THE INFLUENCE OF AEDES AEGYPTI (DIPTERA: CULICIDAE) FEEDING PATTERNS AND ABUNDANCE ON ARBOVIRAL TRANSMISSION

A Dissertation

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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August 2020

Major Subject: Entomology

ABSTRACT

Mosquito-borne viruses are emerging or re-emerging globally, afflicting millions of people around the world. *Aedes aegypti*, the yellow fever mosquito, is the principal vector of dengue, Zika, and chikungunya viruses, and has well-established populations across tropical and subtropical urban areas of the world, including the southern United States. While intense arboviral epidemics have occurred in Mexico and further south in the Americas, local transmission in the United States has been minimal. In Chapter II of this dissertation, I investigated the degree to which South Texas Ae. aegypti feed on sugar sources to help inform vectorial capacity. I analyzed sugar feeding patterns of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes using the cold and hot anthrone test. While this research affirms earlier studies that sugar feeding among female *Ae. aegypti* is limited, I also present evidence that the frequency of fructose feeding is only slightly less than that of male *Ae. aegypti*. In Chapter III, I investigated the host feeding patterns of *Ae. aegypti* to determine the degree of anthropophagy. Surprisingly, only 31% of *Ae. aegypti* blood meals were derived from humans, while 50% were from dogs and 19% from other wild and domestic animals. Using mosquito and human case data on both sides of the border, I modeled the reproductive number of Zika virus to show that wasted bites on non-amplification hosts in South Texas diluted virus transmission. In Chapter IV, I explored the global patterns of 'aegyptism without arbovirus' using geographic information system (GIS) and spatial modeling. I present a global map showing a gradient from high suitability for *Ae. aegypti* but low suitability for dengue to

the other end of the spectrum where areas have similar and higher suitability for both *Ae. aegypti* and dengue. I then use a generalized additive model to assess the correlation between this deviation and population density, gross domestic product, infant mortality rate, temperature, precipitation, and elevation. This analysis yielded several significant relationships between the deviation values and environmental and demographic factors. This dissertation advanced the field in understanding the basic biology of the world's most important mosquito vector of viruses.

DEDICATION

To God, Creator of all things, whose infinite wisdom is displayed in each sunset, every wave that crashes on the shore, every star in this vast universe, every rumble of thunder, and even in the miraculous design of the mosquito. May I never lose the wonder and enjoyment of it all…

To my wife, Tina, who has faithfully followed me around the world on one amazing adventure after another, and for being my greatest fan, supporter, and friend. When I lose my way, you have always been there to point me in the right direction…

And to my children, Hannah, Rachel, Sarah, Abby and Caleb. Your words of encouragement, help in memorizing insect biodiversity, and enthusiasm in catching insects along the way has been such a delight!

ACKNOWLEDGEMENTS

First, I would like to acknowledge and thank my Lord and Savior Jesus Christ, without whom I would never had made it through this program. I was often reminded from God's Word along the way that "nothing will be impossible with God (Luke 1:37)," and "I can do all things through him who strengthens me (Philippians 4:13)."

I would also like to thank my committee chair and academic advisor, Dr. Gabe Hamer. Taking me on as a PhD student was no small undertaking. You have shown me an amazing level of patience and your drive for excellence has truly been inspiring. Also, your love for the outdoors, wildlife, hunting, and fishing, and your willingness to share that with me made this a truly enjoyable time! You and Sarah make an excellent team. On that note, I would also like to thank another member of my PhD committee, Dr. Sarah Hamer. I've learned so much from your leadership and excellence in academia. I am truly amazed at how much the two of you are able to accomplish, all while being great parents to your four young children! Incredible! Dr. Micky Eubanks, I truly appreciate the way you challenged me throughout the course of this program. I needed that! I believe you recognized I had a steep learning curve and you pushed me to go beyond what I thought I was capable of. The most important thing is that you really took the time to thoroughly review my work and made many excellent recommendations. Thank you! Dr. Rudy Bueno, I appreciate your inspiration and guidance along the way.

You are a great example of how to apply knowledge to practice. Having you on my committee has been such a blessing.

Additionally, I would like to thank my fellow students and co-workers in the Hamer Lab. Thank you, Wendy, for all the help and assistance you gave while I was running experiments in the lab. Your kindness is greatly appreciated. Thanks to Selene Garcia-Luna, Cierra Briggs, Helena Hopson, and Victoria Kamilar for their assistance with mosquito processing and PCRs. Estelle Martin, thank you for spending many hours helping me develop and troubleshoot the protocol. I truly enjoyed working with you. Thanks to another fellow student, Dr. Karen Poh. Karen, you were a great source of encouragement, especially as I bemoaned the classes I thought I was failing. You always said "B's get degrees!" You were correct; I'm living proof! Willy, I would have never been able to complete Chapter 4 without your help in QGIS. Thank you for taking the time to share your wisdom and skills. Also, thanks for making me laugh, so often. Every Ph.D student needs a 'Willy' to keep things light in a stressful academic program like this!

CONTRIBUTORS AND FUNDING SOURCES

Contributors

The research, data collection, experimentation and data analysis comprising this work was supervised by a dissertation committee consisting of the chair of the committee, Dr. Gabriel L. Hamer of the Department of Entomology, and the members of the committee, Dr. Micky Eubanks, Department of Entomology, Dr. Rudy Bueno, Department of Entomology, and Dr. Sarah A. Hamer, Department of Veterinary Integrative Biosciences.

The mathematical modeling of reproductive rate depicted in Chapter 3 was provided by Dr. Martial Ndeffo-Mbah, Department of Veterinary Integrative Biosciences. Statistical analyses of mosquito wing length and sugar content depicted in Chapter 2 were conducted by Jose Juarez.

All other work conducted for the dissertation was completed by the student independently.

Funding Sources

Graduate study was supported by the Air Force Institute of Technology, Civilian Institution Program.

This work was also made possible in part by National Institutes of Health under grant number R21AI128953 and K01AI128005, Texas A&M AgriLife Research Insect-Vectored Diseases Seed Grant, and the U.S. Department of Energy by Lawrence

Livermore National Laboratory under Contract DE-AC52-07NA27344. Additional funding for the blood meal analysis described in Chapter 3 was supported by Cooperative Agreement Number U01CK000512 and contract 200-2017-93141, funded by the Centers for Disease Control and Prevention. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the United States Air Force, the Department of Defense, the U.S. Department of Energy, the National Institutes of Health, the Centers for Disease Control and Prevention or Texas A&M AgriLife Research.

The views expressed in this dissertation are those of the author and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government.

NOMENCLATURE

AGO	Autocidal Gravid Ovitrap
BGS2	Biogents Sentinel 2 trap
CHIKV	Chikungunya virus
DEN	Dengue
DENV	Dengue virus
DNA	Deoxyribonucleic acid
LRGV	Lower Rio Grande Valley
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
R_0	Reproductive rate
RNA	Ribonucleic acid
SEM	Standard Error of the Mean
WNV	West Nile virus
ZIKV	Zika virus

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1. INTRODUCTION

1.1. Problem Background and Significance

Despite a century of research and a wide array of strategies to minimize mosquito-borne diseases, the transmission of pathogens vectored by mosquitoes is occurring globally at an accelerating rate. For example, in 2018 there were an estimated 228 million cases of malaria worldwide, resulting in about 405,000 deaths [1]. Additionally, over half the world's population is at risk of dengue and approximately 10,000 deaths per year are attributed to this arbovirus which is primarily transmitted by *Aedes aegypti* mosquitoes [2]. In the continental United States, West Nile virus (WNV) is the preeminent mosquito-borne disease afflicting approximately 7 million persons between 2000 and 2016[3].

The U.S. Centers for Disease Control and Prevention recently announced that vector-borne disease cases in the U.S. from mosquito, tick and flea bites have more than tripled from 2004 to 2016 [4]. This is in part due to an exponential increase of movement of people and goods internationally, but also heterogeneity in vector host interactions which influence vectorial capacity and the likelihood of arthropod-borne pathogen transmission. Mosquitoes and the pathogens they carry are indifferent to international and political boundaries, thus, pathogens that are endemic in one region can easily spread to neighboring countries [5]. *Aedes aegypti* and *Cx. quinquefasciatus* are two globally important species of mosquito responsible for the spread of a variety of human diseases such as Zika, dengue, chikungunya and WNV. These two species are commonly

found in the Lower Rio Grande Valley (LRGV), a region with high risk for arbovirus transmission.

1.2. Mosquito classification and biology

Mosquitoes are found virtually everywhere, on every continent except Antarctica and are comprised of approximately 3,500 species spanning 41 genera in the family Culicidae within the dipteran order, hexapod class, in the arthropod phylum of the animal kingdom [6]. The Culicidae family is further divided into two subfamilies, the Anophelinae which contain *Anopheles, Bironella,* and *Chagasia* genera and the Culicinae containing all other genera [6]. Mosquitoes can be differentiated by distinct physical and morphological characteristics. For example, the strongly recurved shape of the proboscis of *Toxorhynchites septentrionalis* and its ability to produce eggs without a blood meal differentiates it from all other mosquito species. Other features, such as wing venation, palp length, leg, proboscis and abdominal banding, as well as scale patterns also aid in taxonomy. For species that are morphologically indistinguishable, DNA extraction and amplification by PCR is now widely used for accurate identification.

Although both *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes are ubiquitous world-wide, the pathogens they carry and the rate of spillover into human populations vary from place to place and season to season. This heterogeneity of vector-borne pathogen transmission can be attributed to many environmental and biological factors, of which sugar feeding and blood feeding are two essential elements. Sugar feeding provides the necessary fuel for flight [7] and has also been shown to have a positive

association with survival and mating success [8,9]. The frequency of sugar feeding, which depends upon nectar and sugar source availability, could impact biting rates and survival, two main components affecting the ability of mosquitoes to transmit viruses, or the vectorial capacity [10]. Furthermore, there is strong evidence for heterogeneity among mosquito species in host preference for blood meals. *Culex* mosquitoes often exhibit ornithophilic feeding preference [11,12] whereas *Aedes* mosquitoes tend to feed more frequently on mammals [13]. *Aedes aegypti*, the yellow fever mosquito, is universally considered anthropophilic and some researchers have even suggested it may forego sugar feeding in favor of a blood meal [14,15].

Currently, very little data exists on sugar and blood feeding for these species in South Texas which is necessary to identify conditions that promote or inhibit virus amplification. By studying the feeding patterns of these two species, a greater understanding of their biology and host preference can be gained, informing new or revised strategies for mosquito surveillance and control thereby improving our ability to better manage mosquito populations and reduce viral spillover to human populations.

1.3. Rationale

Viral transmission within the endemic or enzootic cycle, and the involvement of humans in the amplification process or through spill-over transmission is closely linked to the feeding patterns of mosquito vectors. Many studies have described the feeding activity of *Ae. aegypti* as it relates to reproductive behavior [9,16], feeding periodicity and frequency [17-19], patterns of blood meal host selection [19-21], fructose feeding patterns

[14,22], and host biting as it relates to sugar feeding and climate [23]. Likewise, *Cx. quinquefasciatus* blood feeding patterns have also been described [24-30] from various locations around the world. Although blood feeding and sugar feeding are inextricably linked [8], a comprehensive look at sugar and blood feeding of *Cx. quinquefasciatus* and *Ae. aegypti* mosquitos has rarely been conducted in the same study site and at the same time. Establishing this baseline information for the LRGV in South Texas will allow us to compare data with surveillance across the border in Mexico, potentially informing drivers of arbovirus transmission in Mexico and the southern U.S.

1.4. Study location for chapters II and III

The Lower Rio Grande Valley (LRGV) is a region in South Texas along the US-Mexico border (Figure 1.1). The LRGV and has an estimated population of 1,370,424 with about 92% being Hispanic (US Census Bureau, 2017).

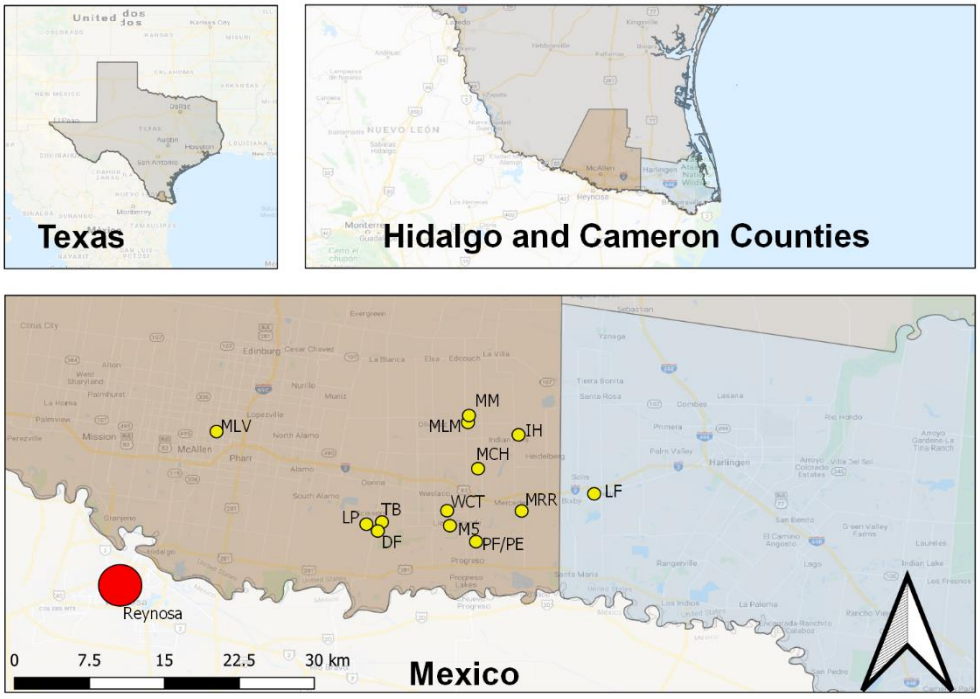

Figure 1.1 Map of study area and sugar study trapping locations.

Temperatures in the LRGV range from an average low of 50º F (10º C) in winter months (December/January) to and average high of 85º F (29.4º C) in the summer months (http://city-data.com). Relative humidity stays fairly constant throughout the year between 60 and 90%. Precipitation is variable with the wettest month being September (average 5 inches). Wind speeds between 9 and 14 mph are typical in this region.

The LRGV has diverse socio-economic communities ranging from lower income 'colonias' to middle and upper-income neighborhoods. Retail trade and construction are the main industries. The topography of the LRGV is flat and predominately agriculture

(sugar cane, cotton, citrus, vegetables) with some publicly and privately-owned natural areas.

1.5. Research Question

How does *Aedes aegypti* and *Culex quinquefasciatus* sugar feeding patterns and blood meal host utilization affect arboviral transmission?

1.6. Objectives

This dissertation explores the spatial and temporal heterogeneity of arboviral disease transmission by harnessing field-based research, chemical analyses, molecular biological techniques and geographic information system (GIS).

The three major objectives of this dissertation are:

1. Quantify sugar feeding in male and female *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes of South Texas.
2. Identify the vertebrate host feeding patterns and host selection of *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes of South Texas.
3. Analyze global patterns of 'aegyptism without arbovirus'.

The outcome of this dissertation research will fill knowledge gaps in our understanding of disease transmission among these two species of mosquito in the

region of South Texas bordering Mexico. Furthermore, this course of study will help explain the phenomenon of 'aegyptism without arbovirus' by exploring various landscape conditions that either enhance or limit arboviral transmission around the world.

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2. SUGAR FEEDING PATTERNS FOR AEDES AEGYPTI AND CULEX QUINQUEFASCIATUS (DIPTERA: CULICIDAE) MOSQUITOES OF SOUTH TEXAS[*]

2.1. Introduction

Mosquito-borne diseases continue to emerge and re-emerge globally causing significant public health concern. For some viral pathogens, commercially licensed vaccines are not available or in short supply placing a greater emphasis on disease prevention through effective mosquito management and control [1]. Two important vector species involved in arbovirus transmission are *Ae. aegypti* (L.) (Diptera: Culicidae), the yellow fever mosquito, and *Cx. quinquefasciatus* (Say) (Diptera: Culicidae), the southern house mosquito. *Aedes aegypti* is the principal vector of dengue (DENV) [2], chikungunya (CHIKV) [3], yellow fever [4], and Zika viruses (ZIKV) [5]. *Culex quinquefasciatus* is a member of the *Culex pipiens* complex that is an important vector of West Nile virus (WNV) and other arthropod-borne viruses including Japanese encephalitis virus, Saint Louis encephalitis virus and Rift Valley fever [6].

As early as 1873, adult *Culex* mosquitoes were observed sucking nectar from the flowers of *Ramnus frangula* [7] and in 1958 both sexes of *Aedes* and *Culex* mosquitoes were observed frequently visiting flowers for nectar [8]. However, we also know that nearly all female mosquitoes require a blood meal to develop eggs, and in some

[*] Olson, M. F., Garcia-Luna, S., Juarez, J. G., Martin, E., Harrington, L. C., Eubanks, M. D., ... & Hamer, G. L. (2020). Sugar Feeding Patterns for *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) Mosquitoes in South Texas. *Journal of Medical Entomology*.

environments, *Ae. aegypti* have adapted to seldom feed on sugar, deriving needed energy from blood meals alone [9-12]. Mosquitoes can become vectors of disease when they acquire blood from an infected host, but the frequency of biting may be allayed by sugar feeding. Hence, the choices mosquitoes make in obtaining food resources greatly impact pathogen transmission dynamics.

Sugar feeding is thought to be common among most mosquito species, providing the necessary fuel for flight [13] and is linked to survival and successful mating [14]. Some studies have suggested that sugar-poor environments effectively limit the population or survivorship of adult mosquitoes [14-17], but Klowden [18] demonstrated that host-seeking is not inhibited by sugar deprivation in female *Ae. aegypti* mosquitoes, differing from the typical pattern seen in other mosquito species of one blood meal taken per gonotrophic cycle. Further research with *Ae. aegypti* has suggested that they have evolved to become highly anthropophilic by feeding almost exclusively on humans with minimal feeding on sugar sources in nature [12,19]. Moreover, Costero et al. [10] observed a reproductive advantage in *Ae. aegypti* fed only human blood versus blood with sugar. This may partly explain *Ae. aegypti* mosquitoes' increased role in arboviral transmission in many locations.

Alternatively, *Culex* spp. mosquitoes exhibit enhanced survivorship with increasing sucrose meal concentrations but their ability to transmit WNV actually decreases [20]. A different study observed a significant difference in sugar and host feeding between diapausing and non-diapausing female *Cx. pipiens* [21]. Significant differences in longevity and fecundity of *Cx. pipiens pallens* were observed in

mosquitoes reared on different flowering plants and seed pods [22]. Accordingly, heterogeneity in sugar resources could have a profound effect on when and where populations of mosquitoes can support arbovirus transmission among *Culex* species as well.

Our ability to conduct vector surveillance and control is inextricably linked to the feeding strategies and biology of these mosquitoes. For example, host-seeking traps are often baited with CO_2 or octenol and gravid oviposition traps use water with organic material whereas DNA preservation cards and attractive toxic sugar baits (ATSBs) exploit sugar consumption for surveillance and control. In Australia, researchers demonstrated the efficiency of detecting arboviruses on honey-soaked nucleic acid preservation cards placed in CO_2 baited box traps [23,24]. In California, cotton dental wicks soaked with scented sugar baits detected WNV activity in areas where conventional surveillance of mosquito pools reported no WNV activity [25,26]. Sugar-baited stations proved to be more sensitive in detecting arboviral activity because they can be deployed continuously for 6 to 7 days at a time as opposed to traditional CO_2-baited light traps which are typically deployed overnight, usually only 1 night per week and tend to not capture optimal numbers of *Ae. aegypti* or *Ae. albopictus* mosquitoes.

For male *Ae. aegypti*, sugar-feeding positively influences probability of survival, longevity, male reproductive physiology including excitation of the antennal fibrillae, and insemination rates [27] and thus is an important factor in effective deployment of the Sterile Insect Technique and other genetically modified mosquito control strategies. The lethality of ATSB against female *Ae. aegypti* mosquitoes has been demonstrated in

laboratory and field settings [28,29], but the components which attract mosquitoes are still being studied. For example, Scott-Fiorenzano et al. found *Ae. aegypti* more attracted to ATSB with the host kairomones lactic acid and octenol added as opposed to fruit-based attractants [30]. This concurred with Fikrig et al. [31] who found floral-based attractants and sugar mixtures previously identified in literature to be ineffective lures to ATSB stations or Gravid *Aedes* Traps. Many contemporary mosquito management tools exploit mosquito sugar feeding behavior.

The amount of sugar feeding by populations of *Ae. aegypti* and *Cx. quinquefasciatus* in the United States is poorly understood. The state of Texas has experienced large epidemics of WNV (Chung et al. 2013, Poh et al. in review) and the Lower Rio Grande Valley (LRGV) in South Texas has now experienced autochthonous transmission of DENV, CHIKV, and ZIKV [32-36]. The objective of this study is to document the degree to which *Ae. aegypti* and *Cx. quinquefasciatus* utilize sugar resources in South Texas as a pre-requisite to considering different surveillance and vector control techniques and estimating the importance of sugar feeding for pathogen transmission.

2.2. Materials and Methods

To quantify total sugar content of all mosquitoes we utilized the hot anthrone test developed by Emile Van Handel [13,37]. We validated the assay on laboratory colonies of *Cx. quinquefasciatus* (Sebring) and *Ae. aegypti* (Liverpool) reared from eggs in larval trays stored in a 37°C incubator with a 12:12 L:D cycle. Larvae were fed a mix of liver

powder and Brewer's yeast in a 12:8 gram ratio per 100 ml sterile, deionized water [38]. Baseline values were established by rearing ten male and ten female unfed mosquitoes of both species. Approximately 24 hours post emergence, the mosquitoes were euthanized at -20° C and analyzed with the hot anthrone test.

To facilitate comparison with prior studies, the mosquitoes captured in 2019 were analyzed by both the cold and hot anthrone tests on each sample [13,37,39,40]. Furthermore, additional lab-reared mosquitoes were analyzed using both cold and hot methods allowing us to quantify fructose and total carbohydrate values for unfed, 24-hours post sugar feeding (10% sucrose), blood-fed, gravid, and post-oviposition mosquitoes (Table A-1).

2.2.1. Study area

Wild *Ae. aegypti* and *Cx. quinquefasciatus* were collected from five residential sites in the Lower Rio Grande Valley (LRGV) of South Texas from September 20, 2017 through December 6, 2017, June 12-14, 2018, and October 9-16, 2019. The neighborhoods are Indian Hills (26º12'43"N, 97º54'36"W ± 0.5 km), Tierra Bella (26º07'44"N, 98º03'07" W ± 0.5 km) La Piñata (26º07'44"N, 98º03'25"W ± 0.5 km), Mercedes La Mesa (26º13'51"N, 97º57'29"W ± 0.5 km) and Mile 5 (26º07'37"N, 97º58'08"W ± 0.5 km). This region along the US-Mexico border is home to approximately 1.4 million residents (US Census Bureau, 2017). Temperatures in the LRGV range from an average low of 50º F (10º C) in winter months (December/January) to and average high of 85º F (29.4º C) in the summer months

(http://city-data.com). Relative humidity stays fairly constant throughout the year between 60 and 90%. The LRGV has diverse socio-economic communities ranging from lower income 'colonias' to middle and upper-income neighborhoods [41]. Retail trade and construction are the main industries (city-data.com) and the topography of the LRGV is flat and predominately agricultural (sugar cane, cotton, citrus, vegetables) with some publicly and privately-owned natural areas.

2.2.2. Collection and identification

Collection techniques included Biogents Sentinel 2 (BGS2) (Biogents, Inc., Moorefield, WV), CDC Resting Traps (BioQuip® Products, Rancho Dominguez, CA) and Prokopack aspirators (John W. Hock Co., Gainesville, FL). BGS2 traps were placed outside the home, within one to three meters of the residence. Likewise, CDC resting traps were placed outside the home and separated from the BGS2 by one to three meters. Traps were allowed to operate for 24 hours. All aspiration was conducted outside of the home for a 10-minute period at each location in the natural vegetation as well as in and around sheds, abandoned vehicles, and wood piles. Mosquitoes were collected in the mornings between 8:00 and 11:00 AM. Mosquitoes were collected from 30 unique residences in Indian Hills, 22 residences in La Pinata, 11 residences in Tierra Bella, 2 residences in Mercedes La Mesa, and 1 residence at the Mile 5 location. Mosquito specimens were transported to the Texas A&M AgriLife Research & Extension Center in Weslaco, TX, alive on ice packs in coolers, sorted by sex and identified morphologically using *The Illustrated Key to Common Mosquitoes of Louisiana* [42].

Individuals were placed in microcentrifuge tubes and then stored in a -80° C freezer until transported to College Station, TX, on dry ice and stored at -80° C until further analysis.

2.2.3. Sugar quantification

After species and sex were confirmed each mosquito was placed into a disposable, 75 mm glass test tube (VWR) and heat fixed for 30 minutes at 100° C to ensure that enzymatic activity ceased (Techne Dri-Block®, Techne Ltd., Cambridge, UK). The entire mosquito (minus the right wing for those samples used as a proxy for mosquito size) was then homogenized in the test tube using a glass pestle. To each tube containing the homogenized mosquito, 200 µl of 2% sodium sulfate ($NaSO_4$), followed by 1.5 ml of 1:2 chloroform methanol solution was added and stirred. The glycogen was absorbed to the $NaSO_4$ precipitate. Sample tubes were then centrifuged at 450 x *g* for one minute. Being careful not to disturb the pellet containing glycogen, the supernatant was carefully transferred to a new test tube and allowed to evaporate to approximately 200 µl by leaving the tubes open inside the fume hood for approximately 48 hours at room temperature, or with the assistance of a heating block set to 95° C.

For the hot anthrone analysis, sugar standards were prepared by dissolving 25 mg of glucose in 25 ml of 25% ethanol to produce an initial 1:1 (50 µg/50 µl) concentration. From this, the following dilutions were prepared: 1:2, 1:5, 1:10, 1:20. A comparative blank was prepared with only 25% ethanol. Standards were run in duplicate for each 96-well plate with samples. For the cold anthrone analysis, fructose standards were prepared

in the same manner with the exception of using 25 mg of fructose instead of 25 mg of glucose.

Anthrone reagent was prepared in advance by putting 150 ml deionized water into a 1L Erlenmeyer flask under a hood and then slowly adding 380 ml sulfuric acid. Subsequently, 750 mg of anthrone was mixed in by swirling. The reagent was allowed to cool and stored at 10° C. To each sample tube (containing ~200 µl of supernatant) and each of the 12 standard tubes, 3 ml of anthrone reagent was added. At this point, for the cold anthrone test, the samples and fructose standards were allowed to remain at room temperature for 75 minutes. Following this, each standard and sample tube was vortexed thoroughly and 100 µl of the resulting mixture was pipetted into a 96-well spectrophotometer plate. For greater accuracy, technical duplicates were analyzed and the average was taken. All tubes were then heated at 95° C for 17 minutes, allowed to cool for ten minutes and vortexed to thoroughly mix. The presence of total carbohydrates was indicated by a greenish blue color that tended to be most intense at the top of the tube, thus mixing was imperative. Using a fresh tip for each sample, 100 µl was transferred into the designated well on the 96-well spectrophotometer plate, in duplicate as was performed with the cold assay. The plate was then analyzed on a spectrophotometer (Epoch^{TM}, BioTek Instruments, Inc.) set for 625 nm. The quantity of fructose or total carbohydrates in the mosquito samples was determined by taking the optical density (OD), subtracting the value obtained for the blank (25% ETOH and anthrone) and then dividing the result by the slope obtained from the standard curve generated using the fructose or glucose standards.

2.2.4. Wing measurements

To determine if adult mosquito body size influenced the amount of sugar detected in specimens, the wings were used as a proxy for body size [43]. Before the heat fixing step, the right wing of each adult was removed and measured from the axillary incision to the apical margin excluding fringe hairs [44] with the aid of a digital microscope (Dino-Lite, Torrance, CA) . Samples collected in 2019 were measured with a USB digital microscope (Bysameyee, China), calibrated with the same calibration tool used previously.

2.2.5. Statistical analysis

To evaluate the effect of sugar content on wing length of female and male *Ae. aegypti* and *Cx. quinquefasciatus* we used a generalized linear model for count data on JMP 14 (SAS Institute Inc., NC, USA) [45]. For both the hot and cold anthrone tests a Poisson distribution with a log link function was used, with a Maximum Likelihood estimation method. The residuals were used to evaluate normality by Q-Q plots and the Shapiro-Wilk test.

We used the Mann Whitney U test in GraphPad Prism 8.1.2 (GraphPad Software, San Diego, CA) to detect differences in mean sugar content between male and female mosquitoes of both species, and also to detect differences in season. To compare percentages of mosquitoes containing ≥ 3.5 μg sugar content, a Chi-square analysis was performed. We also used Fisher's exact test to compare percentage of fructose-positive

mosquitoes. A one-way ANOVA was conducted to compare trapping method for each species and sex. Finally, we performed a Kruskall-Wallis, one-way ANOVA for each species and sex to detect significant differences between the mean sugar content at each location.

2.3. Results

2.3.1. Laboratory study

Unfed male and female *Ae. aegypti* mosquitoes had a mean fructose value of 0.46 µg (± 0.32 SEM) and 0.79 µg (± 0.28 SEM) respectively (Table A-1). Conversely, male and females 24-hours post sugar feeding had mean fructose values of 1.19 µg (± 0.49 SEM) and 4.65 µg (± 0.48 SEM) respectively. Mean fructose content for blood-fed female *Ae. aegypti* was 3.72 µg (± 0.31 SEM) and 3.59 µg (± 0.53 SEM) for gravid females, decreasing to a mean of 2.33 µg (± 0.42 SEM) for females, post-oviposition.

The total sugar content for unfed male and female *Ae. aegypti* mosquitoes was 0.27 µg (± 0.09 SEM) and 0.79 µg (± 0.28 SEM) respectively (Table A-1). In sugar-fed mosquitoes, the hot anthrone test detected 4.28 µg (± 1.73 SEM) in males and 10.45 µg (± 1.62 SEM) in females. Blood-fed females had a mean total sugar value of 5.86 µg (± 0.39 SEM), gravid females had 7.47 µg (± 1.18 SEM), and females post-oviposition had 5.96 µg (± 1.12 SEM).

2.3.2. Field-captured mosquitoes

The mean sugar content for *Ae. aegypti* females was 8.63 µg (± 1.03), compared to 15.02 µg (± 1.98) for *Cx. quinquefasciatus* female mosquitoes (Table A-2). Among the male mosquitoes, *Ae. aegypti* had higher levels of sugar than *Cx. quinquefasciatus*, 17.28 µg (± 1.46) and 11.82 µg (± 1.19), respectively. After removing mosquitoes containing < 3.5 µg sugar from the dataset, *Ae. aegypti* females had significantly less mean total sugar content of 17.00 µg (± 1.90), compared to 24.40 µg (± 3.06) for *Cx. quinquefasciatus* females ($p = 0.0050$). Significant difference was also found in males with *Ae. aegypti* having mean total sugar content of 26.11 µg (± 2.03), compared to 15.81 µg (± 1.48) for *Cx. quinquefasciatus* ($p = 0.0032$). The difference in mean sugar content between male and female *Ae. aegypti* was found to be significant ($p < 0.0001$) but there was no significant difference ($p = 0.207$) between male and female *Cx. quinquefasciatus* (Figure 2.1).

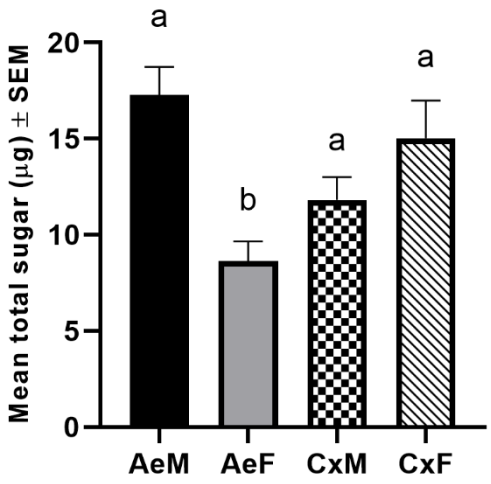

Figure 2.1 Mean sugar content (± SEM) for all *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes collected between September 20, 2017 and October 16, 2019 from all study site locations.
Includes samples with zero sugar detected.
'a' indicates no statistical difference between means.
'b' indicates statistical significance between means ($p < 0.0001$).

We also analyzed the percentage of mosquitoes deemed 'positive' for total carbohydrates, containing ≥ 3.5 µg of sugar, based upon baseline values + 2 standard deviations for unfed, laboratory-raised female *Ae. aegypti* mosquitoes (Table A-1). Using the hot anthrone test, we found 47.91% (172/359) *Ae. aegypti* females, 63.87% (198/310) *Ae. aegypti* males, 60% (114/190) *Cx. quinquefasciatus* females, and 72.33% (115/159) *Cx. quinquefasciatus* male mosquitoes positive for sugar consumption (Figure 2.2). Significant difference between *Ae. aegypti* females and all other groups was observed ($X^2 = 32.99$, df = 3, $p < 0.0001$). Using the cold anthrone test, we found 43.31% (68/157) *Ae. aegypti* females and 51.05% (73/143) *Ae. aegypti* male mosquitoes

positive for sugar consumption. Significant difference between male and female *Ae. aegypti* was not observed (p = 0.2032) (Figure 2.3).

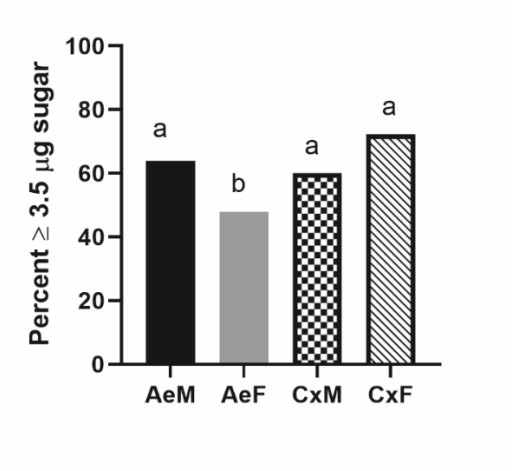

Figure 2.2 Percentage of male and female *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes containing ≥ 3.5 µg of sugar (hot anthrone test).

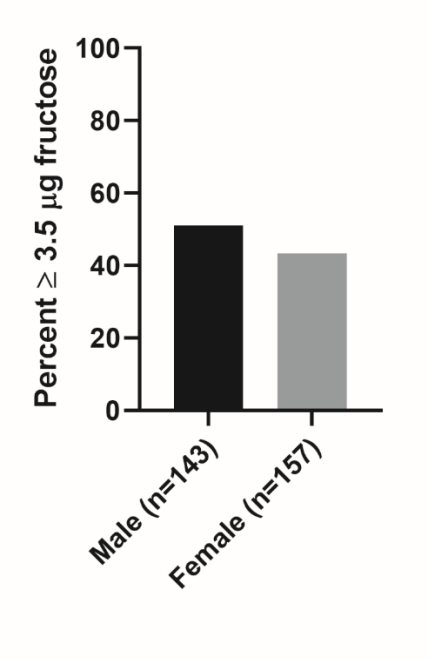

Figure 2.3 Percent male and female *Ae. aegypti* containing ≥ 3.5 µg fructose (cold anthrone test).

2.3.3. Season and trapping method

When compared by season and trapping method, the data follows a similar pattern. *Ae. aegypti* females had significantly less sugar content than *Ae. aegypti* males in fall (September - December, 2017 and October, 2019) (9.29 ± 1.17 µg, 18.11 ± 1.58 µg, respectively) ($p < 0.0001$), and in summer (June, 2018) (4.71 ± 1.51 µg, 9.58 ± 2.72 µg, respectively) ($p < 0.0001$) No significant difference was observed between female and male *Cx. quinquefasciatus* for fall (11.32 ± 1.75 µg, 11.84 ± 1.39 µg, respectively) ($p = 0.0518$) or summer (22.85 ± 4.80 µg, 11.74 ± 2.03, respectively) ($p = 0.6337$) (Figure 2.4). Male and female *Ae. aegypti* captured in fall had significantly higher levels of total sugar from those captured in summer, but this was not observed in *Cx. quinquefasciatus* (Figure 2.5). *Ae. aegypti* samples collected in October, 2019 were also analyzed by trapping method and location, but no significant differences were observed.

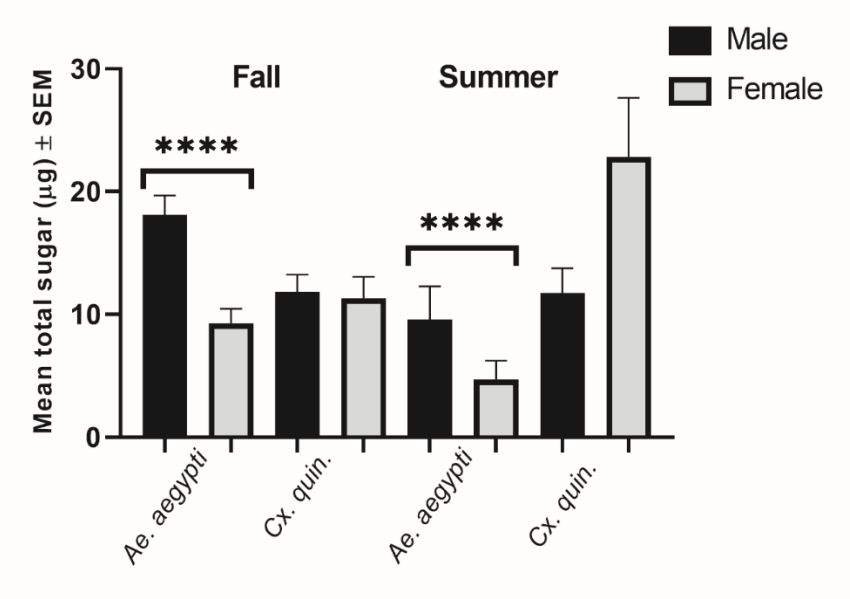

Figure 2.4 Comparison of mean total sugar content by mosquito species and sex, grouped by season.

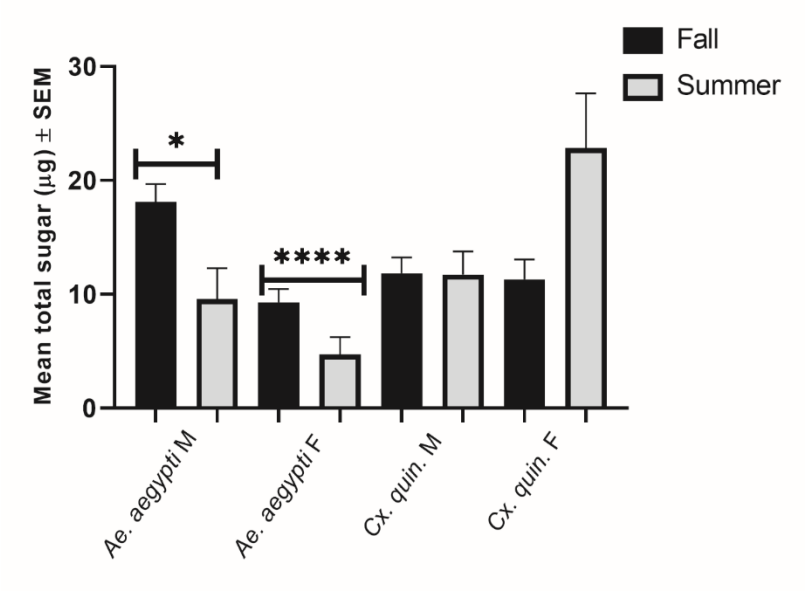

Figure 2.5 Comparison of mean sugar content by season, for each mosquito species and sex.

Both *Aedes* and *Culex* females caught in summer 2018 with the resting trap had higher mean sugar content than their male counterparts. Additionally, female *Cx. quinquefasciatus* caught in summer with an aspirator had the highest mean sugar content at 50.78 ± 19.52 µg (Table 2.1). *Cx. quinquefasciatus* captured by CDC resting traps had significantly more sugar content than those captured by BGS2 for females (CDC resting: 23.86 ± 8.00 µg vs. BGS2: 13.73 ± 3.21 µg; $p = 0.0094$) but not for males (CDC resting: 7.19 ± 1.85 µg vs. BGS2: 13.60 ± 2.90 µg; $p = 0.4109$) (Figure 2.6). The mean sugar content for female *Ae. aegypti* was higher for specimens captured by resting traps and aspirator than for specimens captured by BGS2, although the difference was not statistically significant ($p = 0.2761$).

Table 2.1 Mean total sugar content (±SE) of *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes collected in Fall 2017 and Summer 2018.

Season	Species	Sex	All	Trap type		
				BGS2	CDC Resting	Aspirator
			µg ± SE (*n*)	µg ± SE (*n*)	µg ± SE (*n*)	µg ± SE (*n*)
Fall '17	*Ae. aegypti*	Male	15.67 ± 2.05 (137)	15.67 ± 2.04 (138)	N/A	N/A
		Female	3.54 ± 0.69 (156)	3.54 ± 0.69 (159)	N/A	N/A
	Cx. quin.	Male	11.84 ± 1.39 (129)	11.84 ± 1.38 (130)	N/A	N/A
		Female	11.32 ± 1.75 (129)	11.31 ± 1.74 (130)	N/A	N/A
Summer '18	*Ae. aegypti*	Male	9.58 ± 2.72 (30)	10.45 ± 3.09 (26)	2.12 ± 1.95 (2)	5.72 ± 3.60 (2)
		Female	4.71 ± 1.52 (53)	3.75 ± 1.51 (42)	11.56 ± 8.09 (6)	4.47 ± 2.80 (5)
	Cx. quin.	Male	11.74 ± 2.03 (30)	13.60 ± 2.90 (16)	7.19 ± 1.85 (8)	12.85 ± 6.19 (6)
		Female	22.85 ± 4.80 (61)	13.73 ± 3.21 (38)	23.86 ± 8.00 (11)	50.79 ± 19.52 (12)
Fall '19	*Ae. aegypti*	Male	20.45 ± 2.39 (143)	19.20 ± 2.61 (103)	27.93 ± 14.09 (12)	21.83 ± 4.82 (28)
		Female	15.00 ± 2.13 (157)	13.31 ± 2.52 (117)	2.95 ± 1.05 (4)	21.85 ± 4.21 (36)

Table 2.2 Mean fructose content (±SE) of *Ae. aegypti* mosquitoes collected in Fall 2019.

Season	**Species**	**Sex**	**All**	**Trap type**		
				BGS2	CDC Resting	Aspirator
			µg ± SE (*n*)	µg ± SE (*n*)	µg ± SE (*n*)	µg ± SE (*n*)
Fall '19	*Ae. aegypti*	Male	8.78 ± 1.05	8.02 ± 1.05	15.24 ± 7.59	8.79 ± 1.87
			(143)	(103)	(12)	(28)
		Female	6.74 ± 0.78	5.94 ± 0.82	4.40 ± 2.99	9.60 ± 1.99
			(157)	(117)	(4)	(36)

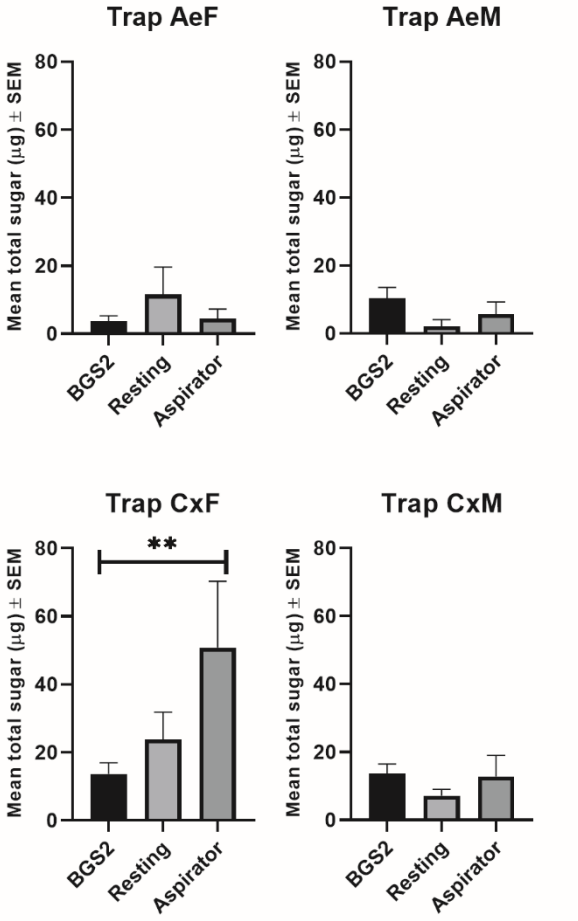

Figure 2.6 Comparison of mean sugar content by mosquito species and sex, grouped by trapping method.
This data only includes Summer 2018 mosquitoes. AeF = *Ae. aegypti* female; AeM = *Ae. aegypti* male; CxF = *Cx. quinquefasciatus* female; CxM = *Cx. quinquefasciatus* male
** indicates statistical significance between means (p = 0.0094)

2.3.4. Location

Overall, mean total sugar content ± SEM on field-collected *Ae. aegypti* and *Cx. quinquefasciatus* adult mosquitoes varied by location. The difference between locations was significant for both *Cx. quinquefasciatus* and *Ae. aegypti* males (p = 0.0472 and p =

0.0268, respectively) but not for females (p = 0.0575 and p = 0.4449, respectively) (Figure 2.7).

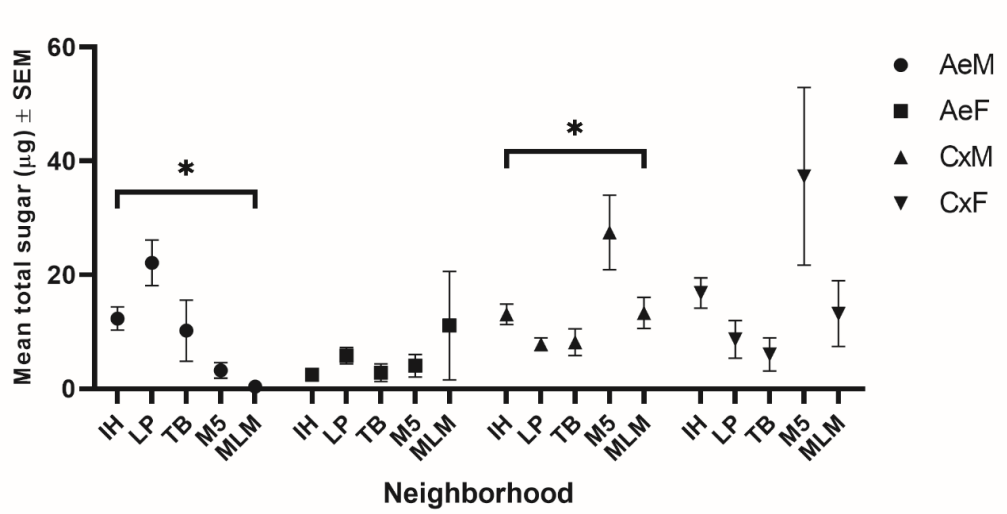

Figure 2.7 Mean sugar content of species and sex, by location.
IH = Indian Hills, LP = La Piñata, TB = Tierra Bella, M5 = Mile 5, MLM = Mercedes La Mesa. AeM = *Ae. aegypti* (male), AeF = *Ae. aegypti* (female), CxM = *Cx. quinquefasciatus* (male), CxF = *Cx. quinquefasciatus* (female). Includes mosquitoes from 2017 and 2018.
* indicates statistical significance between means (p = 0.0268 for *Ae. aegypti* males; p = 0.0472 for *Cx. quinquefasciatus* males)

2.3.5. Wing measurements

Wing measurements were successfully obtained from 139 of 174 (79.9%) mosquitoes captured in summer, 2018 and 285 of 300 (95.0%) mosquitoes captured in fall, 2019. The mean wing length (± SEM) for *Ae. aegypti* male and female mosquitoes was 2.01 ± 0.02 (*n* = 147) and 2.54 ± 0.02 (*n* = 183), respectively. For *Cx. quinquefasciatus* male and females, the mean wing length (± SEM) was 2.55 ± 0.04 (*n* = 26) and 2.81 ± 0.05 (*n* = 48), respectively. A statistically significant correlation between wing length and sugar content was not observed for *Ae. aegypti* males nor females using

either cold (p = 0.202) or hot (p = 0.739) anthrone tests (Tables 2.3 and 2.4; Figure A-1 and A-2). We also evaluated *Cx. quinquefasciatus* wing length compared to sugar content using the same criteria and found no significant relationship (p = 0.373) using the hot anthrone test (Table 2.5 and Figure A-3).

Table 2.3 Generalized linear model estimates of the cold anthrone test on wing length of male and female *Ae. aegypti*.

Variable	Estimate	Std. Error	95% CI	Chi^2	p-value
Intercept	1.694	0.76	0.19 – 3.18	4.89	0.027
Wing length	0.433	0.33	-0.23 – 1.09	1.62	0.202
Sex (Female)	-0.166	0.11	-0.39 – 0.06	2.06	0.151

Table 2.4 Generalized linear model estimates of the hot anthrone test on wing length of male and female *Ae. aegypti*.

Variable	Estimate	Std. Error	95% CI	Chi^2	p-value
Intercept	2.791	0.80	1.22 – 4.36	12.35	
Wing length	0.118	0.35	-0.58 – 0.79	0.11	0.739
Sex (Female)	-0.190	0.12	-0.44 – 0.05	2.23	0.135

Table 2.5 Generalized linear model estimates of the hot anthrone test on wing length of male and female *Cx. quinquefasciatus*.

Variable	Estimate	Std. Error	95% CI	Chi^2	p-value
Intercept	1.644	1.55	-1.42 – 4.70	1.10	0.293
Wing length	0.498	0.49	-0.48 – 1.46	0.79	0.373
Sex (Female)	0.147	0.17	-0.17 – 0.49	1.00	0.315

2.4. Discussion

Previous studies indicate female *Ae. aegypti* mosquitoes feed preferentially on human blood, and rarely on sugar [12,19,46,47]. Our results support these observations showing that sugar content in *Ae. aegypti* females was significantly lower than males in both seasons, and significantly lower than both male and female *Cx. quinquefasciatus* mosquitoes. Female *Ae. aegypti* mosquitoes contained 2 -approximately 3 times less sugar than their male counterparts, or both male and female *Cx. quinquefasciatus* in Fall of 2017 and Summer of 2018. We also compared the percentage of mosquitoes deemed 'positive' for sugar feeding and while the average sugar content of female *Ae. aegypti* mosquitoes was significantly lower than males, a substantial percentage of females (48.91%, n=366) had a total sugar content ≥ 3.5 µg, (~ 2 standard deviations above mean baseline value), suggesting sugar consumption was common in this region during these time periods. In a previous study, Costero et al. [48] adjusted this baseline for *Ae. aegypti* females to 7 µg based on the fact that females fed only blood had a constant background detection of sugar that was higher than those fed only water, and also with the assumption that most field-caught females contained some blood in their abdomens. While we did not observe any blood in our specimens, if we were to apply this cutoff for considering a 'positive' result, only 99 out of 366 (27.05%) *Ae. aegypti* females would remain positive for total sugar.

The results from our laboratory study of *Ae. aegypti* mosquitoes at various physiological states confirmed what Costero et al. [48] suggested about background detection of sugar in blood-fed mosquitoes. While our mean fructose value for blood-

feds was only 3.72 µg, adding two standard deviations to this would make it 7.41. However, it should be noted that we tested fully engorged mosquitoes for this lab analysis while none of our field-collected mosquitoes appeared to be blood-fed at the time of analysis. The hot anthrone test, which detects all carbohydrates, had higher mean values for most of the laboratory-reared groups, as expected. Interestingly, the mean sugar content for both male and female starved *Ae. aegypti* actually decreased slightly. It was also interesting to see higher values for females than males in both unfed and sugar-fed cohorts. In the unfed mosquitoes, perhaps body size difference (females are on average larger than males) was enough to give a higher reading, but with the mosquitoes that were analyzed 24-hours after feeding on 10% sucrose, differences in rate of digestion and levels of activity may account for this distinction where females had four times the fructose and over twice as much total sugars compared to males. Perhaps males burn ingested sugars at a faster rate as they are seeking mates immediately after obtaining a sugar meal. Gravid females had a slightly lower mean fructose compared to blood-feds, but were higher than blood-feds with the hot anthrone test. Finally, post oviposition females appeared to have slightly less fructose than blood-fed or gravid females, and less total sugars than gravid females, but slightly higher total sugar than blood-fed mosquitoes. Among females, the range of values was greatest in this physiological category. Further study of post-oviposition females would be useful to better understand why some show negligible sugar while others in this category appear to have significant sugar reserves.

We collected additional *Ae. aegypti* mosquitoes in October, 2019 and performed both 'hot' and 'cold' anthrone tests. The 'hot anthrone' test is a quantitative assay for total carbohydrates, while the 'cold anthrone' test demonstrates the presence of fructose and fructose-yielding carbohydrates [13,37,39]. Using the 'hot' and 'cold' anthrone tests on a subsample of mosquitoes allowed us to compare our results with prior studies which only utilized the 'cold' test. Using 7 µg as the baseline value as recommended by Costero et al. [48], we found more than four times the percentage of females with detectable amounts of fructose (27.39%) than *Ae. aegypti* females in Puerto Rico (6%) as reported by Costero et al., and over eight times the percentage (3%) found in Thailand by Edman et al. [19]. To the best of our knowledge, this is the first study to document frequency of fructose feeding among female *Ae. aegypti* that is only slightly less than that of male *Ae. aegypti*. Our observations indicate sugar feeding by female *Ae. aegypti* is occurring in South Texas suggesting surveillance and control methods that utilize sugar could be effective. Furthermore, increased sugar feeding could be allaying blood feeding frequency, as demonstrated by Foster and Eischen [49] and may decrease pathogen transmission compared with nutritionally stressed mosquitoes, which Vaidyanathan [20] demonstrated with *Culex* mosquitoes and WNV.

An unexpected, but interesting observation was the difference in overall mean sugar content between male *Ae. aegypti* and *Cx. quinquefasciatus* (17.28 µg (± 1.46) and 11.82 µg (± 1.18), respectively); *Ae. aegypti* had 32.8% more sugar. This difference between the species could be linked to variability in the time that feeding, swarming, and resting occurs. Reisen et al. discovered mosquitoes captured early in the morning were

more likely to test positive for fructose than those captured after swarming [50]. Additionally, these differences could also be influenced by the male mosquitoes' ability to discover and exploit sugar resources [51].

For Summer 2018 and Fall 2019 samples, we considered factoring mosquito wing length as a proxy for body size into our analysis. Using 3.5 µg as a baseline, we did not observe a significant relationship between wing length and sugar content for *Ae. aegypti* or *Cx. quinquefasciatus.* Therefore, we did not incorporate body size into the analysis of sugar feeding in this study.

Another important consideration is the time since the last sugar meal. Presumably, mosquitoes caught at the time of feeding would have greater sugar content compared with those who had fed 2-3 days previously. This would likely explain why our *Cx. quinquefasciatus* female which was aspirated from vegetation had 214.65 µg sugar. Rate of digestion would also influence the quantity of sugar detected. Edman et al. [19] released sugar-fed *Ae. aegypti* and were unable to detect sugar in the recaptured females after 4 days, suggesting they were not actively consuming more sugar in the wild. This concurs with Costero et al. who were able to detect sugar in *Ae. aegypti* up to 4 days post feeding on a 10% sucrose solution [48]. Other researchers have demonstrated nectar-fed mosquitoes can be anthrone-negative in as little as 20 hours of digestion [52]. Therefore, further study comparing the rate of sugar digestion between *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes in a controlled laboratory environment is also warranted. If *Ae. aegypti* females are taking more frequent sugar meals in this location, the result could be fewer blood meals as demonstrated in the laboratory by Klowden [18].

This is also the first study to compare sugar content in *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes collected by different methods in Texas. A similar study in California by Reisen et al. [50] considered variation of sugar positivity in *Cx. tarsalis* mosquitoes between four methods of collection and demonstrated similar differences in the number of sugar-positive mosquitoes collected from resting traps as opposed to CO_2-baited host-seeking traps, melon-baited carbohydrate-seeking traps, and aerial netting. For the current study, both *Ae. aegypti* and *Cx. quinquefasciatus* females caught in CDC Resting traps had a higher mean sugar content than those caught in the BGS2 trap, suggesting that the physiological state of the mosquito (resting or host-seeking) influences the amount of sugar detected. Aspirated *Cx. quinquefasciatus* females had the highest mean sugar content (50.79 µg ± 19.52) of all groups and collection methods. However, the differences in mean sugar content between methods of collection was only statistically significant for female *Cx. quinquefasciatus* and not for males, or for male and female *Ae. aegypti*. We suspect statistical significance between trapping method for both sexes of both species would be observed with larger sample sizes. These results suggest that collection technique could greatly influence the results of any mosquito sugar feeding quantification study.

Spatial heterogeneity in the availability of sugar sources is likely to influence that ability of mosquitoes to find and feed on sugar. In this study the analysis of mean sugar content by location showed significant variation between the neighborhoods for male *Cx. quinquefasciatus* and *Ae. aegypti*, perhaps indicating sugar-rich and sugar-poor environments. Regional variation in sugar availability was studied by Martinez-Ibarra et

al. [53] in Southern Mexico. They found a significantly higher proportion of fructose-positive *Ae. aegypti* mosquitoes in sampling areas that had higher numbers of flowering plants (particularly bougainvillea and hibiscus) per house [53]. An interesting observation from our study was a difference between the two mosquito species as to which neighborhood had the highest mean sugar content. For male and female *Cx. quinquefasciatus*, mosquitoes collected at the Mile 5 location had the highest average sugar content, but for male and female *Ae. aegypti*, La Piñata seemed to have richer sugar resources. Perhaps *Aedes* and *Culex* mosquitoes differ in their preference for certain types of plant sugars or their location of finding sugar (*e.g.*, endophily vs. exophily). A more detailed examination of the types of sugar in mosquitoes, such as those using liquid chromatography, mass spectrometry, or DNA barcoding, could improve our ability to determine sources of sugar in nature [54,55].

2.5. Conclusion

This study from South Texas confirms that sugar feeding by *Ae. aegypti* females is limited compared to their male counterparts, or when compared with male and female *Cx. quinquefasciatus*. This idiosyncrasy helps explain the high propensity for vertebrate host seeking in *Ae. aegypti* females as blood meals are sought for both reproductive facilitation and energetics, thereby increasing its capacity for vector-borne pathogen transmission. In spite of this, detectable amounts of fructose were found in over 27% of the *Ae. aegypti* females that we collected. This apparently higher rate of sugar feeding by *Ae. aegypti* females in South Texas compared with other locations could be one factor

resulting in lower human biting rates and therefore lower rates of arbovirus transmission. From our data, *Cx. quinquefasciatus* consistently took sugar meals in both fall and summer. However, we observed that the mean sugar content of mosquitoes was significantly influenced by trapping method. Future studies should examine how physiological condition and time since sugar meal influences results from wild populations. Sugar is an important component in many surveillance and control strategies for both *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes, but determining preferred sugar sources is the critical next step to improving the effectiveness of these tools.

2.6. Acknowledgements

This study would not have been possible without the support and willing participation of the residents in our study sites of the Lower Rio Grande Valley. Additionally, we would like to thank the members of the team based at the Texas A&M AgriLife Research and Extension Center in Weslaco, TX, for field collections including Ester Carbajal, Edwin Valdez, and Courtney Avila. We would like to also thank Dr. Wendy Tang, Helena Hopson and Victoria Kamilar for their invaluable assistance in the College Station laboratory at Texas A&M University. Funding was provided by National Institutes of Health R21AI128953 and Texas A&M AgriLife Research Insect-Vectored Diseases Seed Grant.

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3. HIGH NON-HUMAN FEEDING BY AEDES AEGYPTI REDUCES ZIKA VIRUS TRANSMISSION IN SOUTH TEXAS[*]

3.1. Introduction

Mosquito-borne viruses driven principally by *Aedes aegypti*, have emerged and re-emerged globally, resulting in a large burden of human disease [1]. Globalization and other anthropogenic factors have allowed this mosquito to thrive in diverse landscapes and facilitate urban transmission cycles of dengue virus (DENV), chikungunya virus (CHIKV), Zika virus (ZIKV), and others [2]. In the Americas, all four serotypes of DENV have re-emerged causing consistent epidemics from South America to Mexico and the Caribbean [3]. The Asian lineage of CHIKV first arrived in the Caribbean in 2014 and spread throughout the Americas in just a few years [4], resulting in 338,963 confirmed human cases [5]. ZIKV invaded Brazil in 2013 [6] and rapidly swept through the Americas in a similar fashion, resulting in an estimated 8.5 million cases in Brazil alone [7].

In the continental United States, *Ae. aegypti* is found throughout the southern states, and recent enhanced surveys of *Stegomyia* mosquitoes have documented the presence of this species in 26 states [8]. Despite this wide distribution of the primary vector and the Asian tiger mosquito (*Ae. albopictus*), a secondary vector for these viruses in many locations, the only regions experiencing autochthonous transmission of

[*] Olson, M. F., Ndeffo-Mbah, M. L., Juarez, J. G., Garcia-Luna, S., Martin, E., Borucki, M. K., ... & Molina-Gamboa, G. D. J. (2020). High Rate of Non-Human Feeding by Aedes aegypti Reduces Zika Virus Transmission in South Texas. *Viruses*, *12*(4), 453.

DENV, CHIKV, and ZIKV by mosquito exposure, are South Florida and South Texas [9,10]. While the Mexico cities along the U.S.—Mexico border have experienced consistent epidemics of *Ae. aegypti*-driven viruses, markedly fewer human cases have occurred in the communities of the Lower Rio Grande Valley (LRGV) on the Texas side of the border. For example, the state of Tamaulipas, Mexico, recorded an estimated 11,760 probable cases of DENV and 2677 cases of Dengue Hemorrhagic Fever between 2009 and 2019 [11]. In contrast, in the LRGV, local mosquito-borne DENV epidemics occurred only in 2005 and 2013 [9], and the outbreaks were associated with relatively small numbers of human cases. For example, in 2005 the LRGV documented three symptomatic cases and six asymptomatic cases of DEN with no travel history [12], compared to 7062 reported DEN cases in Tamaulipas the same year [13]. In contrast, these viruses are recorded with only isolated cases of local transmission in South Texas, including a single CHIKV case in Brownsville, TX in 2015 (Texas Department of State Health Services, 2016) and 11 cases of locally acquired ZIKV in the LRGV between 2016–2017 [14].

Aedes aegypti-driven viruses continue to have intense epidemics in the Americas, resulting in high rates of viremic humans entering the U.S. [15], but minimal local transmission has occurred [16]. This discrepancy in the magnitude of virus transmission along geo-political boundaries of the U.S.—Mexico border has attracted research attention to identify the mechanisms responsible for these patterns. This is especially perplexing given that *Ae. aegypti* in U.S. border communities has comparable relative

abundances in residential neighborhoods to areas with a much higher burden of human disease across the border in Mexico [14,17]. Prior studies have identified several factors contributing to this discrepancy, which have identified social-ecological factors, such as window screens and air conditioning, that reduce the risk of exposure to the viruses [13,18]. However, despite evidence that housing quality is associated with virus transmission [18], there remains limited knowledge of how this influences the ability of *Ae. aegypti* to feed on humans and how proportional human feeding might drive virus transmission potential on both sides of the border.

This study quantifies *Ae. aegypti* host feeding patterns and vertebrate host availability in residential environments in South Texas, to compare the observed frequency of blood meals relative to the expected frequency in the study location. We rely on empirical data on *Ae. aegypti* abundance, human population density, and epidemiological data of epidemics in South Texas and in Tamaulipas, Mexico, to evaluate the risk of human-amplified urban arbovirus transmission. We present evidence contrary to the most commonly reported observation that *Ae. aegypti* feeds mostly on humans by showing a high utilization of dogs and other non-human hosts in South Texas, and that this contributes to a lower risk of human exposure to ZIKV, which reduces epidemic potential.

3.2. Materials and Methods

3.2.1. Study site and mosquito collection

Blood-engorged mosquitoes were collected from several neighborhoods in the Lower Rio Grande Valley (LRGV) on the U.S. side of the U.S.–Mexico border (see Figure 3.1) from September, 2016 through December, 2018. The climate of Weslaco, Texas, which was used as a representation of the general climate for the LRGV, includes an average annual high and low temperature of Weslaco (28.7 and 17.4 °C) and Reynosa, Mexico (29.2 and 17.3 °C; climate-data.org). Average annual precipitation (in mm) is 609 for Weslaco and 532 for Reynosa. We sampled mosquitoes from eight lower-income (15,000–29,999 USD annual household income) neighborhoods (Mercedes, Mesquite (MM); Donna, Figueroa (DF); Mercedes, Chapa (MCH); Progreso Fresno/Progreso Encino (PF/PE); Indian Hills East (IHE); Indian Hills West (IHW); La Piñata (LP); Tierra Bella (TB)) and four middle-income (30,000–40,000 USD annual household income) neighborhoods (La Feria, La Bonita (LF); Mercedes, Rio Rico (MRR); McAllen, La Vista (MLV); Weslaco, Christian Court (WCT)) as described by Martin et al. [14]. We used Biogents Sentinel 2 traps (BGS2; Biogents, Germany) with BG lures at IHE, IHW, LP, TB, and DF neighborhoods, placing one trap outside homes once per week for a 24-h trapping period. We also used Autocidal Gravid Ovitraps (AGO) at PF/PE, DF, LF, MCH, MLV, MM, and MCH. One AGO was placed inside the home and an additional trap was placed outside the home, serviced weekly. More details about AGO preparation and deployment are described in Martin et al. [14]. The selection

of homes was largely dictated by obtaining permission from the homeowners to place traps in and around the property. Upon collection, mosquitoes were identified morphologically based upon illustrations and dichotomous keys found in *The Illustrated Key to Common Mosquitoes of Louisiana* [19]. While processing mosquitoes to identify species and sex, all bloodfed mosquitoes were placed individually into nuclease-free, 1.5 mL micro-centrifuge tubes, labeled with species, sex, date and house identification number and stored at −20 or −80 °C until further processing.

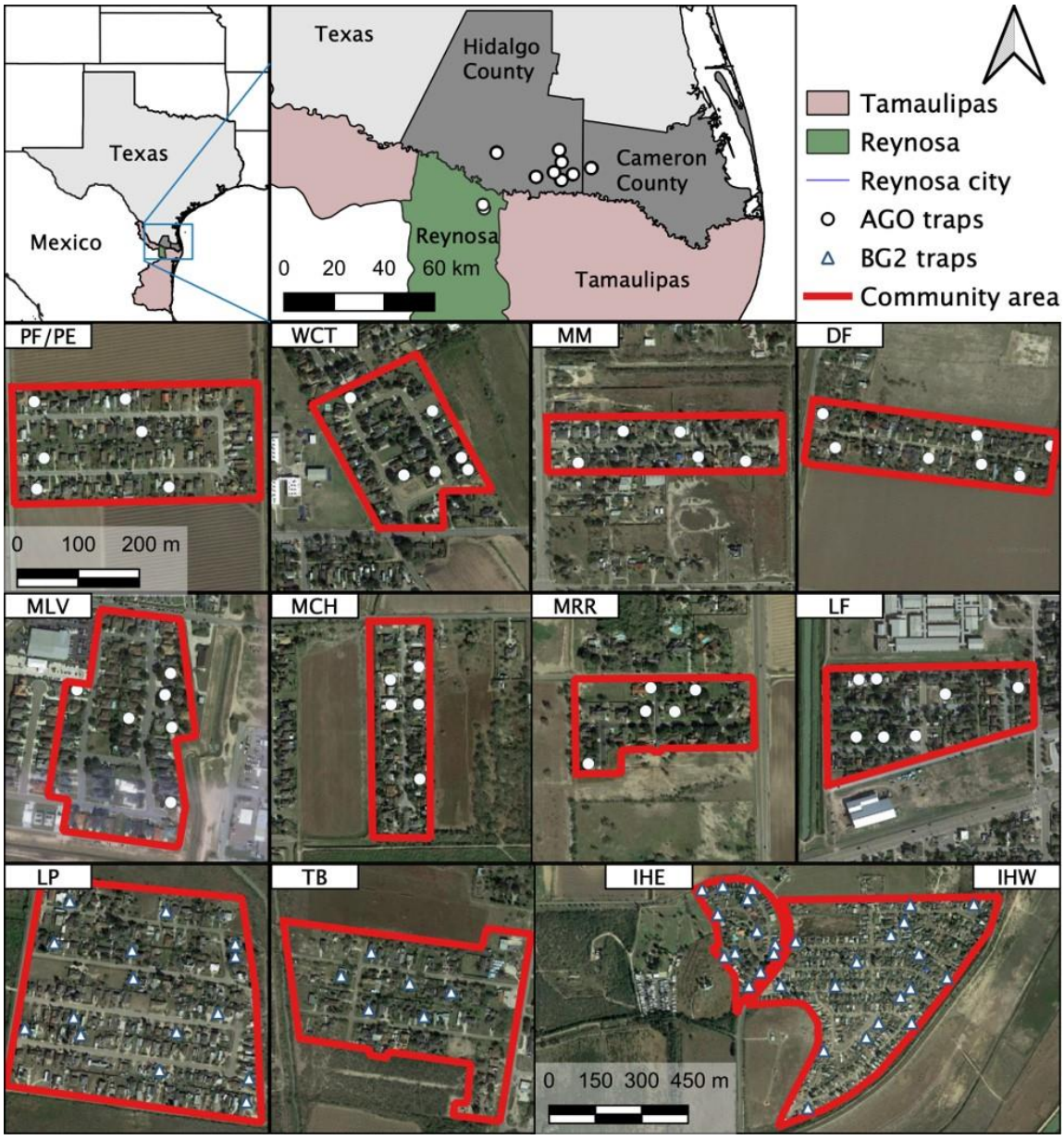

Figure 3.1 Study sites and location of traps in LRGV neighborhoods. PF/PE = Progreso Fresno/Progreso Encino; WCT = Weslaco, Christian Court; MM = Mercedes, Mesquite; DF = Donna, Figueroa; MLV = McAllen, La Vista; MCH = Mercedes, Chapa; MRR = Mercedes, Rio Rico; LF = La Feria; LP = La Piñata; TB = Tierra Bella; IHE = Indian Hills East; IHW = Indian Hills West.

3.2.2. Blood meal analysis

Mosquito samples were identified under microscope, photographed, and given a Sella score (stages of blood digestion and ovary development) based upon observation of the engorged abdomen [20]. The Sella score was used to identify the engorged mosquitoes that had the highest likelihood of yielding a DNA sequence. To minimize exogenous DNA on the mosquito exoskeleton, each whole mosquito was washed in 10% bleach followed by two rinses with nuclease-free water [21–24]. On a clean, chilled microscope slide, the abdomen was carefully separated from the rest of the mosquito body, and the abdominal contents were expressed into a new, labeled, DNA-free 1.5 mL microcentrifuge tube. A homogenizing bead and 200 µL of lysis solution were added to the tube with blood and shaken for 1 min at 30 Hz in the Qiagen Tissue Lyser (Qiagen, Germantown, MD, USA). DNA was extracted using the Thermo Scientific™ Kingfisher™ Flex Purification System, along with the MagMAX Core Nucleic Acid Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.

We adopted previously-published protocols to conduct a PCR-Sanger sequencing blood meal analysis [25–27]. Three primer pairs were used in a tiered approach: (I) A vertebrate cocktail targeting a 648 base pair region of the cytochrome c oxidase 1 (COI) gene, (II) blood meal (BM) primers targeting a 358 base pair region of the cytochrome b gene, and (III) 'Herp' primers that target a 228 base pair region of the cytochrome b gene (Table B-1) [27]. This three-tiered approach has the benefits of cost efficiency, maximizing the number of identified blood meals, and increasing reliability of results [25–27]. First,

every sample was tested using the vertebrate cocktail primers. Samples producing an amplicon of 648 bp were cleaned using ExoSAP-IT (Thermo Fisher Scientific) and submitted to Eton Bioscience (San Diego, CA, USA) for Sanger sequencing. Sequencing results were analyzed using Geneious R9 software (Biomatters, Ltd., Auckland, New Zealand). Only samples with ≥ 95% pairwise identity match to a vertebrate host sequence in NCBI, and ≥ 95% grade (a weighted score comprised of e-value, pairwise identity, and the coverage) were accepted as a confirmed result.

Based upon the outcome of the initial PCR, we continued the iterative bloodmeal analysis PCR process if there was (I) match to human basic local alignment search tool (BLAST), (II) no PCR amplicon, (III) poor sequence quality, or (IV) evidence of mixed DNA (double-nucleotide peaks in chromatograph) [27]. If we obtained any of these four outcomes, a second PCR utilizing the BM1:BM2 primers was conducted [25,27]. Finally, using the same criteria, the analysis was either concluded or subjected to a third primer pair and PCR thermal profile, the 'Herp' primers [25,27].

We followed the protocol of Medeiros et al. [27] for the vertebrate cocktail reaction, but modified the thermal cycling conditions as follows: after denaturation we ran nine cycles of 94 °C for 30 s, a gradient from 45 to 54 °C for 40 s, and 72 °C for 1 min. The remaining thermal cycling conditions were identical to the Medeiros protocol. For the BM and 'Herp' reactions, we followed the protocol of Hamer et al. [25] with the following modification: we lowered the annealing temperature for the 'Herp' reaction from 50 to 47 °C. The vertebrate cocktail, BM1:BM2, and 'herp' PCR reactions used the following

reagents and quantities per reaction: 8.59 µL Nuclease-free H2O, 12.5 µL FailSafe™ PCR 2X Premix E (Lucigen, Middleton, WI, USA), 0.83 µL forward primer, 0.83 µL reverse primer, 0.25 µL FailSafe™ PCR Enzyme Mix (Lucigen), and 2 µL DNA template.

The protocol was tested using lab-raised, Aedes aegypti and Culex quinquefasciatus mosquitoes fed on defibrinated sheep blood (HemoStat Laboratories, Dixon, CA, USA). Adult female mosquitoes were offered artificial blood meals using a Hemotek membrane feeder (Hemotek Ltd., Blackburn, UK). Each specimen was observed under a dissecting light microscope to confirm species, give a Sella score, and capture a digital photograph. We also extracted DNA directly from the blood of several controls including iguana (*Iguana iguana*), white-tailed deer (*Odocoileus virginianus*), tiger (*Panthera tigris*), sandhill crane (*Grus canadensis*), and sheep (*Ovis aries*). These vertebrate species were selected because they are unlikely to be found in mosquito blood meals from this region and would thus minimize the risk of downstream amplicon contamination.

3.2.3. Molecular verification of mosquito species

While most mosquitoes captured via BGS2 traps could be taxonomically classified from morphological features, those collected from the glue boards of the AGO traps are often damaged and more difficult to identify morphologically. Therefore, molecular identification of mosquito species was confirmed using a modified version of the protocol designed by Folmer et al. [28]. Briefly, a primer pair that amplifies a 710-bp

fragment of the cytochrome c oxidase subunit I gene (LCO 1490 and HCO 2198; Table B-2) was used with the following reagents and quantities per reaction: 8 µL Nuclease-free H2O, 12.5 µL FailSafe™ PCR 2X Premix E (Lucigen), 1 µL forward primer (LCO 1490), 1 µL reverse primer (HCO 2198), 0.5 µL FailSafe™ PCR Enzyme Mix (Lucigen), and 2 µL DNA template. The PCR thermal cycling profile included initial denaturation for three minutes at 95 °C followed by 35 cycles of 95 °C for 1 min, 45 °C for 1.5 min, and 72 °C for 2 min, followed by a final extension at 72 °C for 5 min. Amplified PCR products were purified using Exo-SAP-IT™ (ThermoFisher Scientific) and sent to Eton Bioscience (San Diego) for Sanger sequencing.

3.2.4. Quantitative synthesis of published literature

We compiled all the published data on *Ae. aegypti* host feeding patterns from around the world. To systematically review the literature, we searched PubMed, Web of Science, and Google Scholar for published literature using keywords "*Aedes aegypti* host feeding", and a second search of "*Aedes aegypti* blood meal analysis". These queries in Web of Science yielded 50 and seven results, respectively, in PubMed yielded 13 and four results, respectively, and in Google Scholar yielded 1970 and 450 results, respectively. We also tracked references from key review papers and other primary literature. Inclusion criteria included blood meal results from wild-caught mosquitoes. Studies using laboratory-raised mosquitoes, as well as studies which indicated samples were likely from the form *Ae. aegypti formosus* [29–31], were excluded. The form

formosus was excluded to allow a focus on the urban form of *Ae. Aegypti*, which is globally distributed.

3.2.5. Mosquito relative abundance

Female Ae. aegypti relative abundance was estimated in the LRGV and the city of Reynosa, Tamaulipas, Mexico, using AGO traps that were deployed concurrently on both sides of the border in 2017 (Figure 3.1). Eighty AGO traps were deployed outside residential homes in Reynosa and checked weekly between May 7 and Aug 12 (trapping data were unavailable for two weeks in this period due to adverse weather or trap failure) [32]. In the LRGV, 30 AGO traps were deployed outside residential homes during the same weeks as a subset of the data presented in a previous study [14].

3.2.6. Vertebrate surveys

In order to estimate mosquito host selection, a questionnaire related to vertebrate availability was developed and conducted in the four primary communities where blood-fed mosquitoes were collected. Project personnel visited all the homes containing BG Sentinel 2 traps in these communities: 14 (out of 307 total homes present) in Indian Hills East, 10 (96) in Indian Hills West, 13 (160) in La Piñata, and seven (49) in Tierra Bella. An adult from each home was asked for the number of persons living at each residence (further categorized by age group < 5; 5–17; 18–65; > 65), the number of dogs, cats, pet birds, chickens, pigs, horses, and other animals. Of the dogs and cats, the number of

them roaming outside of the property was also noted. From previous observations, we suspected that a large number of stray dogs and cats live in some of these neighborhoods, therefore a final question regarding the number of strays that the adult resident is aware of was also asked. Results from the surveys were tabulated and relative abundance calculated with 95% confidence intervals for selected vertebrates, using the Wilson/Brown method (Table B-3) [33].

Populations of human, dog, cat, chicken, pig, and opossum were estimated by extrapolating the vertebrates documented from survey homes to create estimates of the number of each vertebrate per unit area in the entire community. To achieve this, the average number of vertebrates in the surveyed homes was multiplied by the total number of homes in the defined community to arrive at an estimated density of each vertebrate per unit area. This number was divided by the total estimated number of all potential hosts to obtain relative abundance. Population estimates of wild birds and wild mammals (rodents, meso-predators, etc.) were not obtained.

3.2.7. Human density estimation

We used remote sensing satellite imagery (Google Earth, California, USA) to map the communities within the LRGV using QGIS 3.4 (QGIS Development team 2019). We estimated household densities using the 2010 US census blocks shape file and extracted the information regarding number of houses, number people/house and area of the community. We also quantified household density in Nuevo Amanecer as a

representative community in the city of Reynosa with prior DENV transmission activity (Rodríguez-Pérez Mario A, A. M. A., Russell Tanya L, Olguin-Rodriguez Omar, Laredo-Tiscareño Stephanie V, Garza-Hernandez Javier A, Reyes-Villanueva Filiberto. Host-seeking Aedes aegypti linked to dengue seropositive households at northeastern Mexico. Journal of Vector Borne Diseases (in press)). We used satellite imagery (Google Earth, California, USA) to quantify homes manually and census block information to identify the boundaries of the neighborhood. Nuevo Amanecer was chosen because of its known dengue endemicity.

3.2.8. Host selection indices

We estimated the Forage Ratio (FR), the frequency at which a mosquito selects a vertebrate host over other available vertebrate hosts, by dividing the observed frequency of bloodmeals divided by the expected frequency of bloodmeals of a given species [34]

$$FR = s/a, \quad (1)$$

where s = the percent of female mosquitoes containing blood of a particular host, and a = percent of the total available host population represented by that particular host [35]. A forage ratio of 1.0 indicates mosquitoes are feeding on hosts in equal proportion to availability, whereas values >1.0 indicate over-utilization and values <1.0 indicate under-utilization. We used the Wilson/Brown statistical method to calculate 95% confidence intervals [33].

We also estimated the human blood index (HBI), which measures the frequency at which female mosquitoes feed on human hosts and is the number of human blood meals divided by the number of engorged females [36].

3.2.9. Tamaulipas human disease data

The General Directorate of Epidemiology, Secretariat of Health, México aggregates probable and confirmed empirical cases of DEN, CHIK, and ZIK in the state of Tamaulipas (Figure B-1). Patients with history of travel outside of Tamaulipas in the month prior to onset of symptoms were not included in the modeling of R_0. Physicians of symptomatic patients use a case definition of DEN: fever with >2 signs or symptoms such as retro-orbital or ocular pain, rash, headache, arthralgia, myalgia, leukopenia or hemorrhagic manifestations; CHIK: severe arthralgia, intense asymmetric, debilitating joint pain, swelling associated with tenosynovitis; and, ZIK: pruritic maculopapular rash, for differential clinical diagnosis between the three viruses and are required to report cases to the Secretary of Health of Tamaulipas. Clinical serum samples receiving laboratory confirmation were sent by sanitary jurisdictions of the state to the Molecular Biology Laboratory of the Tamaulipas State Public Health Laboratory. They were stored at −20 °C until further processing.

Nucleic acid extraction was performed using a MagNA Pure LC total nucleic acid isolation kit in a MagNA Pure LC 2.0 Instrument (Roche Applied Science, Germany). The extracted viral RNA was stored at −70 °C. We used RT-PCR to detect the presence of

arboviruses using protocols previously described [37]. We used the SuperScript III Platinum® One-Step qRT-PCR System enzyme (Invitrogen, Carlsbad, CA, USA). A 7500 Fast Real-Time Thermocycler from Applied Biosystems (Foster City, CA, USA) was used, and reportable positive values were below a Ct value of 38.

For ZIKV, the primary patients that received laboratory confirmation using RT-PCR were pregnant females. For the modeling in this study, we used the empirical data of probable cases of ZIKV in the municipality of Reynosa in 2017 with no recent travel history (Figure B-2).

3.2.10. Mathematical modeling

The Ross MacDonald formulation of the basic reproductive number for mosquito-borne diseases [38] is defined as the average number of secondary human cases generated by an index case in an otherwise susceptible population

$$R_0 = \frac{m(af)^2 bce^{-\mu EIP}}{\mu r}, \quad (2)$$

where *m*: the density of female mosquitoes to human, *a*: female mosquito biting rate, *f*: the proportion of mosquito feeding on human, *b*: mosquito-to-human transmission probability, *c*: human-to-mosquito transmission probability, *EIP*: extrinsic incubation period, 1/*r*: human average infectious period, μ: adult mosquito mortality rate [38]. The density of *Ae. aegypti* to humans is a function of mosquito density and human exposure to mosquitoes [38–40] $m = D \times E$ where D is mosquito density and E is the human risk of exposure to *Ae. aegypti*. The risk of exposure to mosquitoes is a function of

socioeconomic variables, such as the availability of air conditioning, which can drastically limit mosquito–human contacts and virus transmission, even when mosquitoes are abundant [18]. Studies have shown that population mobility may also play a role in individuals exposure risk to *Ae. aegypti* [41]. Furthermore, fine-scale variation in population susceptibility, immunity, or social structures may also be factors contributing to vector-borne disease transmission heterogeneity amongst neighboring communities [42]. Though these parameters may be hard to measure empirically, they can play a pivotal role in the risk of mosquito borne disease outbreaks.

Given the geographical proximity and similarities between the city of Reynosa and neighborhoods in the LRGV of South Texas, any difference in the risk of outbreak (R_0), for a newly introduced *Ae. Aegypti*-borne disease such as Zika, between the two communities would be due to the density of mosquito to human (m) or the proportion of mosquito feeding on humans (f). Therefore, R_0 in the LRGV and Reynosa can be written as $R_0^{LRGV} = \frac{m_{LRGV}(af_{LRGV})^2 bce^{-\mu EIP}}{\mu r}$ and $R_0^{Rey} = \frac{m_{Rey}(af_{Rey})^2 bce^{-\mu EIP}}{\mu r}$, respectively. We have $R_0^{Rey} = m_{Rey}(f_{Rey})^2 \frac{(a)^2 bce^{-\mu EIP}}{\mu r}$, which is rearranged as $\frac{(a)^2 bce^{-\mu EIP}}{\mu r} = R_0^{Rey} \frac{1}{m_{Rey}(f_{Rey})^2}$ This implies that

$$R_0^{LRGV} = m_{LRGV}(f_{LRGV})^2 \frac{(a)^2 bce^{-\mu EIP}}{\mu r} = m_{LRGV}(f_{LRGV})^2 R_0^{Rey} \frac{1}{m_{Rey}(f_{Rey})^2}$$

$$R_0^{LRGV} = R_0^{Rey} \frac{m_{LRGV}}{m_{Rey}} \left(\frac{f_{LRGV}}{f_{Rey}}\right)^2, \quad (3)$$

where R_0^{LRGV} and R_0^{Rey} are the basic reproductive numbers in the LRGV and Reynosa, respectively. m_{LRGV} and m_{Rey} are the density of female *Ae. aegypti* to human in the LRGV and Reynosa, respectively; and f_{LRGV} and f_{Rey} are the proportion of *Ae. aegypti* feeding on humans in the LRGV and Reynosa, respectively. We estimated the ratio $\frac{m_{LRGV}}{m_{Rey}} = \frac{D_{LRGV}E_{LRGV}}{D_{Rey}E_{Rey}}$. Female *Ae. aegypti* relative abundance was estimated using AGO data collected in 2017 during the same weeks in the city of Reynosa (5.16 female *Ae. aegypti* per AGO per week) and in the LRGV (4.16 female *Ae. aegypti* per AGO per week) [14]. So $\frac{m_{LRGV}}{m_{Rey}} = 0.8\frac{E_{LRGV}}{E_{Rey}}$. As the proportion of feeding on human in Reynosa is not currently available, we considered a range of values informed by available data from the Americas (Table 3.1): LRGV, Puerto Rico, and Florida.

R_0 in Reynosa municipality was estimated using case data for the 2017 Zika epidemic and the EstimateR function from the EpiEstim R library [43,44] to estimate the time-dependent reproductive number, $R(t)$, based on the method introduced by Cori et al. [43]. We derive R_0 using the fact that R_0 is equal to $R(t)$ at the start of the outbreak, such as Zika, for which we do not have pre-existing immunity in the population [44]. This approach would not be applicable to endemic diseases such as dengue. The instantaneous reproduction number $R(t)$ was computed over 4-week sliding windows using the method introduced by Cori et al. [43]. This approach uses a Bayesian inference method to propagate uncertainty of data and generation time into R_0 estimate. Following Ferguson et al. [44], we assume that the ZIKV generation time is gamma-distributed with

a mean of 20.0 days and a standard deviation (s.d.) of 7.4 days. The incidence data themselves may contain many potential sources of uncertainty such as misdiagnosis, variable time-dependent case detection rate, and asymptomatic cases, which are not explicitly taken into account into our analysis.

Table 3.1 Published studies of *Ae. aegypti* host feeding patterns.

				Feeding Patterns on Vertebrates (%)							
Citation	**Location**	**Method** [a]	**Site** [b]	**Human**	**Mix/Human**	**Dog**	**Cat**	**Other Mammal**	**Avian**	**Unknown**	**Total**
[45]	Nigeria	Ab	In/Out	7 (44%)					1 (6%)	8 (50%)	16
[46]	Tanzania	Ab	In	45 (100%)							45
[47]	Kenya—coast	Ab	In/Out	165 (94%)		1 (0.5%)	1 (0.5%)	9 (5%)			176
[48]	South Africa	Ab	Out	3 (75%)						1 (25%)	4
[49]	India, Poona	Ab	In	17 (81%)						4 (19%)	21
[50]	India	Ab	In	49 (96%)						2 (4%)	51
[49]	Malaya	Ab	In	109 (99%)		1 (1%)					110
[51]	Hawaii	Ab	Out	339 (54%)		117 (19%)	21 (3%)	71 (11%)	3 (0.5%)	80 (13%)	631
[52]	Thailand	Ab	In/Out	789 (88%)	66 (7.4%)	2 (2.2%)	4 (0.5%)	8 (1%)	9 (1%)		896
[53]	Puerto Rico	Ab	In	1483 (95%)	31 (2%)	47 (3%)					1561
[54]	Thailand—single host	Ab	In/Out	658 (99%)			1	4 (0.6%)	1		664
[54]	Thailand—mixed	Ab	In/Out		86 (98%)						88
[55]	E. Australia	DNA	Out	131 (75%)	7 (4%)	23 (13%)	2 (1%)	1 (0.5%)	10 (6%)		174
[56]	Thailand	DNA	N/A	766 (86.1%)	32 (3.6%) *	18 (2%)		39 (4.4%)		35 (3.9%)	890
[57]	Puerto Rico-P	DNA	Out	101 (76.2%)		27 (20.8%)	3 (2.3%)	1 (0.8%)			132
[57]	Puerto Rico-R	DNA	Out	210 (78.9%)	1 (0.4%)	49 (18.4%)	3 (1.1%)		3 (1.1%)		266
[58]	India	Gel precip	In/Out	129 (87.8%)				11 (7.5%)	1 (0.7%)	6 (4%)	147
[59]	India	Gel precip	Out	54 (96.4%)				2 (3.6%)			56
[60]	Mexico	DNA	In/Out	223 (98%)						5 (2%)	228
[61]	Florida—IR	DNA	Out	111 (90.2%)				11 (8.9%)	1 (0.8%)		123
[61]	Florida—M	DNA	Out	8 (61.5%)				5 (38.5%)			13
[62]	Grenada	DNA	Out	22 (70%)		2 (6%)	1 (3%)	6 (18%)	1 (3%)		32

[a] Ab = precipitin test for presence of antibody, DNA = molecular identification, Gel precip = agarose gel precipitin technique. [b] Indoor = In, Outdoor = Out. * Samples were positive for two hosts, but the authors did not reveal which two hosts. It is assumed that one of the hosts is human.

3.3. Results

3.3.1. Blood meal analysis

In total, 230 bloodfed *Ae. aegypti* (Sella score of 2–5) [20] were collected, molecularly confirmed to species and processed for the blood meal analysis (four indoor, 226 outdoor; 181 using BGS2 traps, 49 using AGO). Of these, 186 (81%) yielded a bloodmeal analysis result which include 50% (*n* = 93) from dogs (*Canis lupus familiaris*), 31% (*n* = 57) from humans (*Homo sapiens*), 12% (*n* = 22) from cats (*Felis catus*), 3% (*n* = 6) from chicken (*Gallus gallus*) and 4% from other mammals (Table 3.2). Of the four *Ae. aegypti* collected indoors by AGO traps, three yielded a result (one human, two dogs). Bloodfed *Ae. aegypti* with results came from two different homes in MM, two homes in DF, one home in MCH, four homes in PF/PE, 34 homes in IHE, nine homes in IHW, 19 homes in LP, 10 homes in TB, one home in LF, two homes in MLV, and two homes in WCT. For *Cx. quinquefasciatus*, 124 bloodfed individuals (Sella score of 2–4) were collected, molecularly confirmed to species, and processed for bloodmeal analysis (0 indoor, 124 outdoor; 113 using BGS2 traps, 11 using AGO traps). Of these, 123 (99%) yielded a bloodmeal analysis result which included 67% (*n* = 82) from chicken, 22% (*n* = 27) were from dog, 9% (*n* = 11) from six wild bird species and 2% from other mammals (Table 3.3). Two *Ae. aegypti* samples had mixed bloodmeals including dog and human, while no *Cx. quinquefasciatus* had evidence of mixed bloodmeals. The success of the vertebrate host identification of the blooded abdomen for *Ae. aegypti* was significantly different across Sella scores ($p = 0.0333$; 92% for Sella score of two, 76% for three, 33% for four, and 25% for five). The success of the

vertebrate host identification of the blooded abdomen for *Cx. quinquefasciatus* was not significantly different among Sella scores (p = 0.3333; 99% for Sella score of two, 100% for three, and 100% for four). The quantitative analysis of 18 published studies of *Ae. aegypti* host feeding patterns reveals that humans are the dominant host with an average of 83.1% (Table 3.1). If we only consider prior studies with outdoor mosquito collections, the average percentage of human feeding is 85%. Only two studies, one in Nigeria (Table 3.1) and this current study from South Texas, reveal feeding patterns where humans represent less than half of the bloodmeals.

Table 3.2 Blood meal analysis results and forage ratios for *Ae. aegypti*.

Host	**Count (%)**	**Forage Ratio (95% CI)**
Dog	93 (50%)	1.61 (1.43 - 1.84)
Human	57* (31%)	0.81 (0.73 - 0.91)
Cat	22 (12%)	0.91 (0.73 - 1.13)
Chicken	6 (3%)	0.19 (0.16 - 0.24)
Sheep	3 (1.6%)	2.69** (1.01 - 8.06)
Opossum	2 (1%)	1.19 (0.51 - 2.69)
Pig	3 (1.6%)	2.69 (1.01 - 8.06)
Total	186	

* - includes 2 mixed meals (human-dog)
** - forage ratio estimated based on lowest response from vertebrate surveys (pigs)

Table 3.3 Blood meal analysis results and forage ratios for *Cx. quinquefasciatus*.

Host	Count (%)	Forage Ratio (95% CI)
Chicken	82 (67%)	3.92 (3.33 - 4.87)
Dog	27 (22%)	0.71 (0.63 - 0.81)
House sparrow	6 (5%)	-
Western kingbird	1 (0.8%)	-
Human	1 (0.8%)	0.02 (0.02 - 0.02)
Cat	1 (0.8%)	0.06 (0.05 - 0.08)
Pig	1 (0.8%)	1.36 (0.51 - 4.07)
Plain chachalaca	1 (0.8%)	-
Curvebilled thrasher	1 (0.8%)	-
Northern mockingbird	1 (0.8%)	-
Rock dove	1 (0.8%)	-
Total	123	

3.3.2. Mosquito relative abundance

Mosquito sampling between May 7 to August 13, 2017 using 80 Sentinel AGO traps in Reynosa yielded an average of 5.16 (± 0.43 SEM) female *Ae. aegypti* per AGO per week (Figure B-3). In the LRGV, 30 Sentinel AGO traps during these same weeks yielded an average of 4.16 (± 0.43 SEM) female *Ae. aegypti* per AGO per week [14].

3.3.3. Vertebrate surveys and population density

We conducted a vertebrate questionnaire for 44 homes in four communities asking about all vertebrates living in the home, property, or neighborhood (Table B-3). The average number of occupants per home was 4.7 (± 0.41 SEM) and the total estimated number of homes in all four communities was 612. With our vertebrate surveys, we estimated 5,146 humans per km^2, 4,161 dogs per km^2, 1,751 cats per km^2, 2,299 chickens per km^2, and 75 pigs per km^2 (Table B-4). Independent from the

household questionnaires, our analysis of US Census data using QGIS for the combined communities in the current study where blood-fed individuals were collected and in the eight communities with AGO surveillance [14], we estimate that on average the human density was 3,597 per km^2 (Table B-5). In Nuevo Amanecer, Reynosa, we identified 885 homes in the neighborhood minus the soccer field open space. The area with homes is 0.27 km^2 and using an average occupancy of 4.2 persons per home (based on unpublished data from co-author M. A. Rodríguez-Pérez), the estimated human density for this area is 13,767 per km^2. The human density in Reynosa is between 2.7- and 3.8-fold higher than comparable low-income communities in the LRGV.

3.3.4. Host selection

Forage ratios for *Ae. aegypti* and *Cx. quinquefasciatus* were calculated with host availability estimated from our vertebrate surveys in the neighborhoods where we collected engorged mosquitoes. The *Ae. aegypti* forage ratio (observed frequency of bloodmeals from a given host divided by the expected frequency) for dogs (1.61) was nearly twice the forage ratio for humans (0.81; Table 3.2). In contrast, the highest forage ratio for *Cx. quinquefasciatus* was on chicken (3.92; Table 3.3). The human blood index (total number of human blood meals divided by the total number of engorged females with a confirmed result) for *Ae. aegypti* and *Cx. quinquefasciatus* was 30.7% and 0.8% respectively.

3.3.5. Mathematical modeling

Using the Ross MacDonald equation for the basic reproductive number, R_0, and based on the 2017 cases of ZIKV in Reynosa (Figure B-2) and data on *Ae. aegypti* collected in the LRGV and Reynosa, we estimated ZIKV R_0^{LRGV} in the LRGV. We started by estimating R_0^{Rey} in Reynosa using case data from the 2017 Zika outbreak in Reynosa, where R_0 was 2.2 (95% Confidence Interval: 1.1—3.8) (Figure B-2). A total of 330 cases of Zika were observed in Reynosa in 2017; only a subset were tested by PCR, of which 81 were confirmed positive for ZIKV RNA. Seven Zika cases were not included, given a history of travel in the prior month. Because of the geographical proximity between Reynosa and the LRGV, we assumed that all parameters, except for mosquito abundance and human biting rates, in the Ross MacDonald R_0 equation were equal across the US-Mexico border. We obtained the following expression for R_0 in the LRGV

$$R_0^{LRGV} = R_0^{Rey} 0.8 \frac{E_{LRGV}}{E_{Rey}} \left(\frac{0.31}{f_{Rey}}\right)^2 \quad (4)$$

where 0.8 is the ratio between mosquito abundance in LRGV and Reynosa estimated with AGO traps, and 0.31 is the proportion of *Ae. aegypti* feeding on humans in LRGV. f_{Rey} is the proportion of *Ae. aegypti* feeding on human in Reynosa, and E_{Rey} (E_{LRGV}) is the risk of human exposure to *Ae. aegypti* bites in Reynosa (LRGV). Then, with the R_0^{Rey} estimate and using different combinations for the unknown parameters, f_{Rey} and E_{Rey}/E_{LRGV}, of Equation (4) we studied the conditions for the establishment, i.e., $R_0 > 1$, of a ZIKV epidemic in the LRGV (Figure 3.2). Our analysis shows that if $f_{Rey} = f_{LRGV}$

then ZIKV outbreaks would not occur in the LRGV when the risk of human exposure to *Ae. aegypti* bites in the LRGV is below 60% of the risk in Reynosa (Figure 3.2). Below 60%, there are limited scenarios where Zika outbreaks may occur in the LRGV: for example, when the f_{Rey} is smaller than f_{LRGV} and the human exposure risk to *Ae. aegypti* bites in Reynosa is fives time larger than in LRGV (Figure 3.2).

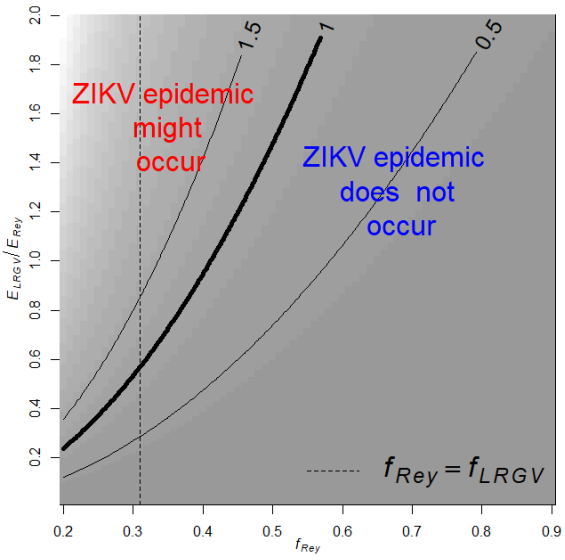

Figure 3.2 Contour plot of R_0 in the LRGV as a function on the relative risk of human exposure to *Ae. aegypti* in the LRGV compared to Reynosa (E_{LRGV}/E_{Rey}), and the proportion of *Ae. aegypti* feeding on humans in Reynosa (f_{Rey}). The dashed vertical line indicates when $\frac{f_{LRGV}}{f_{Rey}} = 1$.

3.4. Discussion

This study documents *Ae. aegypti* feeding on humans only 31% of the time in the sampled communities in South Texas; instead, the majority of bloodmeals (50%) were from domestic dogs. This is an unexpectedly low rate of human feeding given that this species is ubiquitously classified as an anthropophilic species [18,63]. The quantitative

synthesis of 18 published blood meal analysis studies on *Ae. aegypti* shows that the average percent of human blood-feeding was 83.8%. In 1967, MacDonald pointed out that "Although *Ae. aegypti* has been the study of a very large number of papers, there are only a few records of its host preference [49]." A half century later, this observation remains the same, given that only 21 studies have published *Ae. aegypti* host feeding patterns, three of which concerned the subspecies *formosus,* while our review of the published literature identified 86 primary publications that have reported host feeding patterns for members of the *Cx. pipiens* complex. The less attention to *Ae. aegypti* host feeding is likely due to the assumption that this species is largely anthropophilic and reluctance to conduct the expensive bloodmeal analysis for confirmation.

Zooprophylaxis is the concept that the presence of incompetent hosts can 'waste' bites from vector species and reduce the transmission of an infectious agent [64]. Furthermore, prior studies have identified that arboviral transmission potential is impacted by host community composition and competence [65,66]. For human-amplified urban arboviruses like ZIKV, less feeding on humans and more feeding on non-competent hosts (vertebrates with a low duration and magnitude of viremia unable to re-infect *Ae. aegypti*), will have a zooprophylactic effect on transmission, as originally observed with cattle when describing zooprophylaxis in malaria transmission [67,68]. This has principally been considered an important phenomenon in human malaria transmission with *Anopheles* spp. wasting bites on cattle, something that protects humans from malaria infectious bites [69–71]. However, Hess and Hayes [64] determined that potential for zooprophylaxis exists in *Cx. tarsalis, Cx. pipiens, Cx. quinquefasciatus* and *Ae.*

albopictus. Moreover, in East Africa the observation of non-human host feeding by *Ae. aegypti* led to the conclusion that these were likely to be poor vectors of yellow fever virus [29].

Non-human feeding by *Ae. aegypti* may have important consequences for arboviral pathogen transmission. For example, the receptivity of certain regions of the world to *Ae. Aegypti*-driven arboviruses might vary not simply due to the abundance of *Ae. aegypti*, but due to the proclivity and availability of *Ae. aegypti* to feed on humans relative to other hosts. In that sense, threshold indices, such as R_0, that indicate when the transmission of viruses will persist, can guide management activities and even inform urban planning and home modification to further reduce the probability of *Ae. aegypti* feeding on humans.

Collection technique and location can influence the apparent host feeding patterns. While only 2% of the collections in this study were from inside homes, all of the specimens were collected within the residential yard. While our previous study documented *Ae. aegypti* inside homes of the LRGV [14], we did not target indoor collections with aspirators, given the unique socio-demographics and political climate of the LRGV, which makes indoor access challenging. Prior studies on *Ae. aegypti* host feeding patterns are limited, with only ten studies (56%) with indoor collections, seven (39%) with collections from residential yards, and two studies (11%) with collections in non-residential locations (Table 3.1). Of the published studies reporting *Ae. aegypti* blood meal results, most (69%) have been conducted in regions of the world where dengue is endemic (Table 3.1). This identifies a research gap, with a few studies such as

this one reporting *Ae. aegypti* host feeding patterns in areas where the environment may be suitable for arboviral transmission, but risk appears to be diminished by limited access to humans, and, possibly easier access to non-human vertebrates. We also did not process blood-engorged mosquitoes collected in Reynosa in the same laboratory as the current study, which is a priority for future research.

The low rate of *Ae. aegypti* anthropophily in the current study could be explained by several reasons. The most parsimonious explanation is the higher availability of non-human hosts, limited opportunity for human biting, and lower human density [18,63]. In our Texas study sites, humans make up 41% of all domestic hosts in the four study sites combined. Our analysis does not account for wild birds and wild mammals which, if included, would further reduce the relative abundance of humans compared to non-human animals. Although many human-amplified urban arboviruses occur in densely populated settings, a study in Thailand found the largest dengue epidemics occurred in low to moderate population densities, where water storage and the production of mosquitoes is an additional factor driving transmission [72]. Another factor influencing the ability of *Ae. aegypti* to feed on humans is the integrity of the home and frequency of indoor feeding. Our recent study in nearby neighborhoods shows that the outdoor *Ae. aegypti* relative abundance is about eight times that of the indoor population [14]. Prior studies in the Texas–Mexico border region have shown that the presence of air conditioning units and larger lot size are associated with a lower probability of homeowners being exposed to DENV [13,18]. A final hypothesis explaining the low rate of *Ae. aegypti* feeding on humans is that there is a genetic basis. A genetic basis for host

selection has been well documented in *Culex* spp. [73] and *Anopheles* spp. [74]. In 1967, Macdonald [49] reviewed the progress on understanding the ecology of multiple forms of *Ae. Aegypti*, including *Ae. aegypti formosus* found in east Africa which tended to be more exophilic and frequently fed on non-human hosts. More contemporary population genetics studies have confirmed that *Ae. aegypti formosus* is the ancestral form of the domesticated *Ae. aegypti aegypti*, which lives in tight association with human landscapes and is more anthropophilic [75]. The host preference of the ancestral and domesticated forms of *Ae. aegypti* in east Africa is considered to have a genetic basis [76,77]. Although the domesticated form of *Ae. aegypti* has spread around the world, Macdonald [49] postulated that, outside Africa, the plasticity of the species means the potential for non-human feeding and exophily exists, and the current study supports the ability of *Ae. aegypti* to adapt to an environment with lower availability of human hosts [63,78].

With *Ae. aegypti* feeding on non-human hosts about 70% of the time, this study highlights the potential role of *Ae. aeygyti* in contributing to enzootic transmission among animals or even bridge transmission of zoonotic agents to humans. Of the non-human bloodmeals, 50% were from domestic dogs. A recent study testing dogs from animal shelters in the LRGV (Edinburg, TX, USA) identified 20.9% of the dogs to be infected with dog heartworm, *Dirofilaria immitis* [79]. Several studies suggest *Ae. aegypti* as an efficient vector of *D. immitis* in dogs [80,81], and a study in Florida found *Ae. aegypti* infected with *D. immitis* [82]. Given that *Ae. aegypti* is the dominant mammalophilic mosquito species in low- and middle-income LRGV residential communities [14], these observations suggest that *Ae. aegypti* may play a role in *D.*

immitis transmission, which warrants further research. Prior studies have also documented the potential spill-over of human-amplified urban arboviruses into wild or domestic animals [83,84], suggesting that *Ae. aegypti* could play the role of a bridge vector in this context.

The blood meal analysis results for *Cx. quinquefasciatus* yielded 75.6% of the bloodmeals from birds, with chickens being the dominant species (Table 3.3). These results are consistent with prior studies which show that *Culex* are principally ornithophilic [85]. Both the bloodfed *Culex* and *Aedes* were processed with the exact same protocol, and the contrasting results provide more confidence in the accuracy of the identified blood meals. The high host chicken use is consistent with *Cx. quinquefasciatus* host feeding patterns in tropical and subtropical regions [86]. The inclusion of Passerines as hosts by *Cx. quinquefasciatus* would suggest their potential role as an amplification vector for West Nile virus (WNV) in South Texas, while the observation of human feeding would suggest the potential to bridge WNV to humans. In some regions of the world, *Cx. quinquefasciatus* can be highly anthropophilic [87] and it was surprising to not find more human-derived bloodmeals. A good example of a study that analyzed both forage ratio (FR) and the human blood index (HBI) for *Cx. quinquefasciatus* mosquitoes was conducted by Garcia-Rejon et al. in Yucatan State, Mexico [35]. They found an HBI of 6.7, but the FR for humans was < 1 when compared to that of other vertebrate hosts, indicating that *Cx. quinquefasciatus* mosquitoes in this area under-utilized humans as hosts. In fact, species of the Passeriformes and Galliformes orders were the only hosts that had a FR >1 [35]. This ornithophilic pattern

of *Cx. quinquefasciatus* mosquitoes was also demonstrated in College Station, Texas (95.5% blood meals on birds) [34]; and Harris County, Texas (39.1% on birds) [88].

3.5. Conclusions

In conclusion, we identify a potential mechanism explaining how ZIKV resulted in large epidemics in Reynosa, Tamaulipas, Mexico but did not result in widespread transmission in the LRGV of South Texas. The population of *Ae. aegypti* in South Texas fed on humans only 31% of the time, which is likely due to the abundance of non-human hosts in the residential neighborhood, the low human density, and social practices of minimizing risk of exposure to *Ae. aegypti* [18]. The high rate of non-human blood meals of *Ae. aegypti* occurring in the LRGV is likely reducing the risk of human-amplified urban arboviruses such as DENV, ZIKV, and CHIKV. However, the high number of blood meals from dogs and cats is concerning for zoonotic agents such as dog heartworm transmission and the potential for bridge transmission to human populations [89]. The population of *Cx. quinquefasciatus* in the LRGV was ornithophilic, which likely contributes to the local transmission of WNV observed in the region. This study revealed high non-human host utilization in *Ae. aegypti* mosquitoes, which warrants further research to determine factors driving the variation in mosquito–human contact.

3.6. Acknowledgements

We are grateful to the residents of our study locations in the Rio Grande Valley, TX that granted us permission to trap mosquitoes on their property. We thank Ester

Carbajal, Edwin Valdez, and Courtney Avila for assistance in the field and Helena Hopson, Victoria Kamilar, and Wendy Tang for assistance in the lab. We appreciate the helpful review and comments from Sarah Hamer, Roberto Barrera, Ryan Hemme, and Nga Vuong on an earlier version of this manuscript. Three anonymous reviewers provided constructive reviews which improved the manuscript.

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4. GLOBAL PATTERNS OF ‘AEGYPTISM WITHOUT ARBOVIRUS’

4.1. Introduction

Over half the world’s population lives in areas at risk of human-amplified urban arboviruses transmitted by the *Aedes aegypti* mosquitoes [1]. In addition to chikungunya, yellow fever, and Zika viruses, *Ae. aegypti* is the primary vector for dengue virus which infects an estimated 390 million individuals each year [2], with 100 million of those being symptomatic [3]. While great strides have been made in vector surveillance and control through conventional, biological, and genetic approaches, and vaccine development is ongoing [see 4], dengue transmission is expected to persist, and in some regions, expand while other regions contract [1].

Many studies have identified environmental, meteorological, and demographic factors related to vector populations and arboviral transmission such as human population density, climate, normalized difference vegetation index (NDVI), gross domestic product (GDP) [5,6]. More recent research has considered the impact of socio-economic status [7] and urbanization including urban heat islands [8] on risk of increased dengue transmission [9,10]. Understandably, studies tend to be conducted in locations of high endemicity for arboviral disease transmission or where recent outbreaks have occurred. Rarely have studies evaluated landscape influences of *Ae. aegypti* populations or arbovirus transmission in locations representing the margins of endemicity. We recently conducted a study in South Texas where large populations of *Ae. aegypti* occur yet local transmission of human-amplified urban arboviruses is rare,

and we discovered high rates of non-human feeding by *Ae. aegypti* [11]. These wasted bites on non-amplification hosts likely reduced R0 for ZIKV limiting local transmission to 10 human cases between 2016-2017. In contrast, Tamaulipas, the Mexican state across the border, reported 16,835 cases in the same period. The lower availability of humans to *Ae. aegypti* and associated utilization of non-human hosts is one of several mechanisms for a phenomenon we term 'aegyptism without arbovirus'; defined as the occurrence of established *Ae. aegypti* populations without endemic human-amplified urban arboviruses. This context is similar to the long-held observation of 'anophelism without malaria' [12,13], where researchers starting in the 1920s started to notice and understand the mechanisms of some regions having Plasmodium-competent Anopheles spp. mosquitoes but not the associated human malaria. The objective of this study is to explore the global patterns of environmental suitability for *Ae. aegypti* and dengue to characterize the deviations in these predictions. We addressed this objective by developing a map predicting a gradient ranging from higher suitability for *Ae. aegypti* but low environmental suitability for DEN to the other end of the spectrum where areas have similar and higher suitability for both *Ae. aegypti* and DEN. We then identify environmental, meteorological, and demographic factors associated with this gradient in the deviation between *Ae. aegypti* and DEN suitability to explore the social-ecological factors driving 'aegyptism without arbovirus'.

4.2. Materials and Methods

4.2.1. Deviation between the probability of occurrence of *Aedes aegypti* and dengue

This study utilized the 2015 global probability of occurrence for *Ae. aegypti* based on a mosquito database and environmental variables predicting their global distribution [14]. We also used the 2015 global probability of dengue occurrence which was based on an ecological niche model of human cases to predict environmental suitability [1]. To compare the global pattern of *Ae. aegypti* and dengue we performed a raster calculation in QGIS (version 3.10.1-A Coruña). Both the *Ae. aegypti* distribution map and the global probability of dengue occurrence map are at 5 km2 resolution. We removed all cells where either aegypti or dengue suitability were < 0.1. To create a map that illustrates where *Ae. aegypti* and dengue deviate spatially, we calculated "*Ae. aegypti*" - "dengue". This procedure removed all pixels on the map where an interaction between *Ae. aegypti* and dengue did not occur giving us a map of the probability of *Ae. aegypti* presence and dengue environmental suitability. This resulting *Ae. aegypti* minus dengue raster ('uncorrected') produced one end of the spectrum with a suitable environment for *Ae. aegypti* but low dengue and the other end of the spectrum included an equal suitability for both *Ae. aegypti* and dengue. The problem with this later end was that areas of the world with near zero suitability for both *Ae. aegypti* and dengue were indifferent from areas with high suitability for *Ae. aegypti* and dengue. To account for this, we incrementally removed areas with lower DEN environmental suitability (Table 4.1).

Table 4.1 Correction to the deviation in *Ae. aegypti* and dengue map by clipping out respective areas with a lower probability of dengue environmental suitability.

Level	Uncorrected deviation in *Ae. aegypti* and DEN	Clip areas ≤ these values for the probability of DEN suitability	Result
1	- 0.5	0.8	Remaining cells only have stronger DEN suitability
2	- 0.35	0.75	
3	- 0.2	0.7	
4	- 0.05	0.65	Remaining cells have medium DEN suitability
5	0.1	0.6	
6	0.25	0.55	
7	0.4	0.5	Remaining cells have weaker DEN suitability

To create Level 1, we performed this raster calculation: ("deviation layer" ≥ -0.5) * ("dengue_mean_mask layer" ≥ 0.5). The output raster was saved as "Level 1"; this level represents areas with similar and high suitability of both *Ae. aegypti* and dengue. This same procedure was used to create the remaining levels in Table 4.1.

Because our focus is on 'aegyptism without arbovirus', we filtered the deviation raster to only include values ≥ 0 (to exclude areas where dengue risk was greater than probability of *Ae. aegypti* presence).

4.2.2. Socio-ecological patterns in the deviation between *Ae. aegypti* and arbovirus

To identify environmental, meteorological, and demographic factors relating to the deviations between *Ae. aegypti* probability of occurrence and DEN environmental suitability we gathered several global datasets. Population density maps were obtained

through NASA's SEDAC website [15]. A global map of total gross domestic product (GDP) per capita data at 30 arc-sec resolution was obtained from Kummu et al. [16]. Total GDP per cell was estimated by multiplying per capita GDP by gridded population data [16]. A global map of 2015 subnational infant mortality rates was obtained from NASA's SEDAC website. Infant mortality rate is defined as the number of children who die before their first birthday per 1000 live births. Infant mortality rate (IMR) is often used as an indicator for poverty [17] and dengue infection during pregnancy has been linked to increased risk of infant mortality, among other adverse health outcomes [18].IMR data was available from 234 countries, with 143 of those countries reporting subnational units at the 30 arc-second (approximately 1 km^2) resolution [19]. Global precipitation and temperature rasters at 30 arc-sec spatial resolution were obtained from worldclim.org [20]. These rasters represent average monthly data from 1970 to 2000 and are separated by month. We combined the 12 monthly rasters to create one annual mean temperature raster, and a cumulative annual precipitation raster. We hypothesize that temperature and precipitation will have an inverse relationship, where higher annual average temperatures and higher cumulative rainfall will be correlated with a lower deviation value on the scale of equal and greater probability of *Ae. aegypti* occurrence without dengue environmental suitability. We also hypothesize that elevation will be positively correlated with 'aegyptism without arbovirus'. Cells with missing values were removed across all layers before performing the analysis.

To identify the best-fit model, the gradient boosting machine (GBM) package in R was used. We used a learning rate of 0.001, 5-fold cross validation and 10,000 trees to

minimize the mean squared error (MSE) loss function. Subsequently, a generalized additive model was used to detect relationships between the deviation values and the independent variables. Finally, a generalized additive model (GAM) was used to determine the individual effects of each independent variable. All statistical analyses were conducted in R version 3.5.1 [21] using RStudio version 1.1.456 [22]. To import and analyze rasters in R, we utilized the packages of 'raster', 'dplyr', 'mgcv', and 'ggplot2'[23].

4.3. Results

4.3.1. Deviation between the probability of occurrence of *Aedes aegypti* and dengue

A map was generated showing global deviations between the probability of *Ae. aegypti* and dengue occurrence (Figure 4.1). Values range from 0 (equal and higher probability of occurrence of both *Ae. aegypti* and dengue) to 0.48 (higher suitability of *Ae. aegypti* with low suitability of dengue). For example, a 5 x 5 km^2 area that has a 0.78 probability of occurrence for *Ae. aegypti* but only a 0.3 environmental suitability for DEN would have a deviation value of 0.48). A total of 1,209,689 cells with a deviation value were recorded, ranging from 0 to 0.48 with a mean of 0.07 (residual standard error: 0.09 on 1,209,688 degrees of freedom) (Figure 4.2). We report the mean deviation values for each country in Table C-2 which range from 0 to 0.27.

4.3.2. Socio-ecological patterns in the deviation between *Ae. aegypti* and arbovirus

The gradient boosting machine (GBM) for the full model, including population density, GDP, IMR, annual average temperature, annual cumulative precipitation, and elevation resulted in a MSE of 0.084. The independent variable contributing the least to explaining the variation in the dependent variable was elevation (0.067). After removing the elevation data, GBM was conducted again on the remaining variables in a stepwise fashion (Table 4.2). A generalized additive model revealed model 3 to have the best-fit model with an R^2 value of 0.115 (Table 4.3). Statistically significant interactions were found between the dependent variable (amd) and population density with IMR, temperature, and precipitation as the smooth terms. The population density layer had 1,206,335 values that corresponded with each pixel on the deviation raster image, with a range of 0 to 119,921 persons per km^2 and a mean of 135.93 (residual standard error: 0.09 on 1,206,333 degrees of freedom) persons per km^2. Population density had a parametric coefficient estimate of -4.062e-06 (± 1.190e-07 SE; pr (>|t|) = <2e-16) (Table 4.4).

The subnational IMR map contained 1,194,792 values at national and subnational resolution ranging from a low of 0.24 to a high of 142.93 and a mean of 35.63 (infant deaths per 1,000 live births)(residual standard error: 0.09 on 1,194,790 degrees of freedom). Using population density, temperature and precipitation as smoothing terms, the parametric coefficient for IMR was 3.002e-04 (± 3.067e-06 SE; pr (>|t|) = <2e-16). Mean annual temperatures ranged from 6.98°C to 31.21°C throughout the range covered by the deviation raster, with a mean of 25.31°C (residual standard

error: 0.09 on 1,207,056 degrees of freedom). The parametric coefficient for temperature was 1.347e-03 (± 4.233e-05 SE; pr (>|t|) = <2e-16). Precipitation had a range of 4 to 9,083 mm rainfall and a global mean of 1,550.18 mm (residual standard error: 0.09 on 1,207,195 degrees of freedom). The parametric coefficient for precipitation, using population density, temperature and IMR as smoothing terms, was -1.530e-05 (± 1.127e-07 SE; pr (>|t|) = <2e-16).

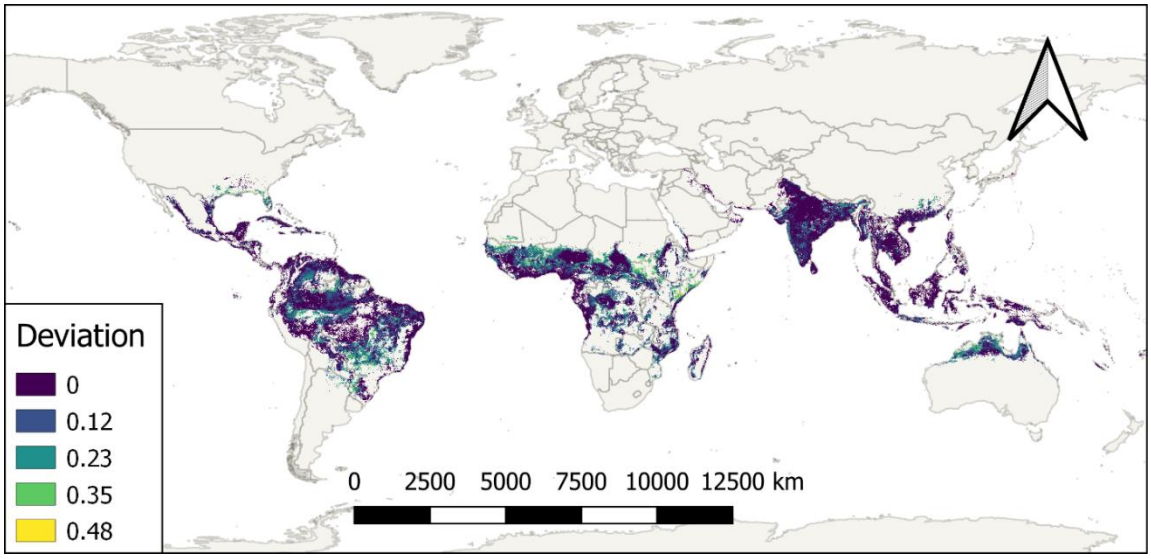

Figure 4.1 Deviation between *Ae. aegypti* probability of occurrence and dengue environmental suitability. Green and yellow indicates areas where *Ae. aegypti* is likely to be found, but the environment is not considered suitable for dengue transmission (e.g. Southern United States, Northern Argentina, Northern Australia). Purple indicates areas where the environmental suitability of *Ae. aegypti* and dengue is similar and higher.

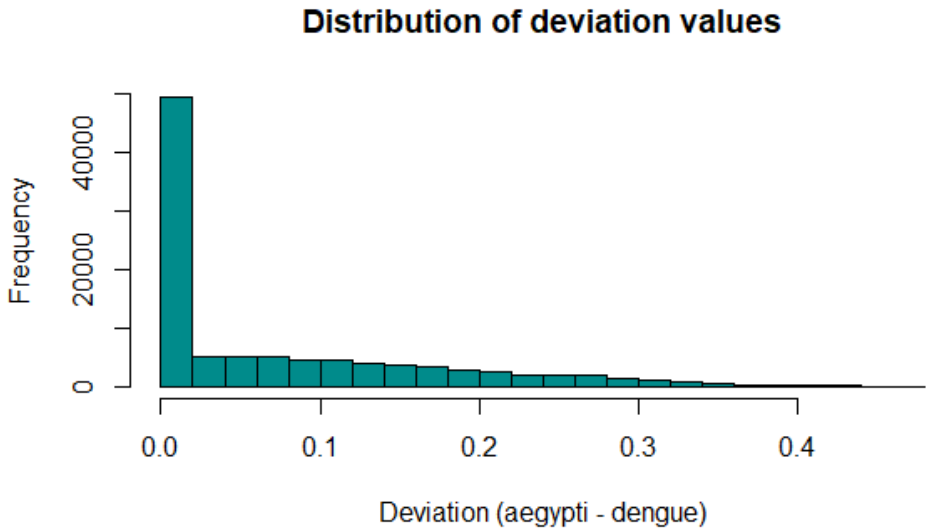

Figure 4.2 Histogram illustrating the distribution of deviation between *Ae. aegypti* probability of occurrence and dengue environmental suitability.

Table 4.2 Gradient Boosting Machine (GBM) to determine best-fit model.

Dependent variable	Independent variables	Greatest relative influence (value)	Least relative influence (value)
amd	pop, gdp, imr, temp, prec, elev	pop (38.475)	elev (0.067)
amd	pop, gdp, imr, temp, prec	pop (38.460)	gdp (2.276)
amd	pop, imr, temp, prec	temp (44.382)	imr (10.905)
amd	pop, temp, prec	temp (48.580)	prec (22.376)
amd	pop, temp	temp (68.744)	pop (31.256)

Table 4.3 Results of Generalized Additive Model (GAM). Family: gaussian; link function: identity.

Model	Formula	Adjusted R^2	Deviance explained
1	amd ~ pop + gdp + s(imr) + s(temp) + s(prec) + s(elev)	0.0406	4.07%
2	amd ~ pop + gdp + s(imr) + s(temp) + s(prec)	0.0424	4.24%
3	amd ~ pop + s(imr) + s(temp) + s(prec)	0.115	11.5%
4	amd ~ pop + s(temp) + s(prec)	0.0984	9.84%
5	amd ~ pop + s(temp)	0.0833	8.33%

Table 4.4 Results of Generalized Additive Model (GAM) for Model 3. Family: gaussian; link function: identity. (Formula: amd_r ~ pop_r + s(imr_r) + s(temp_r) + s(prec_r); n = 1,190,702).

Parametric coefficients:				
	Estimate	**Standard Error**	**t-value**	**pr (>|t|)**
(intercept)	7.223e-02	8.197e-05	881.15	<2e-16 ***
pop	-4.063e-06	1.190e-07	-34.15	<2e-16 ***

Approximate significance of smooth terms:			
	edf	**F**	**p-value**
s(imr)	8.999	2334	<2e-16 ***
s(temp)	8.996	9309	<2e-16 ***
s(prec)	8.998	1477	<2e-16 ***

*** < 0.001

4.4. Discussion

Aedes aegypti has proliferated in urban areas around the globe in the last century. While ubiquitous in many tropical and subtropical urban areas, some locations infested with *Ae. aegypti* do not exhibit high levels of human-amplified urban arboviral transmission as in other areas. This study built on previous studies mapping the global suitability of *Ae. aegytpi* and dengue to generate a map of deviation values including the observation of 'aegyptism without arbovirus'. We produced a global map showing this gradient from high suitability for *Ae. aegypti* but low suitability for dengue to the other end of the spectrum where areas have similar and higher suitability for both *Ae. aegypti* and dengue. We show that some countries on the margins of endemicity of human-amplified arboviruses have a higher deviation value compared to highly endemic countries. For example, the U.S. and Argentina, both countries with occasional

autochthonous transmission of dengue virus [24-26] have mean deviation values of 0.16 and 0.18, respectively (Figure C-2 and Figure C-3). These higher values along this spectrum are more representative of 'aegyptism without arbovirus'. This is also corroborated by empirical data showing that even in areas with high abundances of *Ae. aegypti*, low human feeding diminishes the risk of Zika virus transmission [11]. Countries highly endemic for dengue, such as Honduras and Thailand, have mean deviation values of 0.038 and 0.023, respectively.

We identified a significant association between population density and the deviation in environmental suitability of *Ae. aegypti* and dengue. Locations with higher deviation values had lower population densities. This means that regions of the world with 'aegyptism without arbovirus' are more likely to be lower population densities compared to regions with more equal and higher probabilities of *Ae. aegypti* and dengue. It was surprising to see that GDP did not have a significant effect on the deviation values. Åström et al. modeled various scenarios of dengue distribution according to climate and socioeconomic change, finding a beneficial, protective effect from increasing GDP [27]. Locations with higher GDP would presumably have better access to piped water, screened windows and possibly air conditioning, factors which could reduce arboviral transmission [28]. In addition to GDP, Kummu et al. also mapped a human development index (HDI) which is composed of the achievement of several key development indicators, and this may be a better predictor of deviation. Interestingly, the deviation values for 'aegyptism without arbovirus' were positively correlated to infant mortality rates. We expected to see a higher deviation values representing 'aegyptism

without arbovirus' in places with lower IMR, but this wasn't the case. One potential explanation is reporting bias with some low-income areas having higher dengue burdens that what are reported. For example, Africa has a wide variety of common febrile illnesses with varying etiology, thus a case of dengue fever could be inadvertently misdiagnosed as malaria, especially in places where testing is less than rigorous or non-existent [29]. Regions with notoriously high IMR, but where dengue is underreported could therefore appear to have higher presence of *aegypti* without arbovirus.

Recent studies suggest that climate change, while limiting expansion of *Ae. aegypti* in some locations, will likely increase the risk of human exposure in other areas like North America, Australia and Europe [30,31]. Certainly, temperature plays an important role in its propagation [32]. Interestingly, our study found a significant relationship between temperature and 'aegyptism without arbovirus', where higher average annual temperatures were associated with higher suitability for *Ae. Aegypti* and lower suitability of dengue. This pattern is based on average yearly temperatures and seasonality and diurnal temperature fluctuations were not considered. Carrington et al. found greater potential for DENV transmission in *Ae. aegypti* exposed to large diurnal fluctuations at lower mean temperatures [33]. Further study on the effects of temperature on 'aegyptism without arbovirus' is needed. Precipitation is also a main driver of *Ae. aegypti* populations as a water source is necessary for oviposition. We observed a significant effect on deviation where lower average precipitation was associated with higher probability of *Ae. aegypti* without arbovirus disease. It's interesting to note, however, that many locations with less than 100 mm per year in rainfall were still

considered highly suitable for *Ae. aegypti*. Perhaps places with little to no rainfall such as Phoenix, Arizona, are still capable of maintaining high populations of *Ae. aegypti* due to prolific use of water in the urban landscape and abundant container habitat [34].

Our analysis is built upon predictions of environmental suitability of *Ae. aegypti* [14] and dengue [1], which introduces sources of error and uncertainty. For example, Messina et al. [1] global predictions of dengue includes high risk in regions such as Arkansas, USA, with values around 0.87 (range of 0-1). There is no documented autochthonous transmission of any human-amplified arbovirus in Arkansas in the last two centuries [35,36]. As a result of this model's prediction, our deviation map includes values in Arkansas from 0-0.15, that would falsely indicate that this region has both similar and high levels of *Ae. aegypti* and dengue. These anomalies likely occur elsewhere in the world with these deviation value predictions, especially in developing countries where differential diagnosis of febrile illness is less common.

In conclusion, our study identified several focal points around the globe which appear to exhibit this phenomenon of 'aegyptism without arbovirus'. Parts of South America, Africa, South Europe, and North Australia appear to exhibit this same phenomenon that we find in the United States. While *Ae. aegypti* is found in all of these locations and even expanding in many areas, vector presence does not unequivocally translate to the transmission of human-amplified urban arboviruses such as dengue. A suite of factors such as *Ae. aegypti* vector competence, utilization of humans as hosts, and human social practices reducing contact with mosquitoes are likely to influence the risk of arbovirus transmission. Further research to elucidate the underlying mechanisms

which facilitate ‘aegyptism without arbovirus’ is warranted. The knowledge gained from this research will help guide scientists, public health officials and policy makers in our ongoing battle against mosquito-borne viruses.

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5. SUMMARY AND FUTURE RESEARCH

This study of the sugar and blood feeding patterns of *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes, along with the geospatial analysis of 'aegptism without arbovirus', has added significantly to our current understanding of the interplay between vector, host and landscape. Our results clearly demonstrate spatial-temporal heterogeneity in vector behavior and contact with hosts and how these variations strongly influence the pathogen transmission potential for a given location. Furthermore, this study sheds light on why some locations where these mosquito species are found are endemic for multiple human-amplified urban arboviruses while other places with the vector species are non-endemic.

Chapter II investigated the sugar feeding patterns of both species in South Texas, comparing our results with other locations. Our results supported previous research suggesting sugar feeding among *Ae. aegypti* mosquitoes is limited in comparison to their male counterparts, or both male and female *Cx. quinquefasciatus* mosquitoes. However, the frequency of fructose feeding by *Ae. aegypti* females as determined by the cold anthrone analysis was over four times the frequency recorded in other locations. This is also the first study we know of to document frequency of fructose feeding among female *Ae. aegypti* that is only slightly less than that of male *Ae. aegypti*. This higher than expected sugar feeding frequency could be a factor contributing to lower rates of human biting and therefore, lower rates of arboviral pathogen transmission. Future studies should examine how physiological condition and time since sugar meal influences

results from wild populations. Furthermore, identifying the preferred source of sugar meals will greatly enhance our ability to utilize sugar in surveillance and control activities.

In Chapter III, we analyzed the blood meals of both *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes of South Texas. While the primarily avian results from *Cx. quinquefasciatus* were expected, the low rate of human feeding among *Ae. aegypti* mosquitoes was surprising. Only 31% of the blood meals were from human hosts while 50% were derived from dogs. Our results contrasted greatly with those of 18 previous blood meal analysis studies which found an average of 83.8% human-derived blood meals. The high abundance of non-human hosts in the South Texas study sites appears to reduce the risk of human-amplified urban arboviruses while just across the border in Reynosa, Mexico high rates of dengue, Zika and chikungunya virus transmission are common. While the higher rate of non-human feeding appears to offer a type of zooprophylaxis, the higher rate of feeding on dogs suggest that *Ae. aegypti* may play a role in *D. immitis* transmission, which warrants further research. Future studies should explore the role of non-human feeding in other locations, including places where human-amplified arboviruses are rare.

Given the observation of abundant *Ae. aegypti* populations over much of the southern U.S., and our recent discovery of high non-human feeding by *Ae. aegypti* in South Texas, we explored global patterns of 'aegyptism without arbovirus'. Starting with 5 km^2 resolution global maps of *Ae. aegypti* occurrence and dengue risk, we produced a global map showing a gradient from high suitability for *Ae. aegypti* but low suitability

for dengue to the other end of the spectrum where areas have similar and higher suitability for both *Ae. aegypti* and dengue. We show that some countries on the margins of endemicity of human-amplified arboviruses have a higher deviation value compared to highly endemic countries. Using a linear regression model, we found statistically significant associations between population density, temperature, precipitation and elevation with the deviation map we produced. In particular, regions of the world with 'aegyptism without arbovirus' are more likely to have lower population densities compared to regions with more equal and higher amounts of *Ae. aegypti* and dengue. Further research to elucidate the underlying mechanisms which facilitate 'aegyptism without arbovirus' is warranted.

In summary, the feeding patterns of *Ae. aegypti* are a critical consideration when evaluating the risk of human-amplified urban arboviruses in a given location. Establishing the occurrence of regular sugar feeding among *Ae. aegypti* females has important consequences in the development of surveillance and control techniques and may play a part in reducing human bites. Moreover, the available vertebrate host community may have a zooprophylactic effect in some areas, underlining the importance of ongoing blood meal analysis studies. Finally, other locations around the globe also exhibit 'aegyptism without arbovirus'. Learning more about the factors which drive or inhibit human-amplified urban arboviruses is critical for managing these emerging infectious diseases.

APPENDIX A

SUPPLEMENTAL FIGURES AND TABLES FOR CHAPTER 2

Table A-1 Descriptive statistics of fructose and total sugar content for lab-raised *Ae. aegypti* at various physiological states. (UF = unfed; SF = sugar fed; BF = blood fed; P-Ovi = post oviposition)

Cold Anthrone (fructose)	**Male UF**	**Male SF**	**Female UF**	**Female SF**	**Female BF**	**Female Gravid**	**Female P-Ovi**
Number of values	21	20	19	19	35	13	24
Minimum	0.000	0.000	0.000	2.270	0.8200	1.060	0.5900
Maximum	6.870	8.620	4.810	10.77	7.510	7.110	11.44
Range	6.870	8.620	4.810	8.500	6.690	6.050	10.85
Mean	0.4576	1.189	0.7874	4.650	3.723	3.591	2.326
Std. Deviation	1.483	2.174	1.217	2.074	1.842	1.926	2.048
Std. Error of Mean	0.3236	0.4861	0.2791	0.4759	0.3114	0.5342	0.4181
Hot Anthrone (total sugar)							
Number of values	21	20	19	19	35	13	24
Minimum	0.000	0.000	0.000	4.110	3.170	3.270	2.670
Maximum	1.410	36.60	5.310	28.19	12.99	18.93	30.66
Range	1.410	36.60	5.310	24.08	9.820	15.66	27.99
Mean	0.2738	4.278	0.6789	10.45	5.862	7.474	5.964
Std. Deviation	0.3881	7.725	1.392	7.047	2.295	4.237	5.494
Std. Error of Mean	0.08469	1.727	0.3193	1.617	0.3879	1.175	1.121

Table A-2 Mean total sugar content (±SE) of *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, all collection methods and seasons.

Species	Sex	*n*	Mean µg ± SE	Range µg	Baseline from Lab µg ± SE (*n*)
Ae. aegypti	Male	310	17.28 ± 1.46	0 - 173.3	0.37 ± 0.12 (10)
	Female	366	8.63 ± 1.03	0 - 240.4	1.33 ± 0.31 (10)
Cx. quinquefasciatus	Male	159	11.82 ± 1.19	0 - 106.32	1.19 ± 0.39 (10)
	Female	190	15.02 ± 1.98	0 - 214.65	1.89 ± 0.55 (10)

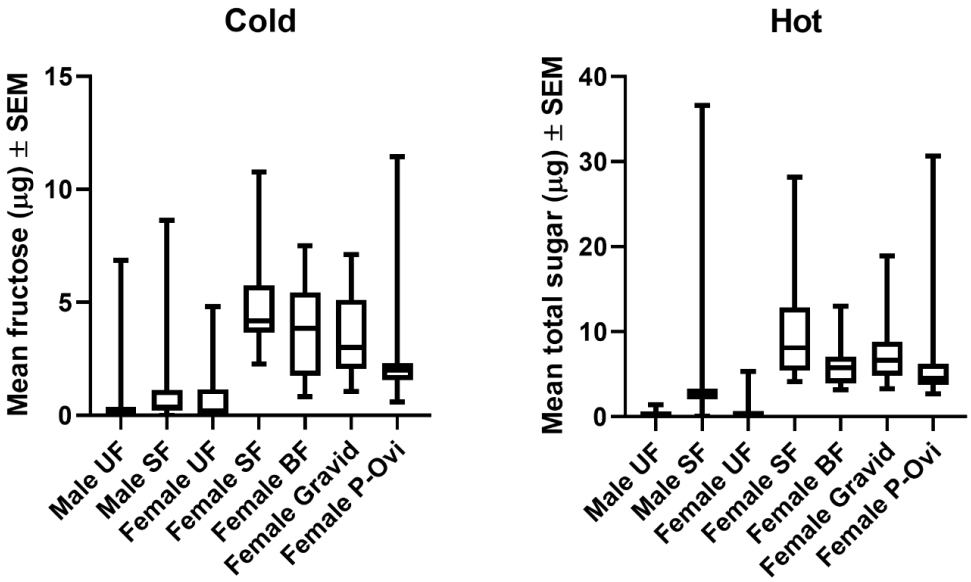

Figure A-1 Mean fructose and total sugar for male and female *Ae. aegypti* at various stages. UF = unfed; SF = 24 hours post 10% sucrose feeding; BF = bloodfed; P-Ovi = post oviposition.

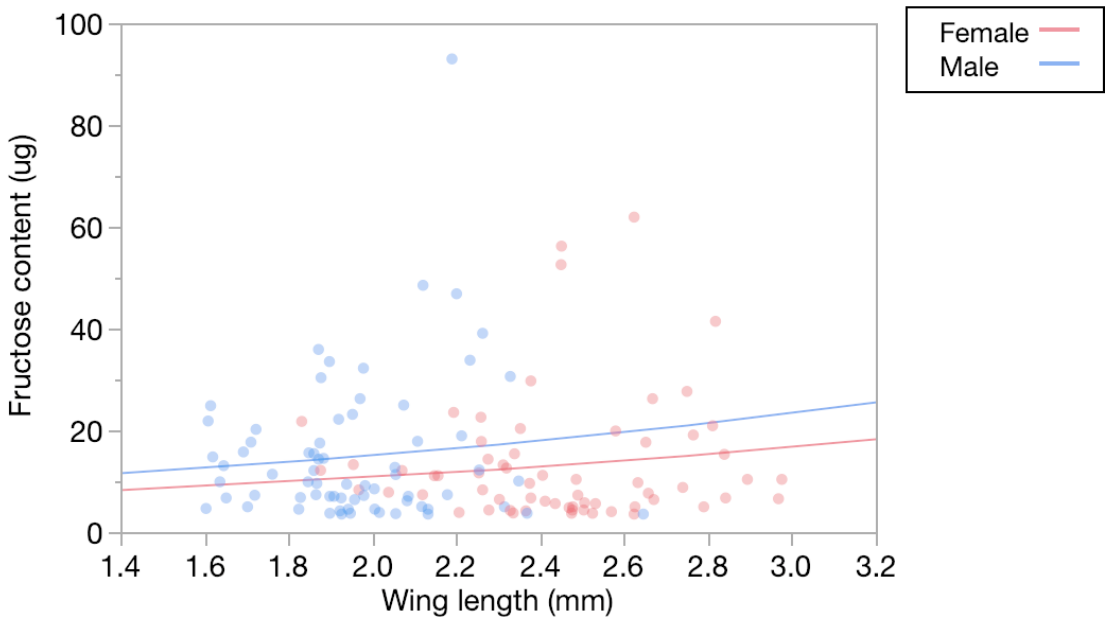

Figure A-2 Generalized linear analysis for fructose content (in µg) by wing length (mm) for male and female *Ae. aegypti*. Samples containing < 3.5 µg were considered 'negative' based upon laboratory baseline values for unfed female *Ae. aegypti* mosquitoes and were excluded from analysis (Table A-1).

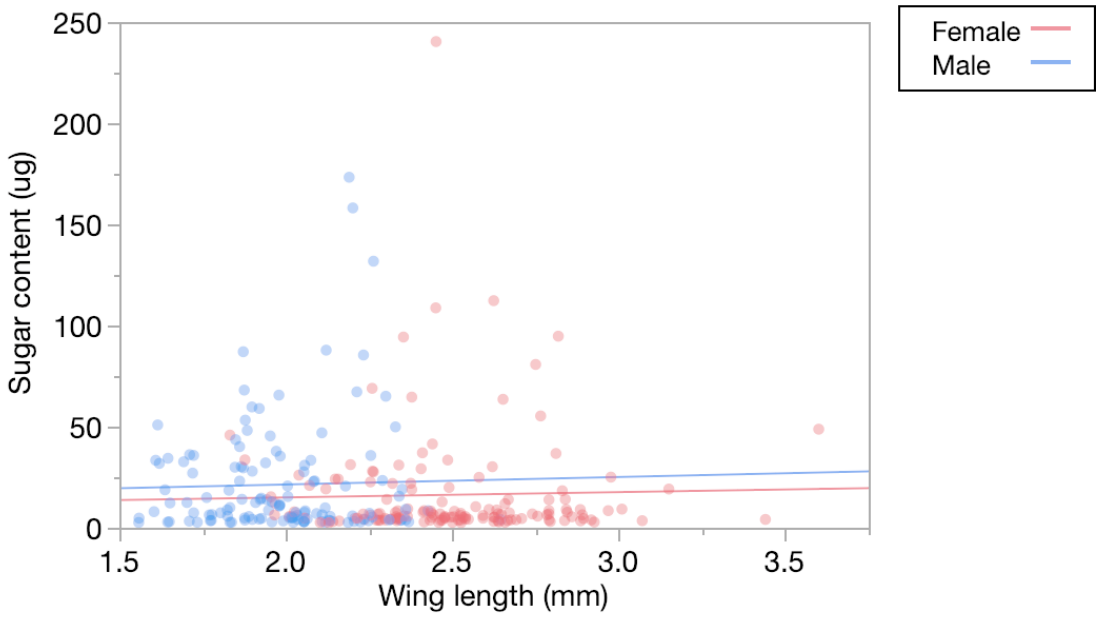

Figure A-3 Generalized linear analysis for total sugar content (µg) by wing length (mm) for male and female *Ae. aegypti*. Samples containing < 3.5 µg were considered 'negative' based upon laboratory baseline values for unfed female *Ae. aegypti* mosquitoes and were excluded from analysis (Table A-1).

APPENDIX B

SUPPLEMENTAL FIGURES AND TABLES FOR CHAPTER 3

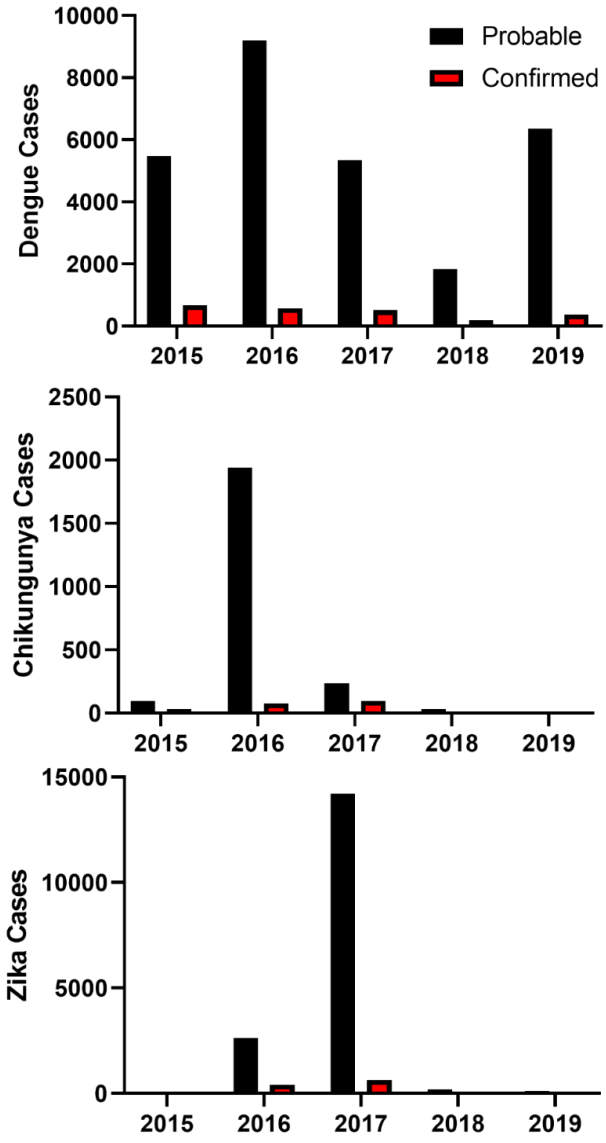

Figure B-1 Probable and confirmed human cases of DENV, CHIKV and ZIKV from 2015 to 2019 in Tamaulipas, México. Probable cases also include those that were ultimately confirmed by PCR.

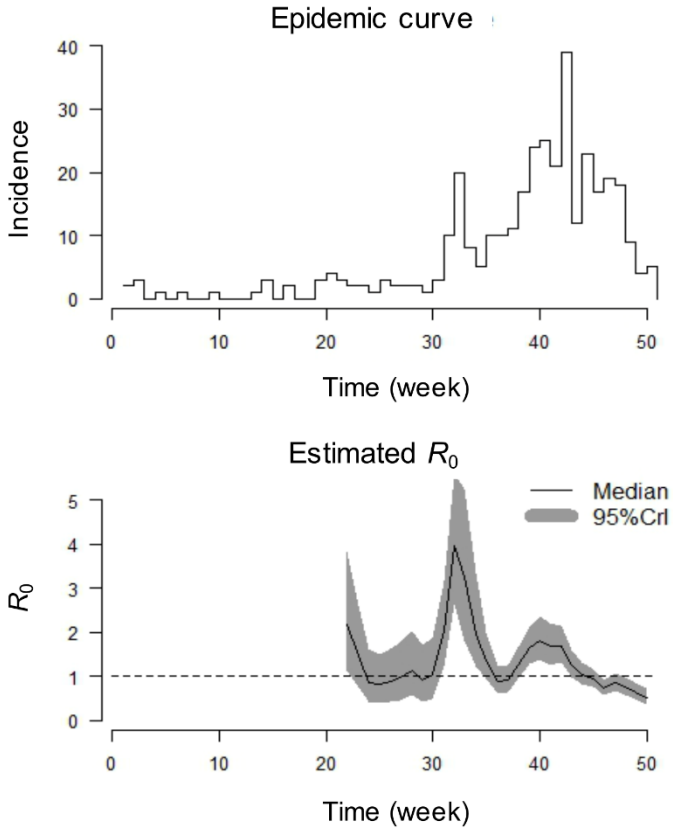

Figure B-2 Weekly human Zika cases in Reynosa in 2017, and estimated effective reproductive number.

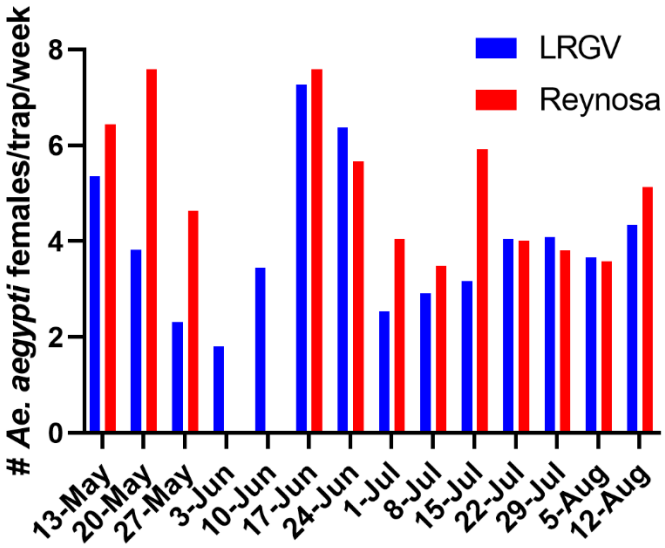

Figure B-3 Weekly Autocidal Gravid Ovitrap (AGO) counts for *Ae. aegypti* in the Lower Rio Grande Valley (LRGV) and Reynosa between 13 May and 12 August, 2017.

Table B-1 Vertebrate-specific primers used in this study.

Primer Name	Ratio	Sequence (5′ -> 3′)	Direction	Reference
VF1_t1	1	TGTAAAACGACGGCCAGTTCTCAACCAACCACA AAGACATTGG	Forward	[1,2]
VF1D_t1	1	TGTAAAACGACGGCCAGTTCTCAACCAACCACA ARGAYATYGG	Forward	[1,2]
VF1i_t1	2	TGTAAAACGACGGCCAGTTCTCAACCAACCAIA AIGAIATIGG	Forward	[1,2]
VR1D_t1	1	CAGGAAACAGCTATGACTAGACTTCTGGGTGGC CRAARAAYCA	Reverse	[1,2]
VR1_t1	1	CAGGAAACAGCTATGACTAGACTTCTGGGTGGC CAAAGAATCA	Reverse	[1,2]
VR1i_t1	2	CAGGAAACAGCTATGACTAGACTTCTGGGTGIC CIAAIAAICA	Reverse	[1,2]
BM1	1	CCCCTCAGAATGATATTTGTCCTCA	Forward	[3,4]
BM2	1	CCATCCAACATCTCAGCATGATGAAA	Reverse	[3,4]
herp*	1	GCHGAYACHWVHHYHGCHTTYTCHTC	Reverse	[3,4]

* Use BM1 as forward primer.

Table B-2 Universal invertebrate primers used in this study.

Primer Name	Ratio	Sequence (5′ -> 3′)	Direction	Reference
LCO 1490	1	GGTCAACAAATCATAAAGATATTGG	Forward	[5]
HCO 2198	1	TAAACTTCAGGGTGACCAAAAAATCA	Reverse	[5]

Table B-3 Vertebrate densities resulting from community surveys.

	Community				*Total*	*Proportion*	*95% CI*	
	IHE	***IHW***	***LP***	***TB***			***Lower***	***Upper***
# Homes Surveyed	14	10	13	7	44			
Human	78	45	59	23	205	.382	0.342	0.424
Dog	31	49	66	22	168	.313	0.275	0.353
Cat	14	9	30	18	71	.132	0.106	0.164
Chicken	27	19	19	25	90	.168	0.138	0.202
Pig	0	3	0	0	3	.006	0.002	0.016

Table B-4 Estimated vertebrate population densities based upon community surveys.

Community	***Area (km^2)***	***Human***	***Dog***	***Cat***	***Chicken***	***Pig***
		per km^2				
IHE	.33	5,209	2,046	930	1,767	0
IHW	.079	5,468	5,954	1,094	2,309	367
LP	.073	9,863	11,178	5,041	3,288	0
TB	.077	2,104	1,974	1,649	2,286	0
Total	.559	5,146	4,161	1,751	2,299	75
% of total vert. pop		38%	31%	13%	17%	0.6%

Table B-5 Estimated number of homes, population sizes and area in the regions of the LRGV receiving mosquito sampling in the current study and Martin et al. 2019. We also present the number of bloodfed mosquitoes with host identification results from each community, how many unique homes had at least one specimen, and what proportion of blood meal results were human.

				Aedes aegypti			***Culex quinquefasciatus***		
Community	#Houses	GIS Pop (2010)	Area (km^2)	# of results	# of unique homes	Proportion human (n)	# of results	# of unique homes	Proportion human (n)
La Piñata	132	572	0.146	56	19	0.304 (17)	27	12	.037 (1)
Tierra Bella	47	191	0.074	22	10	0.318 (7)	6	5	0
Donna	122	510	0.115	8	3	0.250 (2)	-	-	-
Indian Hills W	124	337	0.076	14	9	0 (0)	-	-	-
Indian Hills E	311	1467	0.36	64	34	0.375 (24)	79	23	0
McAllen	67	227	0.116	3	2	0.333 (1)	1	1	0
Mesquite	39	162	0.039	2	2	0 (0)	2	1	0
Rio Rico	20	55	0.041	-	-	-	-	-	-
Donna Fig	49	154	0.042	2	2	0 (0)	1	1	0
Progresso	73	314	0.081	7	4	0.571 (4)	2	2	0
Christian Ct.	34	129	0.059	5	2	0.200 (1)	5	3	0
MCH Chapa	30	127	0.037	2	1	0.500 (1)	-	-	-
La Feria	70	222	0.056	1	1	1 (1)	-	-	-
Total	1118	4467	1.242	186	89		123	48	

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APPENDIX C

SUPPLEMENTAL FIGURES AND TABLES FOR CHAPTER 4

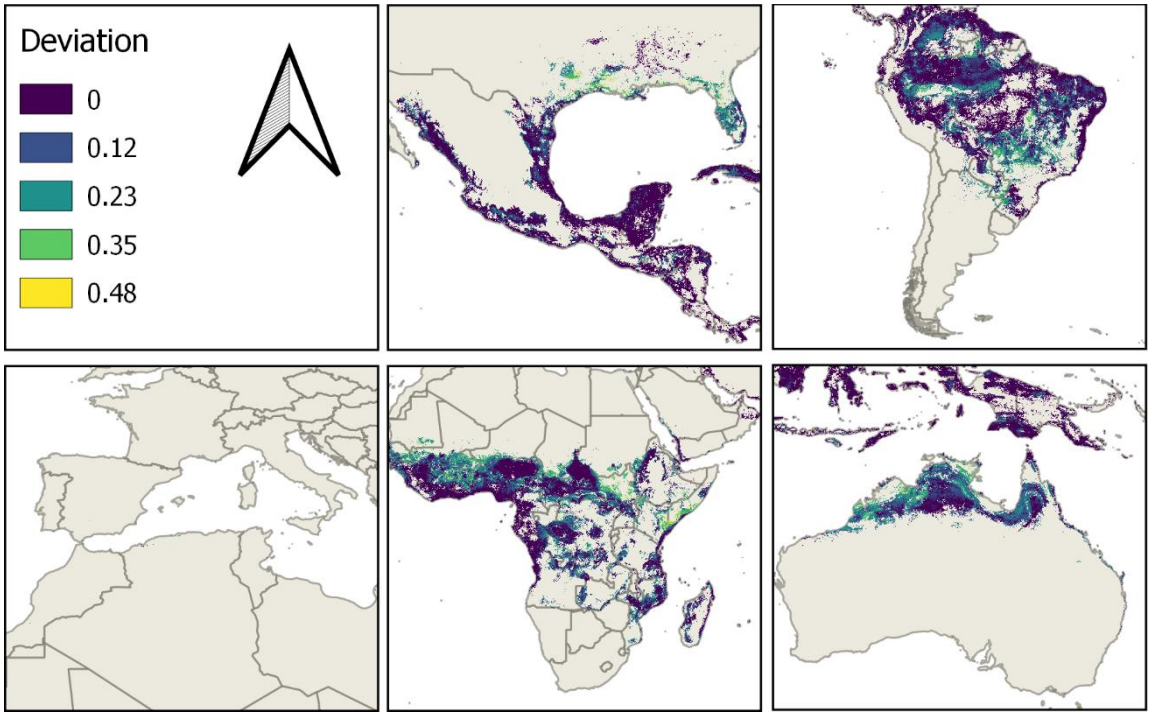

Figure C-1 Deviation between *Ae. aegypti* probability of occurrence and dengue environmental suitability, zoomed in on North America, South America, South Europe and North Africa, Africa, and Australia.

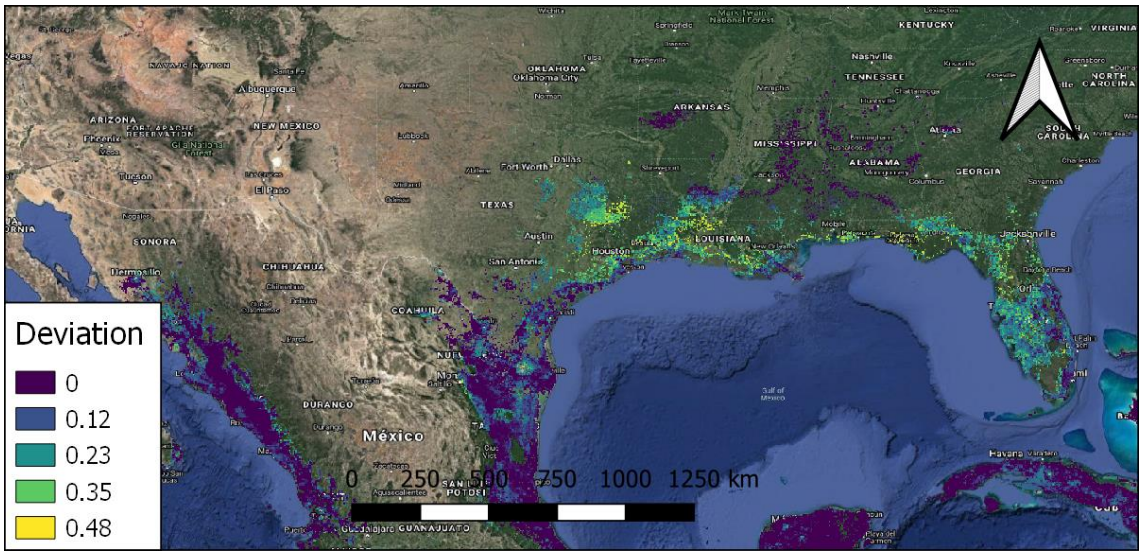

Figure C-2 Deviation between *Ae. aegypti* probability of occurrence and dengue environmental suitability for the Southern United States.

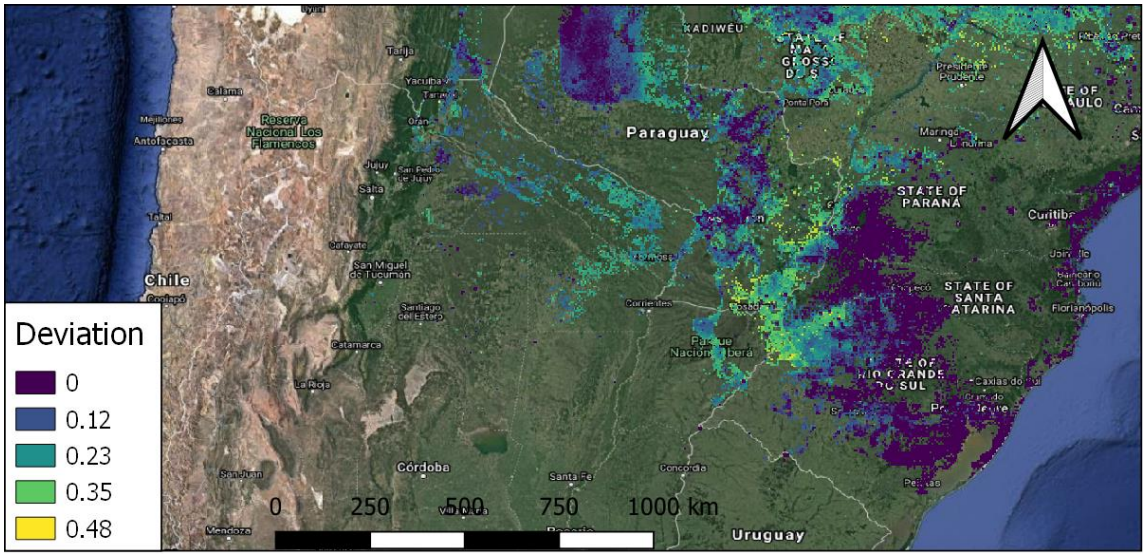

Figure C-3 Deviation between *Ae. aegypti* probability of occurrence and dengue environmental suitability for Northern Argentina, Paraguay, and Southern Brazil.

Table C-1 Data sources for the global rasters used in this paper.

Variable	Scale	Data Source/URL	Reference
Global distribution of *Ae. aegypti*	5 x 5 km	https://datadryad.org/resource/doi:10.5061/dryad.47v3c	[1]
Global distribution of dengue	5 x 5 km	https://figshare.com/s/d7d7871d00afe2870619	[2]
Population Density	1 x 1 km	https://sedac.ciesin.columbia.edu/data/set/gpw-v4-population-density-rev11/data-download	[3]
Gross domestic product	1 x 1 km	https://doi.org/10.5061/dryad.dk1j0	[4]
Infant mortality rate	National /Subnational 1 x 1 km	https://sedac.ciesin.columbia.edu/data/set/povmap-global-subnational-infant-mortality-rates-v2/data-download	[5]
Temperature	1 x 1 km	https://www.worldclim.org/data/worldclim21.html#	[6]
Precipitation	1 x 1 km	https://www.worldclim.org/data/worldclim21.html#	[6]

Table C-2 Statistical summary of *Ae. aegypti* minus dengue deviation, by country. Blue indicates the lower end of the spectrum, where *Ae. aegypti* occurrence and risk of dengue is nearly equal and high, and yellow represents the other end of the spectrum where *Ae. aegypti* can be found without dengue. Countries with 5 or fewer raster cells were excluded from table.

Country	Count	Mean Deviation	SD	Min	Max	Range
Mauritania	1537	0.270822	0.076399	0	0.468075	0.468075
Niger	3212	0.241343	0.075063	0	0.43299	0.43299
Somalia	7144	0.210229	0.140471	0	0.476313	0.476313
Burkina Faso	9555	0.209692	0.08242	0	0.464294	0.464294
eSwatini	25	0.20527	0.030981	0.159627	0.267192	0.107565
S. Sudan	13965	0.189314	0.126751	0	0.461959	0.461959
Senegal	5230	0.182488	0.100429	0	0.449441	0.449441
South Africa	93	0.182341	0.080277	0	0.359835	0.359835
Botswana	15	0.182296	0.057185	0.084908	0.296197	0.211289
Kenya	8705	0.176906	0.130131	0	0.466411	0.466411
Argentina	3983	0.176614	0.105083	0	0.439078	0.439078
Netherlands	9	0.176019	0.089169	0	0.273538	0.273538
United States of America	12322	0.160234	0.127157	0	0.473787	0.473787
Mali	12187	0.143336	0.096475	0	0.444084	0.444084
Zimbabwe	272	0.140671	0.082867	0	0.417163	0.417163
Sudan	8478	0.133645	0.124151	0	0.44961	0.44961
Togo	2485	0.131907	0.111805	0	0.460853	0.460853
Paraguay	7689	0.131267	0.110785	0	0.459871	0.459871
Benin	5000	0.128714	0.099668	0	0.445118	0.445118
Australia	42383	0.125245	0.093226	0	0.449273	0.449273
Chad	12522	0.124446	0.122251	0	0.443918	0.443918
Gambia	423	0.121773	0.076891	0	0.375483	0.375483
Algeria	33	0.120733	0.114471	0	0.311777	0.311777
Zambia	7392	0.119709	0.0742	0	0.319709	0.319709
Ghana	9599	0.115504	0.114871	0	0.455999	0.455999
Singapore	21	0.114384	0.064616	0	0.214392	0.214392
Namibia	351	0.110637	0.074253	0	0.284943	0.284943
Mauritius	84	0.109631	0.099659	0	0.430635	0.430635
Dem. Rep. Congo	49377	0.102295	0.093354	0	0.441369	0.441369
Saudi Arabia	1314	0.098834	0.100066	0	0.42865	0.42865
Guinea-Bissau	1155	0.098167	0.08264	0	0.423113	0.423113
Mozambique	18281	0.095912	0.088501	0	0.441035	0.441035
Afghanistan	29	0.093353	0.105213	0	0.322509	0.322509
Uganda	4281	0.092651	0.087806	0	0.43509	0.43509

Table C-2 continued

Country	Count	Mean Deviation	SD	Min	Max	Range
Djibouti	585	0.091013	0.092261	0	0.380496	0.380496
Ethiopia	14335	0.089882	0.103374	0	0.473594	0.473594
Turkey	125	0.086916	0.103747	0	0.347339	0.347339
Tanzania	12375	0.086016	0.086437	0	0.401491	0.401491
Brazil	251421	0.080467	0.09552	0	0.454705	0.454705
Libya	10	0.076496	0.074334	0	0.208514	0.208514
Angola	13627	0.075843	0.081154	0	0.322925	0.322925
Central African Rep.	23993	0.075756	0.086161	0	0.41307	0.41307
Côte d'Ivoire	13634	0.075138	0.101408	0	0.46961	0.46961
Congo	8203	0.074303	0.080669	0	0.437049	0.437049
Bolivia	20245	0.072595	0.086744	0	0.420899	0.420899
Bangladesh	6589	0.070617	0.080114	0	0.391463	0.391463
Madagascar	10491	0.068162	0.078189	0	0.420165	0.420165
Spain	7	0.067902	0.086906	0	0.252066	0.252066
Malawi	1112	0.066335	0.086921	0	0.360916	0.360916
Guam	12	0.064371	0.060257	0	0.163893	0.163893
Israel	25	0.06422	0.102963	0	0.332563	0.332563
Cameroon	12896	0.062199	0.085394	0	0.421467	0.421467
Hong Kong	29	0.061137	0.077636	0	0.302333	0.302333
Colombia	36374	0.060055	0.075168	0	0.424601	0.424601
China	25740	0.058021	0.078603	0	0.43586	0.43586
Nigeria	40458	0.056554	0.079571	0	0.443585	0.443585
Uruguay	21	0.056547	0.063163	0	0.171148	0.171148
Eritrea	1507	0.056413	0.075275	0	0.437792	0.437792
Aruba	6	0.055947	0.042776	0	0.140137	0.140137
Haiti	1125	0.053313	0.081108	0	0.407427	0.407427
Peru	28758	0.050836	0.0784	0	0.448863	0.448863
Myanmar	18541	0.049784	0.072837	0	0.431414	0.431414
Bhutan	469	0.048846	0.067289	0	0.275594	0.275594
Venezuela	28011	0.047027	0.066544	0	0.426449	0.426449
Brunei	254	0.046476	0.05883	0	0.31676	0.31676
India	131543	0.04542	0.067103	0	0.482278	0.482278
Guinea	10106	0.044463	0.071513	0	0.463709	0.463709
Guyana	7224	0.043455	0.070662	0	0.408294	0.408294
Nepal	2929	0.041801	0.066457	0	0.421316	0.421316
Puerto Rico	371	0.040727	0.057873	0	0.288344	0.288344
Burundi	67	0.038958	0.061973	0	0.255066	0.255066

Table C-2 continued

Country	Count	Mean Deviation	SD	Min	Max	Range
Honduras	3574	0.037542	0.071488	0	0.374007	0.374007
Japan	798	0.03687	0.069031	0	0.45001	0.45001
Taiwan	1023	0.036766	0.05761	0	0.458133	0.458133
Mexico	30091	0.036353	0.062812	0	0.442393	0.442393
Liberia	2271	0.03601	0.063775	0	0.353039	0.353039
Greece	38	0.035851	0.083678	0	0.328919	0.328919
Cuba	5161	0.03374	0.054247	0	0.357021	0.357021
Pakistan	11821	0.033113	0.0626	0	0.435065	0.435065
Cambodia	6908	0.032395	0.057148	0	0.394668	0.394668
Sierra Leone	2137	0.032228	0.050699	0	0.309454	0.309454
Suriname	3890	0.028387	0.04457	0	0.309013	0.309013
Dominican Rep.	1649	0.027419	0.053722	0	0.341377	0.341377
New Caledonia	856	0.026595	0.045528	0	0.27335	0.27335
Tonga	17	0.02625	0.043202	0	0.131397	0.131397
Curaçao	20	0.023596	0.036404	0	0.111911	0.111911
Barbados	18	0.023528	0.032648	0	0.102893	0.102893
Thailand	20176	0.022727	0.044623	0	0.416792	0.416792
Syria	10	0.02266	0.037387	0	0.109027	0.109027
Yemen	1884	0.020873	0.049575	0	0.345343	0.345343
Papua New Guinea	9466	0.020219	0.047637	0	0.414984	0.414984
Gabon	7059	0.020012	0.046014	0	0.277781	0.277781
Belize	817	0.019791	0.043049	0	0.295161	0.295161
Laos	3776	0.018606	0.04681	0	0.302114	0.302114
Turks and Caicos Is.	11	0.017997	0.039329	0	0.134937	0.134937
Comoros	51	0.017759	0.039172	0	0.169279	0.169279
Indonesia	61849	0.017626	0.044067	0	0.458635	0.458635
Philippines	8957	0.017602	0.036593	0	0.339598	0.339598
Nicaragua	2649	0.017339	0.045104	0	0.385047	0.385047
Fr. Polynesia	41	0.017271	0.050807	0	0.27058	0.27058
Cabo Verde	86	0.016027	0.035636	0	0.155874	0.155874
Antigua and Barb.	7	0.016003	0.0392	0	0.112023	0.112023
St. Vin. and Gren.	13	0.015825	0.028461	0	0.100453	0.100453
Sri Lanka	2757	0.014808	0.034071	0	0.325308	0.325308
Guatemala	2846	0.013824	0.040273	0	0.345901	0.345901
Iraq	1334	0.013004	0.040605	0	0.313613	0.313613
U.S. Virgin Is.	9	0.012696	0.024272	0	0.067725	0.067725
Ecuador	4006	0.011557	0.040101	0	0.320929	0.320929

Table C-2 continued

Country	Count	Mean Deviation	SD	Min	Max	Range
El Salvador	661	0.010152	0.0362	0	0.251234	0.251234
Vietnam	11576	0.010046	0.029572	0	0.325532	0.325532
Cayman Is.	6	0.009814	0.021944	0	0.058882	0.058882
Jordan	16	0.009607	0.020189	0	0.079458	0.079458
Malaysia	9712	0.009353	0.028345	0	0.352326	0.352326
Dominica	26	0.008615	0.0257	0	0.10747	0.10747
Jamaica	450	0.008046	0.030807	0	0.262963	0.262963
Trinidad and Tobago	213	0.0052	0.015503	0	0.09955	0.09955
South Korea	23	0.004973	0.019298	0	0.093969	0.093969
Timor-Leste	536	0.004884	0.016336	0	0.112183	0.112183
Costa Rica	692	0.004871	0.023694	0	0.213336	0.213336
United Arab Emirates	303	0.004546	0.021288	0	0.140047	0.140047
Saint Lucia	28	0.004106	0.012973	0	0.055122	0.055122
Panama	1704	0.004076	0.019316	0	0.216352	0.216352
Eq. Guinea	955	0.003257	0.017095	0	0.19235	0.19235
Iran	2937	0.003238	0.020055	0	0.313523	0.313523
Bahamas	425	0.002046	0.010936	0	0.104678	0.104678
Vanuatu	325	0.001953	0.014117	0	0.185129	0.185129
Oman	1695	0.001612	0.012964	0	0.183969	0.183969
Grenada	15	0.001142	0.002923	0	0.009292	0.009292
Solomon Is.	140	0.000664	0.005397	0	0.051616	0.051616
Fiji	434	0.000282	0.002888	0	0.039222	0.039222
Chile	6	0	0	0	0	0
Bahrain	15	0	0	0	0	0
Egypt	24	0	0	0	0	0
Georgia	22	0	0	0	0	0
Rwanda	7	0	0	0	0	0
Montenegro	6	0	0	0	0	0
Samoa	34	0	0	0	0	0
São Tomé and Principe	30	0	0	0	0	0
Niue	7	0	0	0	0	0
N. Mariana Is.	14	0	0	0	0	0

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