Plant sugar feeding patterns of wild-caught *Aedes aegypti* from dengue endemic and non-endemic areas of Kenya

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Running title: Sugar feeding of wild *Aedes aegypti*

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Abstract

A fundamental understanding of plant sugar feeding behaviour in vector populations can lead to the development of ecologically effective vector monitoring and control strategies. Despite previous studies on mosquito-plant interactions, relatively few have been conducted on the dengue vector *Aedes aegypti* (Diptera: Culicidae). Here, we studied *Ae. aegypti*-plant interactions at two sites of varying dengue endemicity in Kenya: Kilifi (endemic) and Isiolo (non-endemic). Using chemical and molecular assays (DNA barcoding targeting the chloroplast ribulose-1,5 bisphosphate carboxylase/oxygenase large chain (rbcL) gene), we show that at the two sites plant feeding in this mosquito species: 1) varies by sex, and season, 2) results in acquisition of diverse sugars, and 3) is associated with diverse host plants in the families Fabaceae, Malvaceae, Poaceae and Rosaceae. These results reveal insights into the plant sugar feeding patterns of wild-caught *Ae.*

aegypti and provide a baseline for future studies on the olfactory basis for host plant attraction for the development of vector monitoring and control tools.

Introduction

Plant sugar is an important dietary requirement for most blood feeding arthropods including mosquitoes and sand flies (Stone & Foster, 2013). It affects longevity, reproduction, flight and even immunity to pathogens thereby significantly affecting their life history and vectorial capacity (Koella & Sørensen, 2002; Okech *et al.*, 2003; Gary *et al*., 2009; Chadee *et al*., 2014). Thus, plant sugar feeding behaviour of disease vectors, and plant species composition are important components of vector-borne disease epidemiology (Stone *et al*., 2012; Ebrahimi *et al*., 2018). In the field, removal of highly preferred plant sugar sources has been shown to negatively impact density and age structure of malaria vector populations (Müller *et al*., 2017). Similar effects on dengue vector populations have been documented with attractive toxic sugar baits (ATSBs) (Sissoko *et al*., 2019). Previous studies have also demonstrated the utility of plant- derived attractants in field surveillance of both malaria and arbovirus disease vectors (Nyasembe *et al.*, 2014; Nyasembe *et al.*, 2015). These examples indicate that plant sugar feeding behaviour in disease vectors is an important target for development of complementary vector monitoring and control strategies. Moreover, male mosquitoes depend entirely on sugar for their survival (Foster, 1995). Thus, sugar feeding is also an important factor for consideration in the development of genetic vector control strategies such as the sterile male insect technique (SIT) (Lees *et al*., 2014).

Compared to malaria vectors, plant sugar feeding behaviour of *Aedes aegypti* is largely understudied, particularly in sub-Saharan Africa. Being highly anthropophilic, this species was thought to derive its protein and energy requirements from blood and seldom fed on plants (Edman *et al*., 1992). However, a few recent studies show that they readily forage on plant sugars although

the degree varies between sites (Nyasembe *et al*., 2018; Olson *et al*., 2020). Whether variation exists in the utilization of this resource by sex, site or season remains poorly described. Moreover, only a few plant species have been definitively identified as candidate *Ae. aegypti* host plants thus far. Improved knowledge on the plant feeding associations in disease vectors has greatly been enhanced through application of molecular based approaches (Junnila *et al.*, 2010; Abbasi *et al.*, 2018; Nyasembe *et al.*, 2018). However, only one study has applied this on *Ae. aegypti* (Nyasembe *et al*., 2018). Using the DNA barcoding approach, Nyasembe *et al*. (2018) identified *Pithecellobium dulce*, *Senna uniflora*, and *Hibiscus heterophyllus* as candidate *Ae. aegypti* host plants in coastal Kenya. However, this study was limited in geographic and seasonal scope as it was only conducted during the rainy season and at only one site. To improve our understanding of how seasonality, sex and site might affect the host plant choices of *Ae. aegypti* we examined: 1) the extent of plant feeding in wild populations from a dengue endemic (Kilifi) and non-endemic (Isiolo) area of Kenya during the dry and wet seasons based on fructose positivity rates, and 2) the composition of sugars obtained from plant feeding activities. Additionally, we identified candidate host plants fed on by both sexes across the sites and seasons.

Materials and Methods

Study site

The study was conducted in Kilifi and Garbatullah towns. Kilifi town (3.63229° S, 39.8569°E) (Figure 1) is the main urban centre in Kilifi County and is located along the Kenyan coast about 61 km north of Mombasa (Kenya's second largest city). As part of the larger coastal region, Kilifi is prone to dengue outbreaks and the *Ae. aegypti* populations shown to be very competent vectors of dengue 2 virus (Chepkorir *et al.*, 2014). The area experiences annual temperatures ranging between 21℃ and 32℃ and has two rainy seasons: March/April to May/ June (long rains), and

October to early December (short rains) (Owino *et al*., 2014), although the pattern of rainfall can vary across years. The dry season falls between July to October and Mid December to March/April with temperatures ranging between 28℃ to 31℃. In 2018, the area experienced a prolonged long rainy season that ended in late July and was marked by extreme flooding. Garbatullah (0.4111° N, 38.5690° E) is one of the major urban centres in Isiolo County, in Northern Kenya and is located about 258 km from the capital city, Nairobi (Figure 1). Annual temperatures in the larger Isiolo County range between 24℃ and 30℃. However, the area is semi-arid to arid and experiences irregular rainfall patterns that sometimes can be absent for an entire year. Due to the erratic rainfall and sparse vegetation cover, Isiolo is also prone to heavy storms and flash floods (Mati *et al*., 2005).

Study design, mosquito trapping and plant survey

In Kilifi, mosquito sampling was conducted for 10 consecutive days between July and August in 2017 and 2018, corresponding to the dry and wet seasons, respectively. In Isiolo sampling was done for 5 consecutive days in the dry season (March 2018). Mosquitoes were trapped using BG-Sentinel traps (Bioquip, Rancho Dominguez, California, USA) baited with carbon dioxide (dry ice). For ten days, traps (10 daily) were set up at dawn (0600 hr) and retrieved at dusk (1830 hr) after which mosquitoes were killed by freezing, sorted, counted and stored in liquid nitrogen before being transferred to the *icipe* laboratories in Nairobi. At both study sites, an observational survey of existing plant species around the mosquito sampling points was also done and an inventory made.

Sample processing

Mosquitoes were identified using taxonomic keys (Jupp, 1996) and individual males and females washed in phosphate buffered saline (PBS) to remove any traces of plant material on their bodies that could potentially contaminate the DNA extracts. The mosquitoes were then transferred individually into sterile microcentrifuge tubes and crushed using individual sterile polypropylene pestles. After crushing, 100 µL of sterile 0.3M sodium acetate and 200 µL of absolute molecular grade ethanol were added and the samples incubated for 30 minutes at -20°C. After, incubation homogenates were centrifuged (Eppendorf 5417r) at 4°C for 10 min at 12000 RPM. The resulting supernatant was then carefully removed and stored for sugar analysis using the cold anthrone test (fructose test) and gas chromatography coupled to mass spectrometry (GC/MS). The remaining pellet was left to air dry for 24 hr in a biosafety cabinet. After drying, the pellet was re-extracted using the RED Extract-N-AMP plant DNA extraction Kit (Sigma-Aldrich, St Louis, MO, USA) following the manufacturer's instructions. Additionally, 0.5 cm leaf disc (from *Lepidium africanum;* a plant on which we observed *Ae. aegypti* probing in the field) was cleaned with double distilled water, placed in a sterile microcentrifuge tube and DNA similarly extracted for use as positive control in subsequent PCR assays.

Detection of recent plant sugar feeding in wild collected mosquitoes

The cold anthrone sugar test (Van Handel, 1985) was used to detect presence of fructose (evidence of recent plant sugar feeding) in the wild mosquito samples. Briefly, 1 mL of anthrone reagent was added to an aliquot of the supernatant (200 µL) obtained in the extraction step above, mixed and left to stand at room temperature for 75 min before being examined for colour change. Where fructose was present, the initial yellow colour of the mixture changed to either a green/blue coloured complex and this recorded as a positive result. A sub set of 20 fructose positive samples (10 males and 10 females) from each cohort of wild collected *Ae. aegypti* (total of 60 samples)

was obtained and the remaining supernatant from the first extraction step analysed for presence of other plant-derived sugars by coupled gas chromatography/ mass spectrometry (GC/MS). Briefly, 40 µL of the supernatant was placed in a GC vial insert and evaporated to dryness. Thereafter, 100 µL of silylation reagent (Tri-Sil HTP (Thermo scientific)) was added and the sample incubated at 85℃ for 15 min. Two microliters of the sample were injected (splitless mode) into the GC/MS system consisting of a gas chromatograph (Agilent technologies- 7890) coupled to inert XL EI/CI mass spectrometer (5975C, EI, 70eV, Agilent, Palo Alto, California, USA) (GC/MS). The GC was equipped with a HP-5MS column (30 m x 250 µm x 0.25 µm) with helium as the carrier gas (flow rate 1.2 mL/min). The oven temperature was held at 70 °C for 3 min then increased to 280 °C at a rate of 10 °C/min. The chemical identities of the trimethysilyl derivatives were determined by comparison of their mass spectra to library data (Adams2.L, Chemecol.L and NIST05a.L) and authentic standards where available (see section on chemicals used).

Plant DNA barcoding assay

DNA of all cold anthrone positive samples were amplified by polymerase chain reaction (PCR) targeting the chloroplast ribulose-1,5 bisphosphate carboxylase/oxygenase large chain gene (rbcL) gene using published primers (Abbasi *et al.*, 2018). From each sample, 200 ng of DNA was used as template in a 20 µL PCR mix comprising 0.4 µM of forward and reverse primer, 10 µL of MyTaqHS mix (Bioline Ltd, UK) (standard volume for a 20 µL reaction) and PCR grade water. Cycling conditions consisted of an initial denaturation of 95℃ for 1 min; 35 cycles of denaturation at 95℃ for 15s, annealing at 50℃ for 40s, and extension at 72℃ for 1min; 1 cycle of final extension at 72℃ for 10 min. PCR products were visualized on a 1.2 % agarose gel and molecular weight determined using a 1Kb DNA ladder. PCR products were cleaned up using EXoSAP IT (Affymetrix Inc, USA) or Isolate II gel purification kit (Bioline Ltd, UK) for multiple bands

following manufacturer's instructions and bidirectional Sanger sequencing outsourced from Macrogen laboratories (Seoul, Korea). Sequences were cleaned using the Molecular Evolutionary Genetic Analysis software (MEGA v. 7) (Kumar *et al*., 2016), and compared to reference sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) (Johnson *et al*., 2008) with default search parameters. Only sequences with > 97% homology to GenBank reference sequences were considered as candidate host plant species.

Chemicals used

Synthetic sugar standards: D-fructose (Sigma Aldrich, 99%), D-glucose (Fischer Scientific, 99.5%), D-(+)-sucrose (Fischer Scientific, 99.5%), D-(+)- xylose (Sigma Aldrich, 99%), D-(+)-galactose (Sigma Aldrich, 99%) L-arabinose (Sigma Aldrich ,99%) and D- turanose (Sigma Aldrich, 98%), L-rhamnose (Sigma Aldrich, 99%)

Data management and statistical analysis

The extent of plant feeding (fructose positivity rates) was computed from the total number of fructose positive samples expressed as a percentage of the total number tested. Chi-square test-of-independence was used to compare the fructose positivity rates between males and females at each site and across seasons. Where significant differences were found, a pairwise comparison of means using the pairwise nominal independence test was done. All tests were done using R statistical software and at 95% significance level (R Core Team, 2020).

Results

Fructose positivity rates of wild caught *Aedes aegypti*

Of the 796 mosquitoes (male = 253 and female = 543) collected and analysed from Kilifi during the dry season the proportion of both sexes that tested positive for fructose was not significantly different; males- 27% (68/253) and females-21 % (112/543) ($\chi^2 = 0.75$, df = 1, $P > 0.05$) (Table 1). However, during the wet season, of the 514 mosquitoes collected and analysed from the same site, two-fold more females (23% (67/286)) than males (11% (25/228)) tested positive for fructose ($\chi^2 = 4.2353$, df = 1, $P < 0.05$) (Table 1). In contrast, the fructose positivity rates of males (36% (79/222)) and females (24% (48/197)) in Isiolo was statistically not significant ($\chi^2 = 2.4$, df = 1, $P > 0.05$). After controlling for site, fructose positivity rates did not vary among females across the seasons (Kilifi (dry) = 21%, Kilifi (wet) = 23%, and Isiolo (dry) = 24%; $\chi^2 = 0.21$, df = 2, $P > 0.05$) (Table 1). However, the fructose positivity rates of males between the seasons was significantly different (Kilifi (dry) = 27%; Kilifi (wet) 11%; Isiolo (dry) = 36%; $\chi^2 = 22.41$, df = 2, $P < 0.05$), with the rates during the dry seasons in Kilifi and Isiolo being 2.4 and 3.2 times higher than the wet season in Kilifi (Table 1).

Diversity of sugars obtained from plant feeding

Using GC/MS, we detected the pyran and furan forms of thirteen monosaccharides: fructose, glucose, xylose, galactose, arabinose, rhamnose, mannose, allulose (psicose), allose, sorbose, talose, ribose, and gulose (Table 2). The identities of the first six monosaccharides was confirmed using authentic standards while the remaining seven were only identified using GC/MS mass spectral data. In addition to the monosaccharides, we also detected two disaccharides: lactose and sucrose; three sugar alcohols: sorbitol, mannitol and myo-inositol; two sugar acids: gluconic acid and arabinonic acid; and one polyol: pinitol (Table 2). Except for sucrose, the remaining sugars were also only tentatively identified using spectral library data. Overall, fructose, glucose, mannose, arabinose, galactose, xylose and sorbose were the most commonly occurring sugars

(Table 2). Fructose and glucose were detected in all male samples at both study sites and across the seasons whereas all the seven sugars were ubiquitous in females (Table 2).

Plant DNA barcoding assay

A total of 394 fructose positive samples were processed by PCR of which 16% (63/394) amplified, producing an ~ 350 bp fragment (Table 3). However, PCR success rates differed between the three cohorts of wild caught *Ae. aegypti* with the highest success rate recorded in samples collected during the wet season from Kilifi (Table 3). Successful sequences were obtained from 40 samples comprising 16 males (Kilifi (9): dry season = 7, wet season = 2; Isiolo (7)), and 24 females (Kilifi (20): dry season = 6, wet season = 14; Isiolo (4)) (Table 3). We also had few instances (n = 4) of multiple bands in PCR indicative of DNA amplification from different plants in a single specimen. However, sequencing was unsuccessful for these samples.

Candidate plant sources of wild caught *Ae aegypti* sugar meals

Of the 40 sequences analysed, 34 plant species belonging to 21 families were identified (Figure 2, Table 4) of which 16 were associated with females and 10 with males. The dominant plant families identified as candidate sugar sources for males were Fabaceae and Malvaceae whereas for females they were Fabaceae, Malvaceae, Poaceae, and Rosaceae. The key plant families associated with both sexes were Fabaceae and Malvaceae. An analysis of the 8 plant species profiles of the Fabaceae detected showed differences by sex, site and season. *Pithecellobium dulce* was commonly fed upon during the dry season at both sites and by both sexes. Of the 3 plant species detected in the Malvaceae, only *Hibiscus-rosa sinensis* was common to both sexes but at one site-Kilifi and in the dry season only. Two other plant species *Azadirachta indica* (Meliaceae) and *Zea mays* (Poaceae) were also common to both sexes and detected at both sites (Table 4). Of the

plant species identified, seven: *P. dulce*, *Z. mays*, *Vigna unguiculata*, *Hibiscus* spp, *Malva parviflora*, *Acacia* spp and *A. indica* had been documented during the observational survey conducted at the time of mosquito sampling.

Discussion

In this study we examined the plant sugar feeding patterns of wild *Ae. aegypti* populations from a dengue endemic and non-endemic area of Kenya. While plant feeding rates of males were higher during the dry season, there was no difference in females across seasons and sites. Several reasons may account for this pattern. Firstly, plant feeding provides not only sugars, but also water (Foster, 1995; Holmes & Benoit, 2019) which cushions mosquitoes against risk of desiccation during the dry season (Holmes & Benoit, 2019). Males being obligate plant feeders and consuming relatively smaller meal sizes than females (Foster, 1995; Nunes *et al*., 2008), may engage more in plant feeding during such adverse conditions. Secondly, under optimal conditions males tend to emerge with high levels of teneral reserves (Briegel, 1990). Therefore, without metabolic constraints and with decreased dispersal activity in search of plant sugars, as it would be during the wet season, reduced plant sugar feeding is not unexpected. Thirdly, female *Ae. aegypti* are highly anthropophilic and can survive solely on blood (Edman *et al*., 1992). However, recent research has shown that plant feeding is widespread in females too (Nyasembe *et al*., 2018; Sissoko *et al*., 2019; Olson *et al*., 2020). Given that a blood meal may provide the same caloric content as a sugar meal (with 15% sugars) (Huestis *et al*., 2011), females may exhibit plasticity in utilizing both resources regardless of season and based on their availability. It is noteworthy, that the samples analyzed for fructose were trapped and retrieved after about 12 h before being frozen. Thus, it is likely that digestion of sugar meals may have occurred in a certain proportion of these samples. Hence, the

fructose positivity rates reported in the current study may be an underestimation of the true value in nature.

GC/MS analysis revealed the detection of diverse plant-derived sugars in trapped mosquitoes. Fructose, glucose, mannose, arabinose, galactose, xylose and sorbose were the most common sugars detected, which was consistent with previous findings (Manda *et al*., 2007). Nonetheless, some of the sugars may also have been of microbial origin (Lenaerts *et al*., 2016) and/or metabolic intermediates from gut microbiota. Mosquitoes may also feed on honey dew leading to the acquisition of sugars that include melezitose, stachyose, raffinose among others (Russell & Hunter, 2002). However, these sugars, including trehalose that is endogenously produced in the fat body (Becker *et al.,* 1996) were not detected because they may have been present in trace levels in our samples. Overall, we detected a higher number of sugars in females than males, possibly indicative of their different feeding habits. Sugar composition also varies between different plant species (Hassaballa *et al*., 2021); the wider range of plants detected in females may contribute to the diversity in sugars detected.

DNA barcoding sequence analysis revealed a wide plant host range for *Ae. aegypti*. Most of the plant species identified belonged to the families Fabaceae, and Malvaceae. Interestingly, previous studies had highlighted the seeming preference for plants in the Fabaceae by mosquitoes (Nyasembe *et al*., 2018) and sand flies (Lima *et al*., 2016; Hassaballa *et al*., 2021). Phylogenetic analysis of extra floral nectaries (EFNs) across different plant taxa show that, the Fabaceae family actually constitutes the highest number of species bearing EFNs (Díaz-Castelazo *et al*., 2005; Weber & Keeler, 2013). Likewise, EFNs have been reported for plant species in the Malvaceae (Weber & Keeler, 2013). Thus, the association found here between *Ae. aegypti* and plants in the Fabaceae and Malvaceae warrants further research. While we found an overlap in plant species

previously detected as host plants of *Ae. aegypti* in Kilifi at the Kenyan Coast *viz*: *Pithecellobium spp* (*P. dulce*, and *P. unguis-cati*) (Nyasembe *et al*., 2018), other plants such as *Senna uniflora* (Fabaceae), and *Hibiscus heterophyllus* (Malvaceae) (Nyasembe *et al*., 2018), were not detected in the profiles of the *Ae. aegypti* we trapped. These plant species were also not detected in our observational study during the time of mosquito trapping. Kilifi, the study site is expansive and *Ae. aegypti* exhibits a short flight range (Honório *et al*., 2003). Thus, site specific differences where the mosquitoes were sampled may account for the observed disparity.

Analysis of plant DNA is evidently a very useful approach in the characterization of plant sugar sources of arthropods in nature (Abbasi *et al*., 2018; Nyasembe *et al*., 2018). However, there is always the possibility of contamination of sugar sources by plant material such as pollen either blown by the wind or introduced by other foragers. To increase confidence in results from DNA analysis, proof of direct feeding and/or the presence of the identified plants at the trapping site is warranted. While direct observation could help to confirm that a plant was fed on, this approach has several limitations: Firstly, extensive surveys would be needed to gather sufficient information about feeding patterns of the insect, which is both labour intensive and time consuming. Secondly, although an insect may probe on a plant, this may not always directly translate to feeding. Therefore, this approach was not employed in the current study. Given our approach, whereby samples were individually surface sterilized to eliminate extraneous plant material and analysed singly, the presence of some of the identified plant species at the study sites, and consistency of our findings with previous studies (Liu *et al*., 2006; Nyasembe *et al*., 2018; Hassaballa *et al*., 2021) strongly confirms plant tissue feeding.

Our DNA barcoding assay showed variations in the PCR success rates and particularly low amplification rates in the dry season and especially in females from Isiolo. This was unexpected,

however, previous studies attribute such low success rates in amplification of plant DNA in insects to possible degradation by gut enzymes (Lima *et al*., 2016; Hasaballa *et al*., 2021). Whether the process varies among the sexes and is influenced by season, would require additional research. Future studies are also needed to correlate the pattern of sugars detected in the mosquito to plant species, location and season. Only fructose-positive specimens were analysed further for sugars and plant DNA. This may have precluded a full appraisal of other important metabolic intermediates in fructose-negative samples. Moreover, possibility of plant DNA detection in such samples needs to also be explored in future studies.

Conclusion

We conclude that seasonality influences plant feeding rates of *Ae. aegypti* populations but the effects are more pronounced in males than females and in the dry season. Plant feeding results in acquisition of diverse sugars essential for various mosquito life processes. The *Ae. aegypti* populations sampled feed on diverse plant species that include food crops. Overall, females are associated with a greater diversity of sugars and host plant species more than males, which possibly reflects their feeding habits in nature. Inspite of the diverse plant feeding profile, this mosquito species appears to have a predilection for Malvaceae and Fabaceae plants, a finding necessitating further evaluation of the properties that make these families highly suitable. Longitudinal surveys and incorporating diverse molecular markers including high throughput sequencing approaches will be helpful to unravel plant interactions with this disease vector. Nonetheless, our findings provide useful insights into *Ae. aegypti* plant feeding and provide the groundwork for investigations into the olfactory cues mediating host plant attraction and how they can be used for monitoring and control of vector populations.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

BT, DPT and CW conceptualized and designed the study; CW collected and analysed the data; CW, DPT, CLS, CWWP and BT wrote the draft manuscript; CW, DPT and BT finalized the draft. All authors read and approved the manuscript.

Conflict of interest

The authors declare that there are neither conflicts of interest nor competing financial interests associated with this work.

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Figures

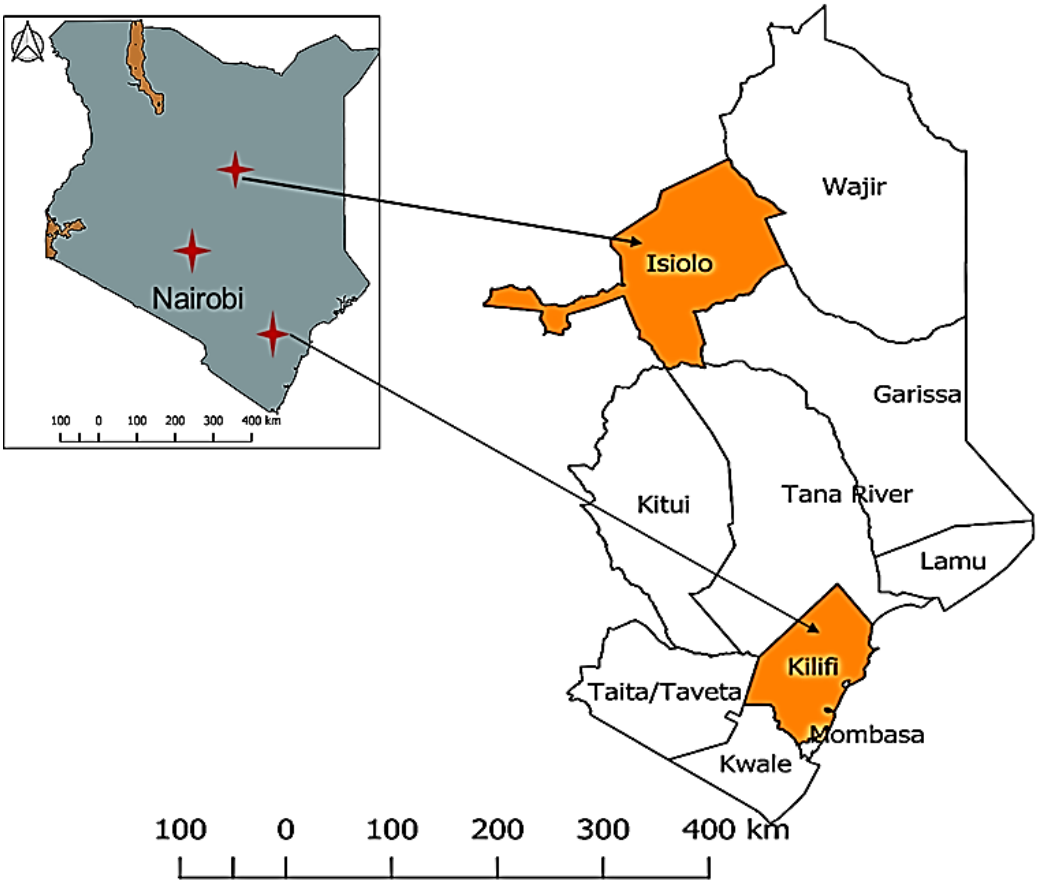

Figure 1 Map of Kenya showing the sites where the study was undertaken

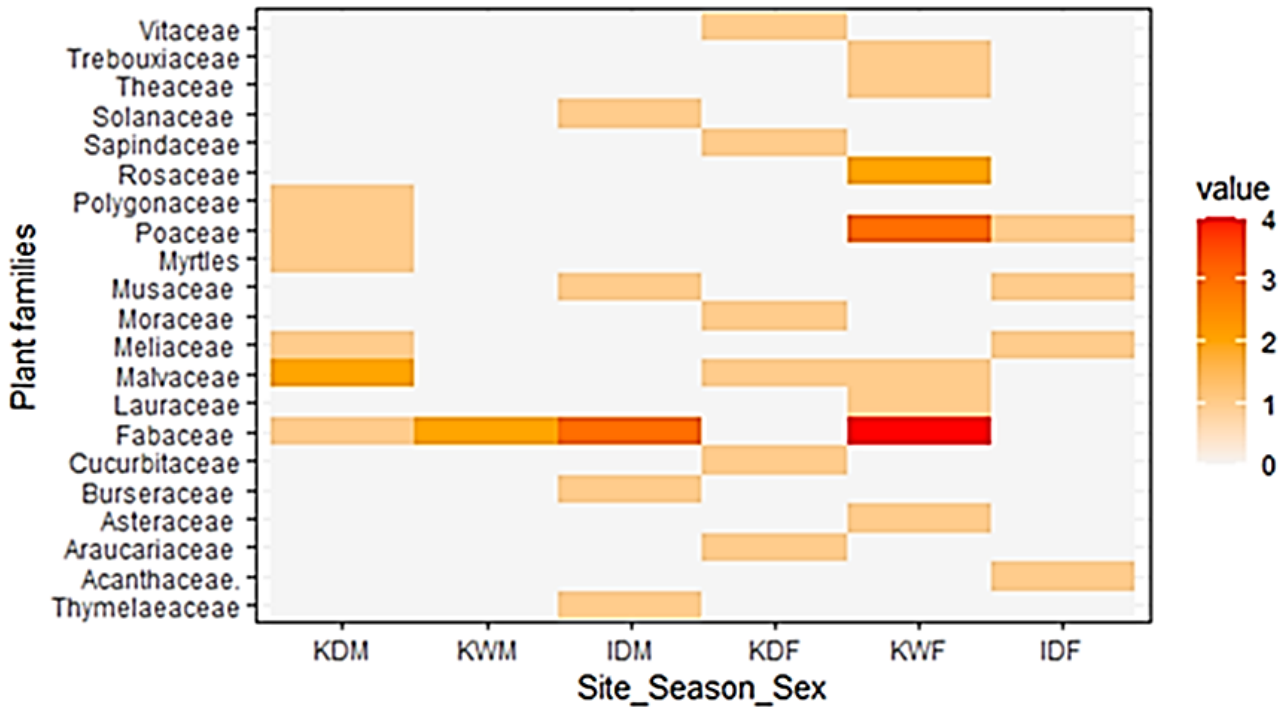

Figure 2 Heat map showing families of the plants detected from the three cohorts of male and female *Ae aegypti* from Kilifi and Isiolo; KDM: Kilifi dry season males, KWM: Kilifi wet season males, IDM: Isiolo dry season males, KDF: Kilifi dry season females, KWF: Kilifi wet season females; IDF: Isiolo dry season females; Colour intensity of bands ranges (1= low (yellow); 2-3 =Mid (orange), 4=high(red)) indicates the number of plant species identified in each family

Tables

Table 1 Fructose positivity rates (%) of male and female Ae. aegypti collected from Kilifi (dry and wet season) and Isiolo (dry season); row and column values denoted with different letters are significantly different at 95% level of significance

	Kilifi (dry) ***(%)***	***Kilifi (wet)*** ***(%)***	***Isiolo (dry)*** ***(%)***	*Chi-square statistic* *(df = 2)*
Males	27^{a}	11^{b}	36^{a}	$\chi2 = 22.41$;
	(*n* = 68/253)	(*n* = 25/228)	(*n* = 79/222)	***P* = 0.002**
Females	21^{a}	23^{a}	24^{a}	$\chi2 = 0.21$;
	(*n* = 112/543)	(*n* = 67/286)	(*n* = 48/197)	*P* = 0.902
Chi-square statistic	$\chi2 = 0.75$;	$\chi2 = 4.24$;	$\chi2 = 2.4$;	
	P = 0.387	***P* = 0.04**	*P* = 0.121	
(df = 1)				

Pairwise comparison of means (pair wise nominal independence test)

Sex	**Comparison**	***p.adj.Chisq***
Males	Kilifi (dry): Kilifi (wet)	**0.014**
	Kilifi (wet): Isiolo (dry)	**0.001**
	Kilifi (dry): Isiolo (dry)	0.257

Table 2 Summary of sugars detected by coupled gas chromatography -mass spectrometry (GC/MS) in the whole insect extracts of wild Ae. aegypti collected from Kilifi (dry and wet season) and Isiolo (dry season); *******, monosaccharides; ‡, disaccharides; †, sugar alcohols; ‡, sugar acids; ŕ, polyol; +, sugar detected; –, sugar not detected*

	Males			Females		
Sugar	**Kilifi dry**	**Kilifi wet**	**Isiolo dry**	**Kilifi dry**	**Kilifi wet**	**Isiolo dry**
Fructose *	+	+	+	+	+	+
Mannose *	+	-	+	+	+	+
Allulose *	-	-	+	-	-	+
Allose *	+	-	-	-	-	+
Glucose *	+	+	+	+	+	+
Arabinose *	+	-	-	+	+	+
Sorbose *	+	-	+	+	+	+
Xylose *	-	-	+	+	+	+
Galactose *	+	-	+	+	+	+
Rhamnose *	-	-	-	-	+	-
Talose *	-	-	+	-	+	-
Ribose *	+	-	-	-	-	-
Gulose *	+	+	-	-	-	-
Lactose ‡	-	-	+	-	-	-
Sucrose ‡	-	-	+	-	-	-
Sorbitol †	-	-	-	-	+	-

Mannitol †	+	-	+	+	+	-
Myo-inositol †	+	-	-	+	+	+
Gluconic acid ‡	-	-	-	-	+	-
Arabinonic acid ‡	-	-	-	+	+	-
Pinitiol †	-	-	-	+	-	-

Table 3 Success rate (%) of Polymerase Chain Reaction (PCR) employing rbcL primers on samples of wild male and female Ae. aegypti collected from Kilifi (dengue-endemic) and Isiolo (dengue non-endemic) during the dry and wet season

Study site (season)	Number of samples processed by PCR (fructose positive)		PCR success rate (%)	
	F	M	F	M
Kilifi (dry)	70	60	10	12
Kilifi (wet)	67	17	30	41
Isiolo (dry)	79	48	6.3	35

*Table 4 Summary of plant families, species and their life growth habits based on plant DNA detected in wild collected male and female Ae. aegypti from the dry and wet season in Kilifi and the dry season in Isiolo; plant denoted with sp = sequence homology < 97%; * plant DNA detected in both sexes of wild collected Ae. aegypti; † plant species detected in both Kilifi and Isiolo; +, plant DNA detected; –, Plant DNA not detected*

Plant family	Identified species (based on > 97% BLAST similarity)	Life growth habits	Kilifi (dry)		Kilifi (wet)		Isiolo (dry)	
			M	**F**	**M**	**F**	**M**	**F**
Fabaceae	*Pithecellobium unguis-cati*	shrub	+	-	-	-	-	-
	Vigna unguiculata	herb	-	-	+	-	-	-
	Senegalia Senegal	tree	-	-	-	-	+	-
	†*Pithecellobium dulce	shrub	-	+	-	-	+	-
	Vachellia sp	tree	-	-	-	+	+	-
	Albizia sp	tree	-	-	-	+	-	-
	Acacia sp	tree	-	-	-	+	-	-
Malvaceae	*Reevesia sp*	tree	+	-	-	-	-	-
	****Hibiscus rosa-sinensis***	shrub	+	+	-	-	-	-
	Malva parviflora	herb	-	-	-	+	-	-
Meliaceae	***†*Azadirachta indica***	tree	+	-	-	-	-	+
Poaceae	***†*Zea mays***	herb	+	-	-	+	-	+
	Triticum sp	herb	-	-	-	+	-	-
	Eragrostis sp	herb	-	-	-	+	-	-
Polygonaceae	*Oxygonum sinuatum*	herb	+	-	-	-	-	-
Myrtles	*Callistemon sp*	tree	+	-	-	-	-	-
Solanaceae	*Solanum sp*	herb	-	-	-	-	+	-
Burseraceae	*Commiphora sp*	tree	-	-	-	-	+	-
Musaceae	*Heliconia sp*	herb	-	-	-	-	+	-
	Musella sp	herb	-	-	-	-	-	+
Thymelaeaceae	*Lachnaea sp*	herb	-	-	-	-	+	-
Cucurbitaceae	*Cucumis melo*	herb	-	+	-	-	-	-
Sapindaceae	*Matayba laevigata*	tree	-	+	-	-	-	-

Vitaceae	*Vitis riparia*	herb	-	+	-	-	-	-
Moraceae	*Ficus sp*	tree	-	+	-	-	-	-
Araucariaceae	*Araucaria sp*	tree	-	+	-	-	-	-
Rosaceae	*Photinia sp*	tree	-	-	-	+	-	-
	Potentilla sp	shrub	-	-	-	+	-	-
Theaceae	*Camellia mingii*	tree	-	-	-	+	-	-
Asteraceae	*Parthenium hysterophorus*	herb	-	-	-	+	-	-
Trebouxiaceae	*Trebouxia sp*	green algae	-	-	-	+	-	-
Lauraceae	*Lindera sp*	tree	-	-	-	+	-	-
Acanthaceae	*Ruttya sp*	shrub	-	-	-	-	-	+