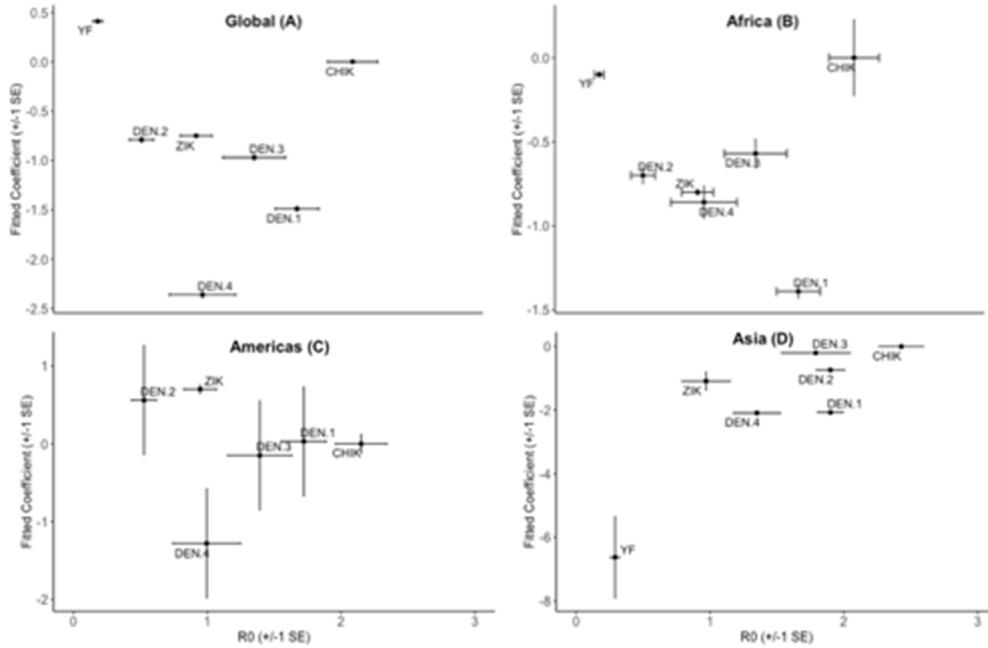
Figure S4. Fitted coefficient of arbovirus seroprevalence plotted against relative R_0 estimates for *A. albopictus*. The relationship was not statistically significant in any location: global (Fitted Coefficient = -0.61 - 0.21 (±0.59)* R_0, (P = 0.74), Africa (Fitted Coefficient = -0.55 - 0.067 (±0.31)* R_0, (P = 0.84), the Americas (Fitted Coefficient = -2.91 + 0.71 (±1.59)* R_0, (P = 0.68), or Asia (Fitted Coefficient = -4.25 + 2.14 (±1.15)* R_0, (P = 0.12). YFV was not included in the Americas due to a lack of serological data.

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