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# Sugar Feeding Patterns for *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) Mosquitoes in South Texas

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## Abstract

Effective mosquito surveillance and management depend on a thorough understanding of the biology and feeding patterns unique to species and sex. Given that a propensity to sugar feed is necessary for some mosquito surveillance and newer control strategies, we sought to document the amount of total sugar in wild *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say) captured from five different locations in the Lower Rio Grande Valley (LRGV) of South Texas over 2 yr. We used Biogents Sentinel 2 (BGS2) traps in year 1 and aspirators, BGS2, and CDC resting traps in years 2 and 3 to collect adult mosquitoes. The hot anthrone test was used to quantify total sugar content in each mosquito. Additionally, the cold and hot anthrone tests were used to distinguish fructose content from total sugars for mosquitoes captured in 2019. Overall, *Ae. aegypti* females had significantly lower total sugar content than *Ae. aegypti* males as well as both sexes of *Cx. quinquefasciatus*. However, the percentage of *Ae. aegypti* positive for fructose consumption was four to eightfold higher than *Ae. aegypti* previously reported in other regions. The difference between locations was significant for males of both species, but not for females. Seasonality and trapping method also revealed significant differences in sugar content of captured mosquitoes. Our results reinforce that sugar feeding in female *Ae. aegypti* is less than *Cx. quinquefasciatus*, although not absent. This study provides necessary data to evaluate the potential effectiveness of sugar baits in surveillance and control of both *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes.

**Key words:** sugar-feeding, *Aedes*, *Culex*, surveillance, collection method

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 (Ramírez et al. 2018). Two important vector species involved in arbovirus transmission are *Aedes aegypti* (L.), the yellow fever mosquito, and *Culex quinquefasciatus* (Say), the southern house mosquito. *Aedes aegypti* is the principal vector of dengue (DENV) (Harrington et al. 2014), chikungunya (CHIKV) (Macpherson et al. 2016), yellow fever (Christ et al. 2017), and Zika viruses (ZIKV) (Weaver et al. 2018). *Culex quinquefasciatus* is a member of the *Culex pipiens* complex that is an important vector of West Nile virus and other arthropod-borne viruses, including Japanese encephalitis virus, Saint Louis encephalitis virus and Rift Valley fever (Vinogradova 2000).

As early as 1873, adult *Culex* mosquitoes were observed sucking nectar from the flowers of *Ramnus frangula* (Knab 1907), and in 1958, both sexes of *Aedes* and *Culex* mosquitoes were observed frequently visiting flowers for nectar (Downes 1958). However, we also know that nearly all female mosquitoes require a bloodmeal to develop eggs, and in some environments, *Ae. aegypti* have adapted to seldom feed on sugar, deriving needed energy from blood meals alone (Scott et al. 1997, Costero et al. 1998a, Naksathit and Scott 1998, Harrington et al. 2001). 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 (Van Handel 1985) and is linked to survival and successful mating (Foster 1995). Some studies have suggested that sugar-poor environments effectively limit the population or survivorship of adult mosquitoes (Foster 1995, Okech et al. 2003, Impoinvil et al. 2004, Gu et al. 2011), but Klowden (1986) 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 bloodmeal 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 (Edman et al. 1992, Harrington et al. 2001). Moreover, Costero et al. (1998a) 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 West Nile virus actually decreases (Vaidyanathan et al. 2008). A different study observed a significant difference in sugar and host feeding between diapausing and non-diapausing female *Cx. pipiens* (Bowen 1992). Significant differences in longevity and fecundity of *Cx. pipiens pallens* were observed in mosquitoes reared on different flowering plants and seed pods (Yu et al. 2016). 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 (Hall-Mendelin et al. 2010, van den Hurk et al. 2014). In California, cotton dental wicks soaked with scented sugar baits detected West Nile virus activity in areas where conventional surveillance of mosquito pools reported no West Nile virus activity (Lothrop et al. 2014, Steiner et al. 2018). Sugar-baited stations proved to be more sensitive in detecting arboviral activity because they can be deployed continuously for 6 to 7 d 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 (Chadee et al. 2014) 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 (Khallaayoune et al. 2013, Qualls et al. 2014), 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 (Scott-Fiorenzano et al. 2017). This concurred with Fikrig et al. (2017), 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 West Nile virus (Chung et al. 2013, Poh et al. 2019) and the Lower Rio Grande Valley (LRGV) in South Texas has now experienced autochthonous transmission of DENV, CHIKV, and ZIKV (Murray et al. 2013, Thomas et al. 2016, Laredo-Tiscareño et al. 2018, Leta et al. 2018, Martin et al. 2019). The objective of this study was 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.

## Materials and Methods

To quantify total sugar content of all mosquitoes, we utilized the hot anthrone test developed by Emile Van Handel (Van Handel 1967, Van Handel 1985). 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) h 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 (Puggioli et al. 2017). Baseline values were established by rearing 10 male and 10 female unfed mosquitoes of both species. Approximately 24 h 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 (Van Handel 1967, Van Handel 1972, Van Handel 1985, Lee 2019). 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-h post sugar feeding (10% sucrose), blood-fed, gravid, and post-oviposition mosquitoes (Supp Table 1 [online only]).

### Study Area

Wild *Ae. aegypti* and *Cx. quinquefasciatus* were collected from five residential sites in the LRGV of South Texas from 20 September 2017 through 6 December 2017, 12–14 June 2018, and 9–16 October 2019. The neighborhoods are Indian Hills (26°12′43″N, 97°54′36″W ± 0.5 km), Tierra Bella (26°07′44″N, 98°03′07″ W ± 0.5 km) La Piñata (26°07′44″N, 98°03′25″W ± 0.5 km), Mercedes La Mesa (26°13′51″N, 97°57′29″W ± 0.5 km) and Mile 5 (26°07′37″N, 97°58′08″W ± 0.5 km). This region along the United States-Mexico border is home to approximately 1.3 million residents (U.S. Census Bureau, 2018). Temperatures in the LRGV range from an average low of 50° F (10°C) in winter months (December/January) to average high of 85°F (29.4°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 (Richardson and Pisani 2017). 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.

### 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 h. All aspiration was conducted outside of the home for a 10-min 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 a.m. 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* (Fox 2007). 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.

## 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 min 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 × *g* for 1 min. 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 h 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 1-liter 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 min. 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 min, allowed to cool for 10 min 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, 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. All total sugar and fructose values are presented as means ± standard error of the mean (SEM).

## 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 (Van Handel and Day 1988). 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 (Nasci 1990) 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.

## 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) (Dobson and Barnett 2008). 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 exact test to compare percentage of fructose-positive mosquitoes. A one-way analysis of variance (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.

# Results

## Laboratory Study

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

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

## 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 (Supp Table 2 [online only]). 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* (Fig. 1). A significant difference ($P = 0.0018$) between male and female *Ae. aegypti* mosquitoes was also observed using the cold anthrone test where we detected 8.78 µg (±1.05) in males and 6.74 µg (±0.78) in females (Table 1).

We also analyzed the percentage of mosquitoes deemed 'positive' for total carbohydrates, containing ≥3.5 µg of sugar, based upon baseline values + 2 SDs for unfed, laboratory-raised female *Ae. aegypti* mosquitoes (Supp Table 1 [online only]). 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 (Fig. 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 ($P = 0.2032$) (Fig. 3).

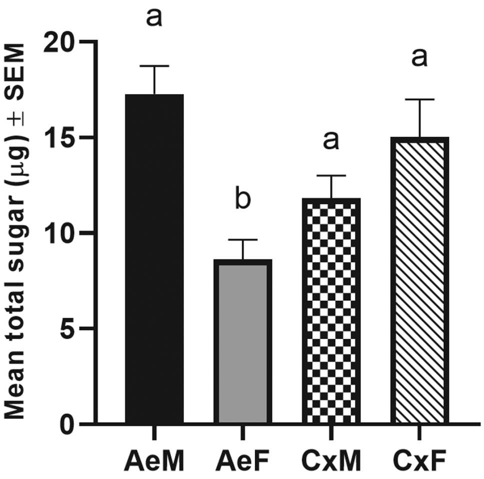

**Fig. 1.** Mean sugar content (±SEM) for all *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes collected between 20 September 2017 and 16 October 2019 from all study site locations. Includes samples with zero sugar detected. 'a' indicates no statistical difference between means. 'b' indicates statistical significance between means ($P < 0.0001$).

## 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) ($P = 0.6337$) (Fig. 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* (Fig. 5). *Ae. aegypti* samples collected in October 2019 were also analyzed by trapping method and location, but no significant differences were observed.

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 50.79 ± 19.52 µg (Table 2). *Culex 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; $P = 0.4109$) (Fig. 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 ($P = 0.2761$).

## 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) (Fig. 7).

## 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 3

**Table 1.** 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 (143) | 8.02 ± 1.05 (103) | 15.24 ± 7.59 (12) | 8.79 ± 1.87 (28) |
| | | Female | 6.74 ± 0.78 (157) | 5.94 ± 0.82 (117) | 4.40 ± 2.99 (4) | 9.60 ± 1.99 (36) |

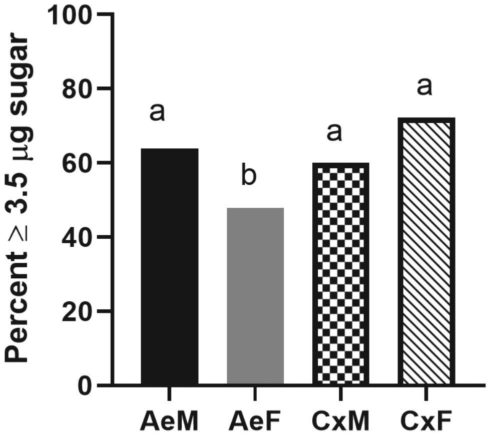

**Fig. 2.** Percentage of male and female *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes containing ≥ 3.5 µg of sugar (hot anthrone test). 'a' indicates no statistical difference between means. 'b' indicates statistical significance between means ($P < 0.0001$).

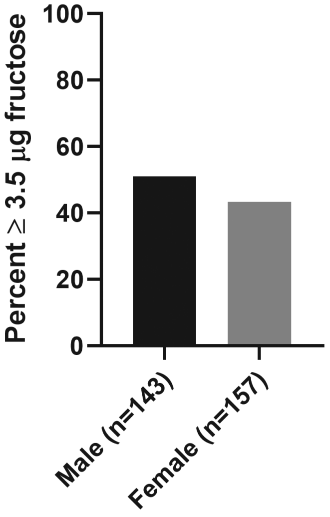

**Fig. 3.** Percent of male and female *Ae. aegypti* containing ≥3.5 µg fructose (cold anthrone test).

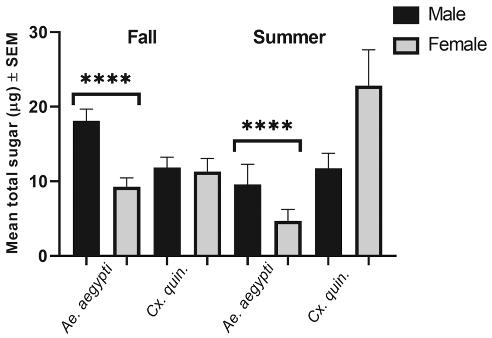

**Fig. 4.** Comparison of mean total sugar content by mosquito species and sex, grouped by season. **** indicates statistical significance between means ($P < 0.0001$).

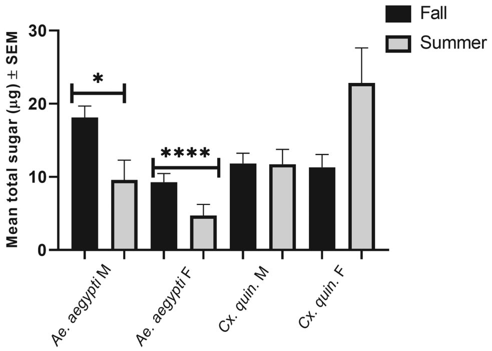

**Fig. 5.** Comparison of mean sugar content by season, for each mosquito species and sex. * indicates statistical significance between means ($P = 0.0310$). **** indicates statistical significance between means ($P < 0.0001$).

and 4; Supp Figs. 2 and 3 [online only]). 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 5 and Supp Fig. 4 [online only]).

## Discussion

Previous studies indicate female *Ae. aegypti* mosquitoes feed preferentially on human blood, and rarely on sugar (Edman et al. 1992, Harrington et al. 2001, Spencer et al. 2005, Scott and Takken 2012). 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 approximately 2 to 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 SDs above mean baseline value), suggesting sugar consumption was common in this region during these time periods. In a previous study, Costero et al. (1998b) 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. (1998b) 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-h 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

**Table 2.** 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. quinquefasciatus* | 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. quinquefasciatus* | 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) |

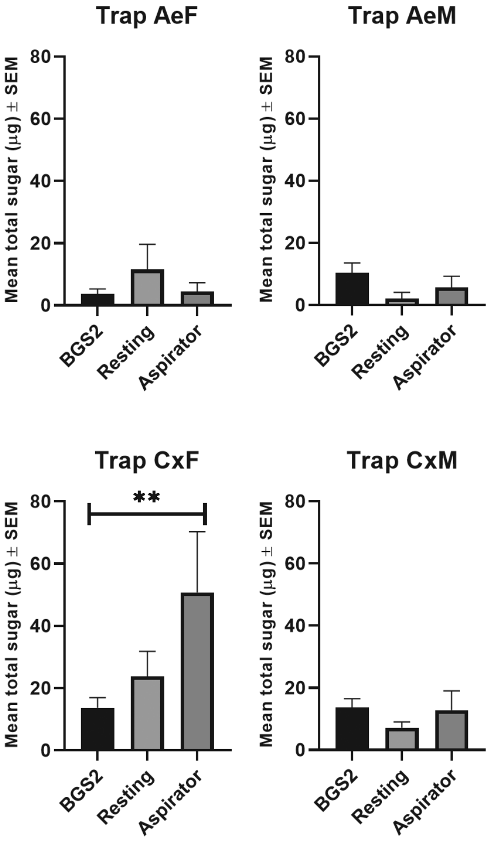

**Fig. 6.** Comparison of mean sugar content by mosquito species and sex, grouped by trapping method. This data only includes Summer 2018 mosquitoes. AeF = *Ae. aegypti* female; AeM = *Ae. aegypti* male; CxF = *Cx. quinquefasciatus* female; CxM = *Cx. quinquefasciatus* male. ** indicates statistical significance between means (*P* = 0.0094).

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 (Van Handel 1967, Van Handel 1972, Van Handel 1985). 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. (1998b), 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. (1992). 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 (1987) and may decrease pathogen transmission compared with nutritionally stressed mosquitoes, which Vaidyanathan (2008) demonstrated with *Culex* mosquitoes and West Nile virus.

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 (Reisen et al. 1986). Additionally, these differences could also be influenced by the male mosquitoes’ ability to discover and exploit sugar resources (Yuval et al. 1994).

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 d 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. (1992) released sugar-fed *Ae. aegypti* and were unable to detect sugar in the recaptured females after 4 d, 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 d post feeding on a 10% sucrose solution (Costero et al. 1998b). Other researchers have demonstrated nectar-fed mosquitoes can be anthrone-negative in as little as 20 h of digestion (Andersson and Jaenson 1987). 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 (1986).

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. (1986) 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.

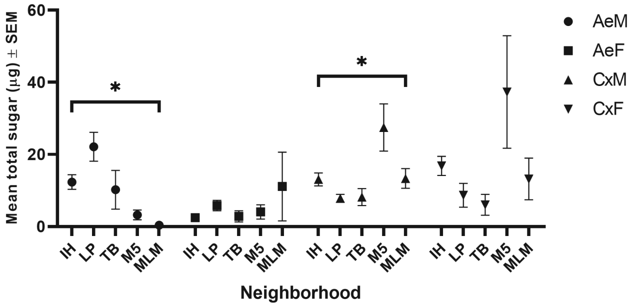

**Fig. 7.** Mean sugar content of species and sex, by location. IH = Indian Hills, LP = La Piñata, TB = Tierra Bella, M5 = Mile 5, MLM = Mercedes La Mesa. AeM = *Ae. aegypti* (male), AeF = *Ae. aegypti* (female), CxM = *Cx. quinquefasciatus* (male), CxF = *Cx. quinquefasciatus* (female). Includes mosquitoes from 2017 to 2018. * indicates statistical significance between means ($P = 0.0268$ for *Ae. aegypti* males; $P = 0.0472$ for *Cx. quinquefasciatus* males).

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. (1997) 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 (Martinez-Ibarra et al. 1997). 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

**Table 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 to 3.18 | 4.89 | 0.027 |
| Wing length | 0.433 | 0.33 | −0.23 to 1.09 | 1.62 | 0.202 |
| Sex (Female) | −0.166 | 0.11 | −0.39 to 0.06 | 2.06 | 0.151 |

**Table 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 to 4.36 | 12.35 | |
| Wing length | 0.118 | 0.35 | −0.58 to 0.79 | 0.11 | 0.739 |
| Sex (Female) | −0.190 | 0.12 | −0.44 to 0.05 | 2.23 | 0.135 |

**Table 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 to 4.70 | 1.10 | 0.293 |
| Wing length | 0.498 | 0.49 | −0.48 to 1.46 | 0.79 | 0.373 |
| Sex (Female) | 0.147 | 0.17 | −0.17 to 0.49 | 1.00 | 0.315 |

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 (Junnila et al. 2010, Nyasembe et al. 2018).

## 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.

## Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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