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

Mark F. Olson,1 Selene Garcia-Luna,1 Jose G. Juárez,1,※ Estelle Martin,1 Laura C. Harrington,2,※ Micky D. Eubanks,1 Ismael E. Badillo-Vargas,3 and Gabriel L. Hamer1,4

1Department of Entomology, Texas A&M University, College Station, TX 77843, 2Department of Entomology, Cornell University, Ithaca, NY 14853, 3Department of Entomology, Texas A&M AgriLife Research, Weslaco, TX 78596, and 4Corresponding author, e-mail: ghamer@tamu.edu

Received 5 September 2019; Editorial decision 5 January 2020

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 South Texas. 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 consumption from total sugars for mosquitoes captured in 2019. Overall, Aedes aegypti females had significantly lower total sugar content than Aedes aegypti males as well as both sexes of Culex quinquefasciatus. However, the percentage of Aedes aegypti positive for fructose consumption was four to eightfold higher than Aedes 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 Aedes aegypti is less common than Culex quinquefasciatus, although not absent. This study provides necessary data to evaluate the potential effectiveness of sugar baits in surveillance and control of both Aedes aegypti and Culex 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 Rhamnus 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, Aedes 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, Oketch 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 CO2 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 CO2-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 6-7 d at a time as opposed to traditional CO2 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 (Khallayoune 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′07″N, 97°36′08″W ± 0.5 km), La Piñata (26°07′44″N, 98°03′25″W ± 0.5 km), La Puente (26°07′44″N, 98°03′25″W ± 0.5 km), 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...
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 (NaSO₄), followed by 1.5 ml of 1:2 chloroform methanol solution was added and stirred. The glycogen was absorbed to the NaSO₄ precipitate. Sample tubes were then centrifuged at 450 × g for 1 min. Being careful not to disturb the pellet containing glycan, 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 µg of glucose in 25 µl of 25% ethanol to produce an initial 1:1 (30 µg/30 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 µg of fructose instead of 25 µg 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 fixating 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 (Bysameeey, 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 was also found in males with *Ae. aegypti* having significantly less mean total sugar content of 17.00 µg (±2.40) compared to 24.40 µg (±1.90), respectively. After removing mosquitoes with >3.5 µg sugar from the dataset, *Ae. aegypti* content was not observed (*P* = 0.207) for *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 2.35 ± 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        | 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 ± 1.59 (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. 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).
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   |
|----------|------------------|-----|----------------------------------|-------------|
|          |                  |     | µg ± SE (n)                      | BGS2        | CDC resting | Aspirator  |
|          |                  |     |                                  | 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)               | N/A         | 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) |

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 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...
have greater sugar content compared to 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 CO2-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. (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      | 0.0739  |
| 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   |

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).
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 feeding by mosquitoes strongly impact malaria transmission potential. PLoS One 6:e1596.

Acknowledgments

This study would not have been possible without the support and willingness 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 the participation of the residents in our study sites of the Lower Rio Grande Valley. 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.

Supplementary Data

Supplementary data are available at Journal of Medical Entomology online.

References Cited

Anderson, I. H., and T. G. Jaenson. 1987. Nectar feeding by mosquitoes in Sweden, with special reference to Culex pipiens and Cx. torrentium. Med. Vet. Entomol. 1: 59–64.

Bown, M. F. 1992. Patterns of sugar feeding in diapausing and nondiapausing Culex pipiens (Diptera: Culicidae) females. J. Med. Entomol. 29: 843–849.

Chadec, D. D., J. M. Sutherland, and J. R. Gilles. 2014. Diet sugar feeding and reproductive behaviours of Aedes aegypti mosquitoes in Trinidad: with implications for mass release of sterile mosquitoes. Acta Trop. 132 Suppl: S86–S90.

Christ, P., A. Reifenrath, J. Kahnt, F. Hauser, S. R. Hill, J. Schachtner, and R. Ignell. 2017. Feeding-induced changes in allatostatin-A and short neuropeptide F in the antennal lobes affect odor-mediated host seeking in the yellow fever mosquito, Aedes aegypti. PLoS One 12: e0188243.

Chung, W. M., C. M. Buseman, S. N. Joyner, S. M. Hughes, T. B. Fomby, J. P. Luby, and R. W. Haley. 2013. The 2012 West Nile encephalitis epidemic in Dallas, Texas. Jama 310: 297–307.

Costero, A., J. D. Edman, G. G. Clark, and T. W. Scott. 1998a. Life table study of Aedes aegypti (Diptera: Culicidae) in Puerto Rico fed only human blood versus blood plus sugar. J. Med. Entomol. 35: 809–813.

Costero, A., G. M. Attardo, T. W. Scott, and J. D. Edman. 1998b. An experimental study on the detection of fructose in Aedes aegypti. J. Am. Mosq. Control Assoc. 14: 234–242.

Dobson, A. J., and A. G. Barnett. 2008. An introduction to generalized linear models, Chapman and Hall/CRC, New York, NY.

Downes, J. 1958. The feeding habits of biting flies and their significance in classification. Annu. Rev. Entomol. 3: 249–266.

Edman, J. D., D. Strickman, P. Kittayapong, and T. W. Scott. 1992. Female Aedes aegypti (Diptera: Culicidae) in Thailand rarely feed on sugar. J. Med. Entomol. 29: 1035–1038.

Frikig, K., B. J. Johnson, D. Fish, and S. A. Ritchie. 2017. Assessment of synthetic floral-based attractants and sugar baits to capture male and female Aedes aegypti (Diptera: Culicidae). Parasit. Vectors. 10: 32.

Foster, W. A. 1995. Mosquito sugar feeding and reproductive energetics. Annu. Rev. Entomol. 40: 443–474.

Foster, W. A., and F. A. Eischen. 1987. Frequency of blood-feeding in relation to sugar availability in Aedes aegypti and Anopheles quadrinaculatus (Diptera: Culicidae). Ann. Entomol. Soc. Am. 80: 103–108.

Fox, M. 2007. Illustrated key to common mosquitoes of Louisiana. Mosquito control training manual. Louisiana Mosquito Control Association, Baton Rouge, LA, p. 86–150.

Gu, W., G. Müller, Y. Schlein, R. J. Novak, and J. C. Beier. 2011. Natural plant sugar sources of Anopheles mosquitoes strongly impact malaria transmission potential. PLoS One 6:e1596.

Hall-Mendelin, S., S. A. Ritchie, C. A. Johansen, P. Zborowski, G. Cortis, S. Dandridge, R. A. Hall, and A. F. van den Hurk. 2010. Exploiting mosquito sugar feeding to detect mosquito-borne pathogens. Proc. Natl. Acad. Sci. U. S. A. 107: 11255–11259.

Harrington, L. C., J. D. Edman, and T. W. Scott. 2001. Why do female Aedes aegypti (Diptera: Culicidae) feed preferentially and frequently on human blood? J. Med. Entomol. 38: 411–422.

Harrington, L. C., A. Fleisher, D. Ruiz-Moreno, F. Vermeylen, C. V. Wa, R. L. Poulson, J. D. Edman, J. M. Clark, J. W. Jones, S. Kittahwee, et al. 2014. Heterogeneous feeding patterns of the dengue vector, Aedes aegypti, on individual human hosts in rural Thailand. Plos Negl. Trop. Dis. 8: e3048.

van den Hurk, A. F., S. Hall-Mendelin, M. Townsend, N. Kurucz, J. Edwards, G. Ehlers, C. Rodwell, F. A. Moore, J. L. McMahon, J. A. Northill, et al. 2014. Applications of a sugar-based surveillance system to track arboviruses in wild mosquito populations. Vector Borne Zoonotic Dis. 14: 66–73.

Impoinvil, D. E., J. O. Kongere, W. A. Foster, B. N. Njuru, G. F. Killeen, J. I. Guthrie, J. C. Beier, A. Hassanali, and B. G. Knols. 2004. Feeding and survival of the malaria vector Anopheles gambiae on plants growing in Kenya. Med. Vet. Entomol. 18: 108–115.

Junnila, A., G. C. Müller, and Y. Schlein. 2010. Species identification of plant tissues from the gut of An. sergentii by DNA analysis. Acta Trop. 115: 227–233.

Khalilayounie, K., W. A. Qualls, E. E. Revay, S. A. Allan, K. L. Arheart, V. D. Kravchenko, R. D. Xue, Y. Schlein, J. C. Beier, and G. C. Müller. 2013. Attractive toxic sugar baits: control of mosquitoes with the low-risk active ingredient dinotefuran and potential impacts on nontarget organisms in Morocco. Environ. Entomol. 42: 1040–1045.

Klowden, M. J. 1986. Effects of sugar deprivation on the host-seeking behaviour of gravid Aedes aegypti mosquitoes. J. Insect Physiol. 32: 479–483.

Knab, F. 1907. Mosquitoes as flower visitors. J. N. Y. Entomol. Soc. 15: 213–219.

Laredo-Tiscareño, S. V., C. Machain-Williams, M. A. Rodríguez-Pérez, J. A. Garza-Hernandez, G. L. Doria-Cobos, R. C. Catina-Trejo, L. A. Bacab-Cab, C. S. Tangud, J. Charles, E. J. De Luna-Santillana, et al. 2018. Arbovirus surveillance near the Mexico-U.S. border: isolation and sequence analysis of chikungunya virus from patients with dengue-like symptoms in Reynosa, Tamaulipas. Am. J. Trop. Med. Hgy. 99: 191–194.

Lee, J. C. 2019. What can we learn from the energetic levels of insects: a guide and review. Ann. Entomol. Soc. Am. 112: 220–226.
