

# Plant nutrient quality impacts survival and reproductive fitness of the dengue vector *Aedes aegypti*

Vincent Odhiambo Nyasembe (✉ [vnyasembe@gmail.com](mailto:vnyasembe@gmail.com))

International Centre for Insect Physiology and Ecology <https://orcid.org/0000-0002-8018-5687>

David Poumo Tchouassi

International Centre for Insect Physiology and Ecology

Martha Njeri Muturi

Kenya Medical Research Institute - Wellcome Trust

Christian W. W. Pirk

University of Pretoria

Catherine L. Sole

University of Pretoria

Baldwyn Torto

International Centre for Insect Physiology and Ecology

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## Research

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# Abstract

**Background:** In a recent study using DNA barcoding, we identified the plants fed upon by four Afro-tropical mosquito species that vector dengue, malaria, and Rift Valley fever. Herein, we have expanded on this study by investigating the role of three of the plants *Pithecellobium dulce* (Fabaceae), *Leonotis nepetifolia* (Lamiaceae), and *Opuntia ficus-indica* (Cactaceae) on survival, fecundity, and egg viability of the dengue vector *Aedes aegypti*.

**Methods:** We tested these effects using females that received a) an initial three rations of bloodmeal, and b) received no bloodmeal at all. Two controls were included; age-matched females fed on glucose solution with or without initial bloodmeal, and those fed exclusively on bloodmeal. Data was collected daily over a 30-day period. The amino acid content of *Ae. aegypti* guts and the amino acid content of their respective diets was detected by coupled liquid chromatography – mass spectrometry.

**Results:** Females fed on *P. dulce* and exclusive bloodmeal had a shorter survival than those fed on glucose. On the other hand, females fed on *L. nepetifolia* survived longer than those fed exclusively on bloodmeal, whereas those fed on *O. ficus-indica* had the shortest survival time. With initial bloodmeal, females fed on *L. nepetifolia* laid 1.6-fold more eggs while those fed on the other diets laid fewer eggs, compared to those fed exclusively on bloodmeal. Hatching rates of the eggs laid varied with the diet. Mass spectroscopic analysis of gut contents of mosquitoes exposed to the different diets showed qualitative and quantitative differences in their amino acid levels.

**Conclusion:** Our findings highlight the central role of plant nutrients in the reproductive fitness of dengue vectors which may impact their disease transmission potential.

## Background

The last two decades has seen the resurgence and spread of arboviruses such as dengue, Zika and chikungunya viruses that are vectored by *Aedes* mosquitoes. Although the number of dengue cases are underreported and misclassified [1], the number of global infections is estimated at 390 million annually, with about 50 - 100 million cases manifesting clinically [2,3]. The geographic expansion of dengue, which is caused by four dengue virus serotypes (DENV 1-4), has been characterized by increase in case incidence, epidemics and super-endemicity, with more frequent severe forms of dengue [4,5]. The recent outbreak of Zika in the South and Central America, and the Caribbean region attests further to the continued geographic spread of arboviral diseases [6,7]. Zika was first identified in *Rhesus* monkey in Uganda in 1947, with the first human cases detected in 1952 in Uganda and Tanzania [8]. The Zika outbreaks in Brazil and Colombia in the year 2015, and its subsequent spread to 13 other countries in the Americas, along with other outbreaks in the Pacific (Yap, 2007; French Polynesia, 2013) and Africa (Cape Verde, 2015), highlights the growing concern of the rapid expansion of arboviral diseases [9]. The overarching commonality among these diseases is that there are no specific drugs currently for their

treatment and no viable vaccines available [10]. This makes effective vector control the mainstay for prevention and control of these diseases.

The geographic expansion of these diseases closely follows the tropical and subtropical distribution of their primary vectors, *Aedes aegypti* and *Aedes albopictus*. The spread has been attributed to a range of factors including climate change, uncontrolled urbanization, globalization, travel, trade, socioeconomics and the ability of these viruses to evolve [5,11]. In addition, factors contributing to the resilience of *Ae. aegypti* populations such as insecticide resistance, the ability of eggs to withstand desiccation, ability of adults to adapt to environmental modifications and their behavioral plasticity have contributed to the sustenance or even expansion of *Ae. aegypti* populations [12,13]. Overall, the continued expansion of the geographic range of these mosquito species and the pathogens that they transmit calls for a detailed understanding of vector and disease ecologies in the renewed effort for innovative management strategies.

Plant feeding is emerging as a key ecological factor in the biology of several mosquito species including *Aedes* species [14–17]. While plant feeding pre-dates blood feeding in insects, blood sucking arthropods are thought to have adopted the latter trait during evolution to enhance the propagation of their progeny [18]. Among different mosquito species, intermittent plant feeding in females has long been documented but its role with respect to reproductive fitness has been downplayed by different studies [19–22]. Central to this dogma are *Ae. aegypti* and *Anopheles gambiae*, the two highly anthropophilic and most important disease vectors. Variably low fructose levels detected in field collected females of these species accompanied by their tendency to have multiple blood meals has led to the proposition that they seldom feed on plants but depend on human blood for both their metabolic processes and reproduction. However, recent evidence where more sensitive trapping strategies and analytical approaches were used shows higher plant feeding frequencies in these two species [16,17,23].

Several studies have demonstrated the central role played by plant sugars in male and female mosquito survival, mating competence and flight activity. In addition, there has been substantial effort to identify plant species fed upon by different mosquito species. These efforts have greatly been boosted by the advent of highly sensitive analytical techniques such as plant DNA barcoding and mass spectrometry which provide secure host plant identification and authenticate their trophic association [24,25]. Molecular approaches have recently been used to identify plant species fed upon by important disease vectors such as *An. sergentii* [23], *An. gambiae*, *Ae. mcintoshi*, *Ae. ochraceus* and *Ae. aegypti* [16], and phlebotomine sand flies [26,27] in their natural habitats. Evidence of more frequent plant feeding among these vectors and the identification of host plant species further augment the proposition of their central role in vector population dynamics. However, beyond a few studies linking plant feeding to mosquito survival, little is known about the nutritional contribution of plants to vector fitness and population dynamics.

Building on our recent identification of natural host plants of four Afro-tropical mosquito species [16], we sought to elucidate the role of plant nutrition using three of the identified plants on survival and

reproductive fitness of *Ae. aegypti*.

## Methods

### Experimental animals

Adult *Ae. aegypti* obtained from eggs collected in Kilifi (3.6333° S and 39.8500° E) in the coastal region of Kenya endemic for dengue (Sang and Dunster, 2001) were used. The eggs were collected by placing black ovicups lined with brown ovistrips in pre-identified *A. aegypti* breeding sites overnight. The collected eggs were either hatched immediately or carefully dried and transported to *icipe* laboratories in Nairobi. The hatching larvae were reared in plastic trays (25 cm long × 20 cm wide × 14 cm high) to adults with a daily ration of Tetramin fish food (Tetramin1, Melle, Germany) of 0.3 g /100 larvae/day. The rearing room was maintained at a temperature of 28 (±1) °C and relative humidity of 80 (±5) % and a photoperiod of 12: 12 (light: dark) hours. The haplotype of the emerging adults was all confirmed to be *Ae. aegypti aegypti* (hereafter referred to as *Ae. aegypti*) as they all had white scales on the first abdominal tergite (McClelland, 1960). The adults, 1-2 days old with no prior exposure to any other nutrient source, were used in survival and fecundity assays.

### Plant materials

Plant species identified as natural host plants of the four mosquito species from our previous study [16] were used in these assays although specific for the vectors from their respective ecologies. These included *Pithecellobium dulce* (Roxb.) Benth (Fabaceae; *Ae. aegypti* host plant), *Leonotis nepetifolia* (L.) R.Br (Lamiaceae; *An. gambiae* host plant) and *Opuntia ficus-indica* (L.) Mill (Cactaceae; *Ae. mcintoshi* and *Ae. ochraceus* host plant).

Studies with *P. dulce* were conducted at the KEMRI-Wellcome Trust laboratories in Kilifi Kenya (3.6333° S and 39.8500° E), where both *Ae. aegypti* and its host plant *P. dulce* co-occur. *P. dulce* is a perennial tree reaching a height of about 10 – 15 m hence we were not able to obtain it as a potted plant. Consequently, fresh cuttings of its leaves, newly blossomed flowers and young pods were used. These were changed daily over the 30-day experimental period.

Studies with *L. nepetifolia* and *O. ficus-indica* were conducted at *icipe* in Nairobi. Wild growing *L. nepetifolia* (obtained from Ahero, western Kenya; 0°10'S, 34°55'E) and *O. ficus-indica* (obtained from Ijara, northeastern Kenya; 1.5988° S and 40.5135° E), were transplanted into pots (D 25 x W 27 x H 30 cm), and transported to *icipe* laboratories in Nairobi. They were used when they started to blossom.

All the experiments were conducted under controlled conditions as described above for mosquito rearing.

### Survival, fecundity, and egg hatchability of *Aedes aegypti* on different host plants

In Experiment I carried out in Kilifi, two assays were conducted. In the first assay, a group of 100 males and 100 females were introduced into a 30 x 30 x 100 cm cage containing *P. dulce* cuttings. In addition to the plant, which was continuously available, they were provided with initial three mice blood meals at day 3, 5 and 7 from the onset of the assay (Fig. 1, Assay 1). The blood meal was provided by placing anaesthetized mouse on top of the mosquito cages and the mosquitoes allowed to feed on them for an hour. Oviposition cups were provided in all the cages 48 h after the first bloodmeal. They were monitored for survival and fecundity daily for 30 days. Mortality and the daily number of eggs laid were recorded and fresh oviposition cups provided. Control experiments comprised 100 females and 100 males *Ae. aegypti* with access to a) 6% glucose solution (the concentration routinely used in mosquito rearing at *icipe*) plus three initial blood meals (Fig. 1, Assay 1) and b) blood meals only on alternating days for thirty days with a total of 15 blood meals. Nine living female mosquitoes were randomly selected from each replicate of all the treatments on day 15 for amino acid analysis as described below. The choice of day 15 for nutrient analysis was informed by the need to allow enough time for all blood-derived nutrients to be absorbed/cleared from the mosquito gut and increase detection of plant derived nutrients. The second assay was the same as the one above, but no blood meal was provided (Fig.1, Assay 2). Nine living female mosquitoes were randomly selected from each replicate of all the treatments on day 15 for amino acid and sugar analysis as described in Additional file 1. A total of three replicates using three different batches of mosquitoes were carried out for all nutrient regimes for both assays.

Similar experimental set up as above was used in Experiment II for survival and fecundity assays using *L. nepetifolia* and *O. ficus-indica*. Newly emerged females and males (100 mosquitoes for each sex) were provided with either *L. nepetifolia*, *O. ficus-indica*, 6 % glucose solution or mice bloodmeal provided on alternate days. Except for the latter group, the mosquitoes in all the other three groups were either provided an initial three mice bloodmeals on days 3, 5, and 7 (Fig. 1, Assay 1), or no bloodmeal at all (Fig. 1, Assay 2). Survival, fecundity, and mosquito sampling for nutrient analysis was done as described above. The collected eggs were wrapped between moist paper towels and put in covered plastic trays for 48 h then transferred to a dark container and allowed to dry slowly under rearing conditions (temperature of 28 ( $\pm$ 1) °C and relative humidity of 80 ( $\pm$ 5) %).

To measure the hatchability of the laid eggs, the eggs were put in 18 x12.5 x 2.1-inch trays and distilled water added to a depth of one inch. The water was first boiled then allowed to cool to before introducing the eggs. The eggs were hatched according to the date laid and nutrient source. The number of larvae were counted daily for up to two weeks after which the unhatched eggs were considered not viable. The counted larvae were promptly removed.

### **Analysis of host plant nutrient content and the ingested equivalence in *Aedes aegypti***

To understand the differences in the performance of *Ae. aegypti* on different nutrient sources, we quantified the amount of amino acids in the three plant species and the corresponding amounts ingested by the mosquitoes. Both plant sap from phloem in the succulent tissues and nectar from the nectaries were collected from the three plants using 20  $\mu$ l micro-capillary tubes (Drummond Scientific Company,

Brumall, PA, USA) tapered on one end using glass puller. Between 10-30  $\mu$ l of plant sap and nectar were separately collected, transferred into 1.5 ml low-binding eppendorf tubes, snap frozen in liquid nitrogen and immediately stored at -80 °C until analysis. Up to 100  $\mu$ l of venous blood was drawn from mouse facial vein into 1.5 ml low-binding eppendorf tubes and immediately snap-frozen in liquid nitrogen and stored at -80 °C. Blood was collected from three mice drawn from different litters for both experiments conducted at KEMRI-Wellcome Trust laboratories and *icipe*. The mosquito samples were prepared by dissecting mosquitoes preserved from survival assays and pooling the midgut plus crop (hereafter referred to as gut) from three mosquitoes.

To detect amino acid content, the pooled guts, 10  $\mu$ l of plant sap + nectar or 10  $\mu$ l of blood samples were hydrolyzed with 6 M HCl for three hours as described by Moran-Palacio *et al* (2014). The product was re-suspended in 200  $\mu$ l mmol 1-1 EDTA solution, pH 7.5 and incubated for 90 min in the dark in a sealed chamber equilibrated at 25 °C with a dish of saturated  $\text{KH}_2\text{PO}_4$  to maintain high humidity. The EDTA samples were subsequently diluted in water - acetonitrile solvent mixture in a ratio of 80:20 and analyzed on a liquid chromatography attached to 6120 quadrupole mass spectrometer Agilent Technologies. Samples were injected via Agilent Technology 1260 Infinity series sample manager, injecting 10  $\mu$ l on to Agilent SB-C18 3.5  $\mu$ m 4.6 x 250 mm column. The run time was 22 min at a flow rate of 0.7 ml/min. The solvent system consisted of A (water + 1% formic acid) and B (acetonitrile + 1% formic acid). The mobile phase used a gradient program, initially 95:5 (A: B), to 70:30 at 3 min, 20:80 at 7.5 min, 0:100 at 13 min, 95:5 at 20 min. The mass spectrometer was operated in positive ion mode, with a capillary voltage of 3 KV, con voltage 50-180 eV, mass range 50-300 *m/z*. The source temperature was 130 °C, desolvation temperature 350 °C, desolvation gas flow 100 ml/min (nitrogen) and con gas flow 0.7 ml/min (nitrogen). The amino acids were identified by comparing their mass spectra with literature data [28].

The sugar content of guts from males and non-blood fed female *Ae. aegypti* fed on different nutrients were analyzed as described in supplementary information (Additional file 1)

## Statistical analyses

For each nutrient regime, survival data from the three replicates was first subjected to GLM to determine if they could be pooled. Since no difference was detected, data from the three replicates were pooled and the difference in survival times of adult *Ae. aegypti* on different nutrient sources detected using Kaplan-Meier and Cox regression survival analyses. Mosquitoes sampled for nutrient analyses and those surviving after the 30-days observation period were treated as censored. The number of eggs laid on each day was corrected to the number of surviving females and differences in fecundity between mosquitoes held on different nutrient sources were detected using zero-inflated GLM. The differences in hatching rate from different nutrient sources were compared using one-way ANOVA and Tukey Post Hoc test. The gut amino acid content was quantified for the different nutrient sources and the differences detected using one-way analysis of variance. The differences in gut sugar content was detected using ANOVA and Turkey Post Hoc test. All statistical analyses were done in R software version 3.6.3 [29].

# Results

## Host plants variably support *Ae. aegypti* survival, fecundity, and egg viability

In Experiment I, *Ae. aegypti* fed on glucose survived significantly longer (23.5 days) than those fed on *P. dulce* and blood (17.7 days) or blood alone (16.1 days) (Log rank = 40.785, df = 2,  $p < 0.001$ ; **Fig. 2a**). With no initial bloodmeal, the mean survival of female *Ae. aegypti* on glucose and *P. dulce* were  $23.6 \pm 0.8$  and  $13.1 \pm 0.8$  days, respectively (Log rank = 48.04, df = 1,  $p < 0.001$ ; **Fig. 2b**). Similar survival patterns were observed in males, with those fed on glucose having a mean survival of  $21.6 \pm 0.7$  days while those fed on *P. dulce* had a median survival of  $14.6 \pm 0.7$  days (Log rank = 25.162, df = 1,  $p < 0.001$ ; **Fig. 2c**).

In Experiment II, survival of *Ae. aegypti* females provided with an initial bloodmeal and fed on the different diets was significantly different (Log rank = 419.727, df = 3,  $P$ -value  $< 0.001$ ; **Fig. 2d**), with mean survival of  $24.0 \pm 0.6$ ,  $17.0 \pm 0.4$ ,  $7.2 \pm 0.4$  and  $16.1 \pm 0.8$  days among glucose, *L. nepetifolia*, *O. ficus-indica* and blood, respectively. With no initial bloodmeal, the mean survivals on glucose solution, *L. nepetifolia* and *O. ficus-indica* were  $23.9 \pm 0.7$ ,  $25.2 \pm 0.5$  and  $7.4 \pm 0.2$  days for females (Log rank = 485.405, df = 2,  $P$ -value  $< 0.001$ ; **Fig. 2e**) and  $21.0 \pm 0.6$ ,  $14.5 \pm 0.5$  and  $6.5 \pm 0.4$  days for males (Log rank = 354.639, df = 2,  $P$ -value  $< 0.001$ ; **Fig. 2f**), respectively.

For fecundity, females fed on *P. dulce* and glucose laid 1.6- and 2.2-fold less eggs, respectively, than those fed exclusively on blood but no significant difference was detected ( $F_{(2, 267)} = 1.985$ ,  $I = 0.139$ ). On the other hand, those fed on *L. nepetifolia* laid 1.6-fold more eggs than those fed exclusively on blood, while mosquitoes fed on *O. ficus-indica* and glucose had 1.7- and 2-fold less eggs than those fed exclusively on bloodmeal, respectively ( $F_{(3, 356)} = 3.495$ ,  $p = 0.0158$ ). Besides having the highest fecundity rate, mosquitoes fed on *L. nepetifolia* had a sustained moderate oviposition throughout the experimental period which was comparable to those that exclusively fed on bloodmeal (**Fig. 3a and b**).

Regarding egg viability, marginal significant differences were detected in the median hatching rates of eggs laid by mosquitoes fed on glucose (0.10), *P. dulce* (0.0.16) and blood (0.65) ( $F_{(2, 31)} = 3.344$ ,  $p = 0.0484$ ; **Fig. 4a**). On the other, a significant difference was detected in hatching rates of eggs from Experiment II ( $F_{(3, 40)} = 4.221$ ,  $p = 0.011$ ); with median hatching rates of 0.13, 0.30, 0.06 and 0.64 for eggs from females fed on glucose, *L. nepetifolia*, *O. ficus-indica* and bloodmeal, respectively, (**Fig. 4b**).

## Variable nutrient quality support observed differences in the fitness matrix of *Ae. aegypti* fed on different host plants

A total of 12 amino acids present in mice blood were detected in variable amounts in the sap plus nectar of three plant species. These included valine, serine, glutamine, proline, glycine, methionine, tyrosine, isoleucine, leucine, phenylalanine, tryptophan, and arginine. Uniquely abundant amino acids detected in the guts of mosquitoes fed on the nutrient regimes included valine, arginine, isoleucine, methionine, and phenylalanine (**Fig. 5a**). Valine was 5-, 13- and 14-fold more abundant in the guts of mosquitoes fed on *L. nepetifolia*, glucose and *O. ficus-indica*, respectively. In addition, arginine was abundant in the guts of

those fed on *O. ficus-indica*, isoleucine in those fed on *P. dulce*, and methionine in those fed on *L. nepetifolia* (Fig. 5b). On the other hand, phenylalanine was 6-, 8- and 11-fold less abundant in the guts of mosquitoes fed on *P. dulce*, *O. ficus-indica* and glucose solution, respectively, relative to those exclusively fed on blood (Fig. 5b). Notably, glutamic acid was present in the guts of mosquitoes fed on mice blood but absent in those from all the other diets. To further confirm that female *Ae. aegypti* indeed were able to imbibe these amino acids from their host plants, we analyzed four of the identified amino acids in the guts of non-blood fed mosquitoes. Besides valine, none of the females from glucose diet had any detectable amino acids in their guts. However, females fed on all the three host plants had variable amounts of methionine, isoleucine, phenylalanine, and arginine in their guts (Fig. 5c).

No significant differences were detected in the amount of sugar ingested by male *Ae. aegypti* ( $F_{(3,93)} = 1.389$ ,  $p = 0.251$ ; Fig. S1). However, females fed on *L. nepetifolia* imbibed significantly higher amount of sugar compared to those fed on *O. ficus-indica* ( $F_{(3,93)} = 2.791$ ,  $p = 0.0449$ ; Fig. S1).

## Discussion

Our findings show that the three plants used in this study differentially impact on the survival and reproductive fitness of dengue vector, *Ae. aegypti*. We previously reported a sugar feeding frequency of 17% in female *Ae. aegypti* collected around vegetations in the coastal Kenya [16]. The study by Olson et al. [17] demonstrated that sugar feeding occurs at a much higher frequency than previously reported, with collection method and season being important in influencing the proportion of fructose-positive females captured. Plant sugars, particularly fructose, have been shown to provide a ready source of energy for various metabolic processes in several mosquito species [17,30–33]. Extended survival time is pivotal in the transmission of vector-borne diseases as it guarantees completion of extrinsic incubation of the causative agents and increases the chances of multiple infective vertebrate host bites [34]. These findings further reinforce the argument of the central role played by plants in the biology of *Ae. aegypti*, contrary to previous beliefs [19 – 22].

While no eggs were laid by mosquitoes exclusively fed on the three plant species, significant difference in fecundity were observed when mosquitoes fed on them with initial bloodmeal rations. Those fed *L. nepetifolia* had slightly higher oviposition than their exclusively blood-fed counterparts, while those fed on *P. dulce*, *O. ficus-indica* and glucose laid fewer eggs than blood fed females. Similar impacts of plant diets on mosquito fecundity have been observed for *An. gambiae* [30,35] and *Culex pipiens* [33]. Although sugar feeding has long been suggested to impact fecundity of *Aedes* mosquitoes [36,37], to the best of our knowledge, this is the first evidence directly linking plant feeding to *Ae. aegypti* fecundity. Plant nectars have been shown to increase mating competence in males of different mosquito species [31,32,38,39]. Sugar has also been shown to be important in inducing egg development in autogenous *Ae. albopictus* and *Cx. pipiens f. molestus* [40,41]. The failure of *Ae. aegypti* to lay eggs without initial bloodmeal in this study is not surprising, although varying degrees of autogeny has been reported among these species in East Africa [42,43]. However, the potential of *L. nepetifolia* to not only boost their overall fecundity but also induce a sustained oviposition long after the last bloodmeal is

noteworthy. Although lower than the hatching rates in eggs from exclusively blood-fed females, a good proportion of eggs laid by females fed on *L. nepetifolia* were viable. The difference in fecundity of mosquitoes held on different nutrient sources observed in this study can be explained under three propositions: 1) males fed on *L. nepetifolia* had sugar-rich diet and therefore increased mating competence and reproductive output in females, compared to males from the exclusive bloodmeal diet which died off within three days; 2) females held on *L. nepetifolia* imbibed sufficient sugar meals/sap from the succulent plant tissues thereby resulting in constant distention of the abdomen and inducing oocyte maturation following initial bloodmeal, as has been reported in the case of *Ae. albopictus* [40]; and 3) mosquitoes feeding on the three plants, especially on *L. nepetifolia*, imbibed not only sugar but also amino acids which supplemented those received from the initial bloodmeal in further boosting their reproduction.

We explored the third proposition further by analyzing amino acid content of female *Ae. aegypti* held on these plant species and comparing the outputs with those from mosquitoes fed exclusively on blood and their plant sources. A total of 12 amino acids present in mice blood were positively identified in the plant sap/nectar and the guts of blood-fed females held on different diets in varying proportions. Notably, mosquitoes held on *L. nepetifolia* had high methionine content in their guts, while those held on *P. dulce* had high isoleucine content. Intriguingly, phenylalanine was significantly low in females held on *P. dulce*, *O. ficus-indica* and glucose solution, with mosquitoes fed on the three plant species and glucose lacking glutamic acid. Non-blood fed females held on the three plant species had similar amino acid profiles as those offered initial bloodmeal. These observations support our proposition that female *Ae. aegypti* imbibed variable amounts of amino acids from these plant species which differentially impacted their fecundity. This was further supported by the detection of these amino acids in the respective host plants the mosquitoes were held on. Different amino acids have been shown to impact differently on mosquito fecundity. Phenylalanine and tyrosine have been shown to be important for the development and tanning of *An. gambiae* and *Ae. aegypti* eggs [44,45], while isoleucine is important in follicular maturation and preventing egg resorption in *Ae. aegypti* [46]. Methionine and leucine have been shown to increase fecundity of green pea aphid, *Cyrtosiphon pisum*, by enhancing target of rapamycin (TOR) signalling pathway [47]. This study represents the first empirical evidence of the possible involvement of plant-derived amino acids in the reproductive fitness of *Ae. aegypti*.

We acknowledge that the study has some limitations that warrant further consideration: 1) in this study, individual females which blood-fed were not separated from those that did not blood-feed 2) we did not include a control with sugar continuously available and blood meal provided every other day to allow males survive longer and improve their mating competence. 3) the use of plant cuttings in the assays with *P. dulce*, and whole potted plants in the case of *L. nepetifolia* and *O. ficus-indica*, could have contributed to the observed differences in the performance of *Ae. aegypti*. Although *L. nepetifolia* significantly improved survival and fecundity of *Ae. aegypti*, it was not identified as a natural host plant of this species in our previous study and is not found within the locality where their eggs were collected. However, it serves as an important pointer to the potential of other plants with similar nutritional value to impact mosquito vector populations.

## Conclusion

We conclude that these findings offer significant insight into the role of plant-derived nutrients in the biology and population dynamics of *Ae. aegypti*. Our study provides the empirical evidence that plant-derived amino acids such as phenylalanine, methionine, isoleucine and arginine could play a role in sustaining reproductive fitness of these disease vectors in situations where vertebrate hosts are scarce and only a few bloodmeal encounters are possible.

## Declarations

### Ethical approval and Consent to participate

Mice (BALB/c strain) used for mosquito blood feeding in these experiments were supplied by *icipé* Animal Rearing and Containment Unit. All blood feeding experiments were conducted according to IACUC approved protocols. Approval for the study sought from Kenya Medical Research Institute Scientific and Ethics Review Unit (KEMRI-SERU) (Project Number SERU 2787).

Consent to participate was not required.

### Consent for Publication

Not applicable.

### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interest

The authors declare that they have no competing interest.

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### Author contribution

Conceived and designed the experiments: VON DPT CLS CP BT. Performed the experiments: VON DPT MNM BT. Analyzed the data: VON DPT BT. Wrote the paper: VON DPT CLS CP BT. All authors approved

the final version for submission.

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## Author information

Not applicable

## References

### References

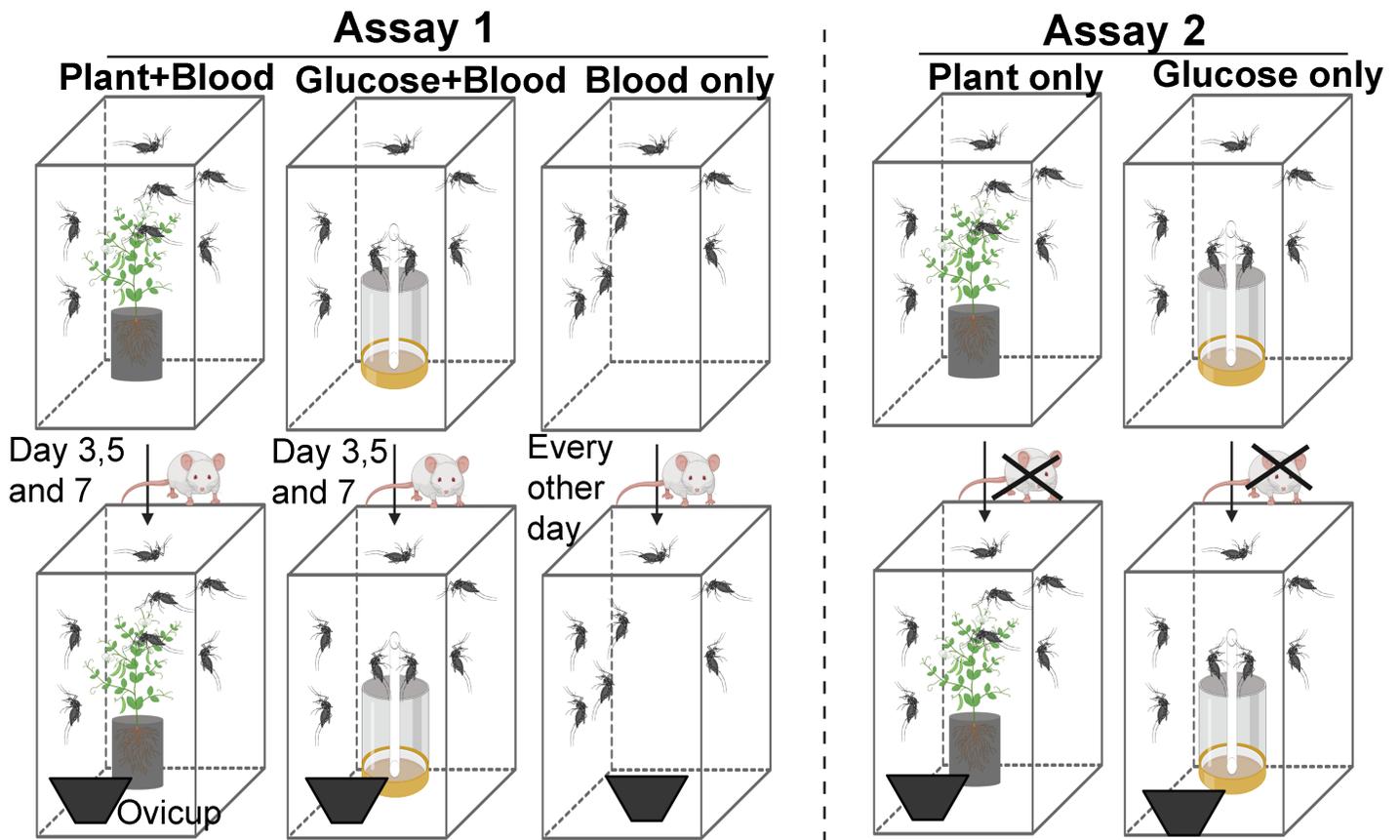
1. Jaensich T, Junghanss T, Wills B, Brady OJ, Eckerle I, Farlow A, et al. Dengue expansion in Africa - not recognized or not happening? *Emerg Infect Dis*. 2014;20:e140487.
2. Guo C, Zhou Z, Wen Z, Liu Y, Zeng C, Xiao D, et al. Global epidemiology of dengue outbreaks in 1990 - 2015: A systematic review and meta-analysis. *Front Cell Infect Microbiol*. 2017;7:317.
3. World Health Organization. Dengue and severe dengue. World Health Organization. 2020 [cited 2020 May 21]. Available from: <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue>
4. Caron M, Grard G, Paupy C, Mombo IM, Bikie Bi Nso B, Kassa Kassa FR, et al. First evidence of simultaneous circulation of three different dengue virus serotypes in Africa. *PLoS ONE*. 2013;8:e78030.
5. Guzman MG, Harris E. Dengue. *The Lancet*. 2015;385:453–65.
6. Attar N. ZIKA virus circulates in new regions. *Nat Rev Microbiol*. 2016;14:62–62.
7. Fauci AS, Morens DM. Zika virus in the Americas - Yet another arbovirus threat. *N Engl J Med*. 2016;374:601–4.
8. Dick GWA, Kitchen SF, Haddow AJ. Zika Virus (I). Isolations and serological specificity. *Trans Royal Soc Trop Med Hyg*. 1952;46:509–20.
9. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika Virus. *N Engl J Med*. 2016;374:1552–63.
10. Benelli G, Mehlhorn H. Declining malaria, rising of dengue and Zika virus: insights for mosquito vector control. *Parasitol Res*. 2016;115:1747–54.
11. Murray NEA, Quam MB, Wilder-Smith A. Epidemiology of dengue: past, present and future prospects. *CLEP*. 2013;5:299–309.
12. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *eLIFE*. 2015;4:e08347.
13. Ebi KL, Nealon J. Dengue in a changing climate. *Environ Res*. 2016;151:115–23.

14. Foster WA. Mosquito sugar feeding and reproductive energetics. *Annu Rev Entomol.* 1995;40:443–74.
15. Nyasembe VO, Torto B. Volatile phytochemicals as mosquito semiochemicals. *Phytochem Lett.* 2014;8:196–201.
16. Nyasembe VO, Tchouassi DP, Pirk CWW, Sole CL, Torto B. Host plant forensics and olfactory-based detection in Afro-tropical mosquito disease vectors. *PLoS Negl Trop Dis.* 2018;12:e0006185.
17. Olson MF, Garcia-Luna S, Juarez JG, Martin E, Harrington LC, Eubanks MD, et al. Sugar feeding patterns for *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) mosquitoes in South Texas. *J Med Entomol.* 2020;1–9.
18. Graca-Souza A, Maya-Monteiro C, Paivasilva G, Braz G, Paes M, Sorgine M, et al. Adaptations against heme toxicity in blood-feeding arthropods. *Insect Biochem Mol Biol.* 2006;36:322–35.
19. Scott TW, Naksathit A, Day JF, Kittayapong P, Edman JD. A fitness advantage for *Aedes aegypti* and the viruses it transmits when females feed only on human blood. *Amer J Trop Med Hyg.* 1997;57:235–9.
20. Costero A, Edman JD, Clark GG, Scott TW. Life table study of *Aedes aegypti* (Diptera: Culicidae) in Puerto Rico fed only human blood versus blood plus sugar. *J Med Entomol.* 1998;35:809–13.
21. Naksathit AT, Edman JD, Scott TW. Partitioning of glycogen, lipid, and sugar in ovaries and body remnants of female *Aedes aegypti* (Diptera: Culicidae) fed human blood. *J Med Entomol.* 1999;36:18–22.
22. Harrington LC, Edman JD, Scott TW. Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? *J Med Entomol.* 2001;38:411–22.
23. Junnila A, Müller GC, Schlein Y. Species identification of plant tissues from the gut of *An. sergentii* by DNA analysis. *Acta Tropica.* 2010;115:227–33.
24. Jurado-Rivera JA, Vogler AP, Reid CAM, Petitpierre E, Gómez-Zurita J. DNA barcoding insect - host plant associations. *Proc R Soc B.* 2009;276:639–48.
25. Jinbo U, Kato T, Ito M. Current progress in DNA barcoding and future implications for entomology: DNA barcoding for entomology. *Entomol Sci.* 2011;14:107–24.
26. Miller MA, Müller GC, Kravchenko VD, Vernon KK, Matheson CD, Hausmann A. DNA-based identification of Lepidoptera larvae and plant meals from their gut contents. *Russian Entomol J.* 2006;15:427–32.
27. Abbasi I, Trancoso Lopo de Queiroz A, Kirstein OD, Nasereddin A, Horwitz BZ, Hailu A, et al. Plant-feeding phlebotomine sand flies, vectors of leishmaniasis, prefer *Cannabis sativa*. *Proc Natl Acad Sci USA.* 2018;115:11790–5.
28. Özcan S, Şenyuva HZ. Improved and simplified liquid chromatography/atmospheric pressure chemical ionization mass spectrometry method for the analysis of underivatized free amino acids in various foods. *J Chromatogr A.* 2006;1135:179–85.

29. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from:<https://www.R-project.org/>
30. Manda H, Gouagna LC, Foster WA, Jackson RR, Beier JC, Githure JI, et al. Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. *Malar J*. 2007;6:113.
31. Gary RE, Cannon JW, Foster WA. Effect of sugar on male *Anopheles gambiae* mating performance, as modified by temperature, space, and body size. *Parasites Vectors*. 2009; 2:19.
32. Bellini R, Puggioli A, Balestrino F, Brunelli P, Medici A, Urbanelli S, et al. Sugar administration to newly emerged *Aedes albopictus* males increases their survival probability and mating performance. *Acta Tropica*. 2014;132:S116–23.
33. Yu B-T, Ding Y-M, Mo X-C, Liu N, Li H-J, Mo J-C. Survivorship and fecundity of *Culex pipiens pallens* feeding on flowering plants and seed pods with differential preferences. *Acta Tropica*. 2016;155:51–7.
34. Vaidyanathan R, Fleisher AE, Minnick SL, Simmons KA, Scott TW. Nutritional stress affects mosquito survival and vector competence for West Nile virus. *Vector-Borne and Zoonotic Diseases*. 2008;8:727–32.
35. Impoinvil DE, Kongere JO, Foster WA, Njiru BN, Killeen GF, Githure JI, et al. Feeding and survival of the malaria vector *Anopheles gambiae* on plants growing in Kenya. *Med Vet Entomol*. 2004;18:108–15.
36. Nayar JK, Sauerman Jr DM. The Effects of nutrition on survival and fecundity in Florida mosquitoes Part 3. Utilization of blood and sugar for fecundity1. *J Med Entomol*. 1975;12:220–5.
37. Anderson HI. The effect of sugar meals and body size on fecundity and longevity of female *Aedes communis* (Diptera: Culicidae). *Physiol Entomol*. 1992;17:203–7.
38. Gouagna L-C, Poueme RS, Dabiré KR, Ouédraogo J-B, Fontenille D, Simard F. Patterns of sugar feeding and host plant preferences in adult males of *Anopheles gambiae* (Diptera: Culicidae). *J Vect Ecol*. 2010;35:267–76.
39. Chadee DD, Sutherland JM, Gilles JRL. Diel sugar feeding and reproductive behaviours of *Aedes aegypti* mosquitoes in Trinidad: With implications for mass release of sterile mosquitoes. *Acta Tropica*. 2014;132:S86–90.
40. Chambers GM, Klowden MJ. Distention and sugar feeding induce autogenous egg development by the Asian tiger mosquito (Diptera: Culicidae). *J Med Entomol*. 1996;33:372–8.
41. Kassim NFA, Webb CE, Russell RC. Is the expression of autogeny by *Culex molestus* Forskal (Diptera: Culicidae) influenced by larval nutrition or by adult mating, sugar feeding, or blood feeding? *J Vect Ecol*. 2012;37:162–71.
42. Trpis M. Autogeny in diverse populations of *Aedes aegypti* from East Africa. *Tropenmedizin und Parasitologie*. 1977;28:77-82.
43. Ariani CV, Smith SCL, Osei-Poku J, Short K, Juneja P, Jiggins FM. Environmental and genetic factors determine whether the mosquito *Aedes aegypti* lays eggs without a blood meal. *Amer J Trop Med Hyg*. 2015.715–21.

44. Li J, Christensen BM. Involvement of l-tyrosine and phenol oxidase in the tanning of *Aedes aegypti* eggs. *Insect Biochem Mol Biol.* 1993;23:739–48.
45. Fuchs S, Behrends V, Bundy JG, Crisanti A, Nolan T. Phenylalanine metabolism regulates reproduction and parasite melanization in the malaria mosquito. *PLoS ONE.* 2014;9:e84865.
46. Chang Y-YH, Judson CL. The role of isoleucine in differential egg production by the mosquito *Aedes aegypti* Linnaeus (Diptera: Culicidae) following feeding on human or guinea pig blood. *Comp Biochem Physiol Part A: Physiol.* 1977;57:23–8.
47. Gao J, Guo H, Sun Y, Ge F. Differential accumulation of leucine and methionine in red and green pea aphids leads to different fecundity in response to nitrogen fertilization. *Pest Manag Sci.* 2018;74:1779–89.

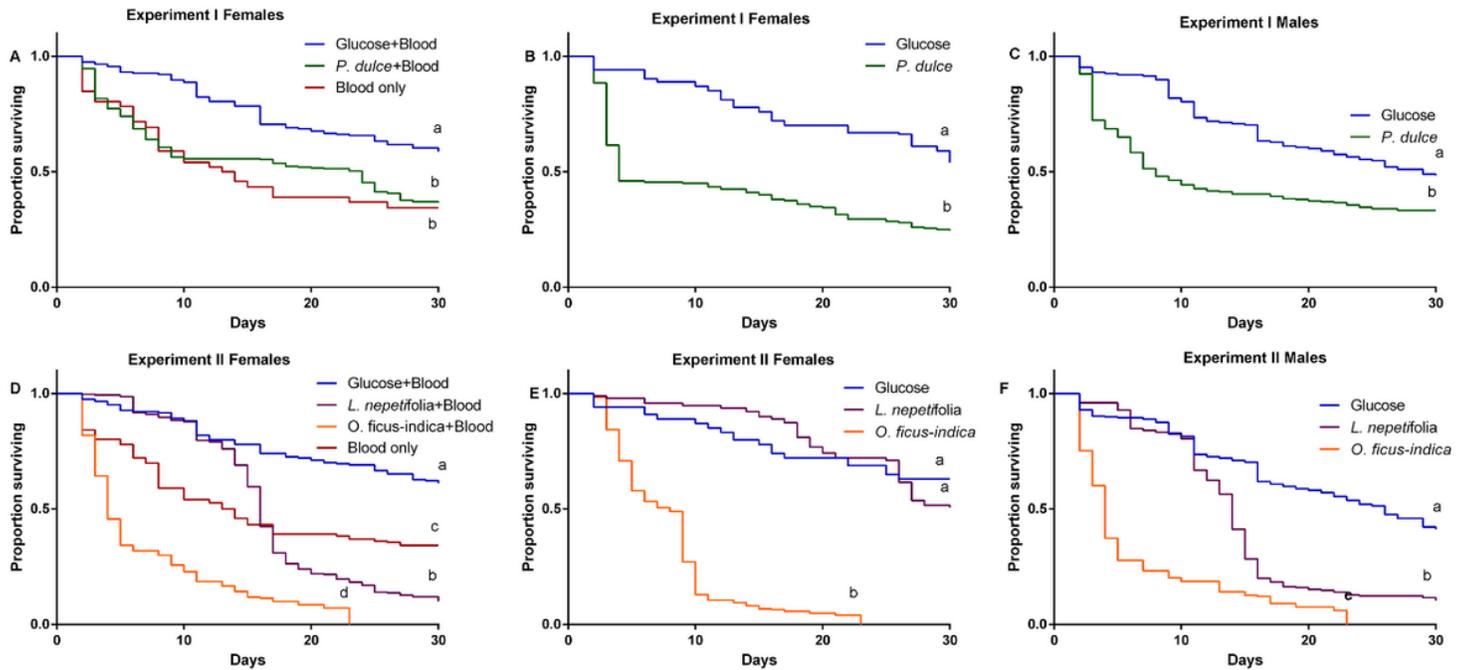
## Figures



**Figure 1**

Schematic representation of the study design. Mosquitoes were either fed on mice blood (Assay 1) or no blood at all (Assay 2). Ovicups were placed in the cages on day 5, monitored daily for eggs over the 30-

day assay period, eggs collected, and the cups replaced with fresh ones every day. Sugar comprised 6% glucose solution. The images were created with BioRender.com.



**Figure 2**

The impact of plant nutrients on *Ae. aegypti* male and female survival. a) Survival curves of female *Ae. aegypti* on *P. dulce*, 6% glucose solution and exclusive mice blood, with initial 3 bloodmeal rations offered to those held on *P. dulce* and glucose solution (P-value < 0.001). (b - c) Survival curves of female and male *Ae. aegypti*, respectively, fed on *P. dulce* and glucose without initial bloodmeal (P-value < 0.001). d) Survival curves of females held on *L. nepetifolia*, *O. ficus-indica*, 6% glucose solution and exclusive mice blood, with the former three offered three initial bloodmeal rations (P-value < 0.001). (e - f) Survival curves of female and male *Ae. aegypti*, respectively, fed on *L. nepetifolia*, *O. ficus-indica* or glucose solution without initial bloodmeal (P-value < 0.001). Survival curves denoted with different letters are significantly different. Differences in survival curves was detected using Kaplan Meyer analysis and Cox regression analyses.

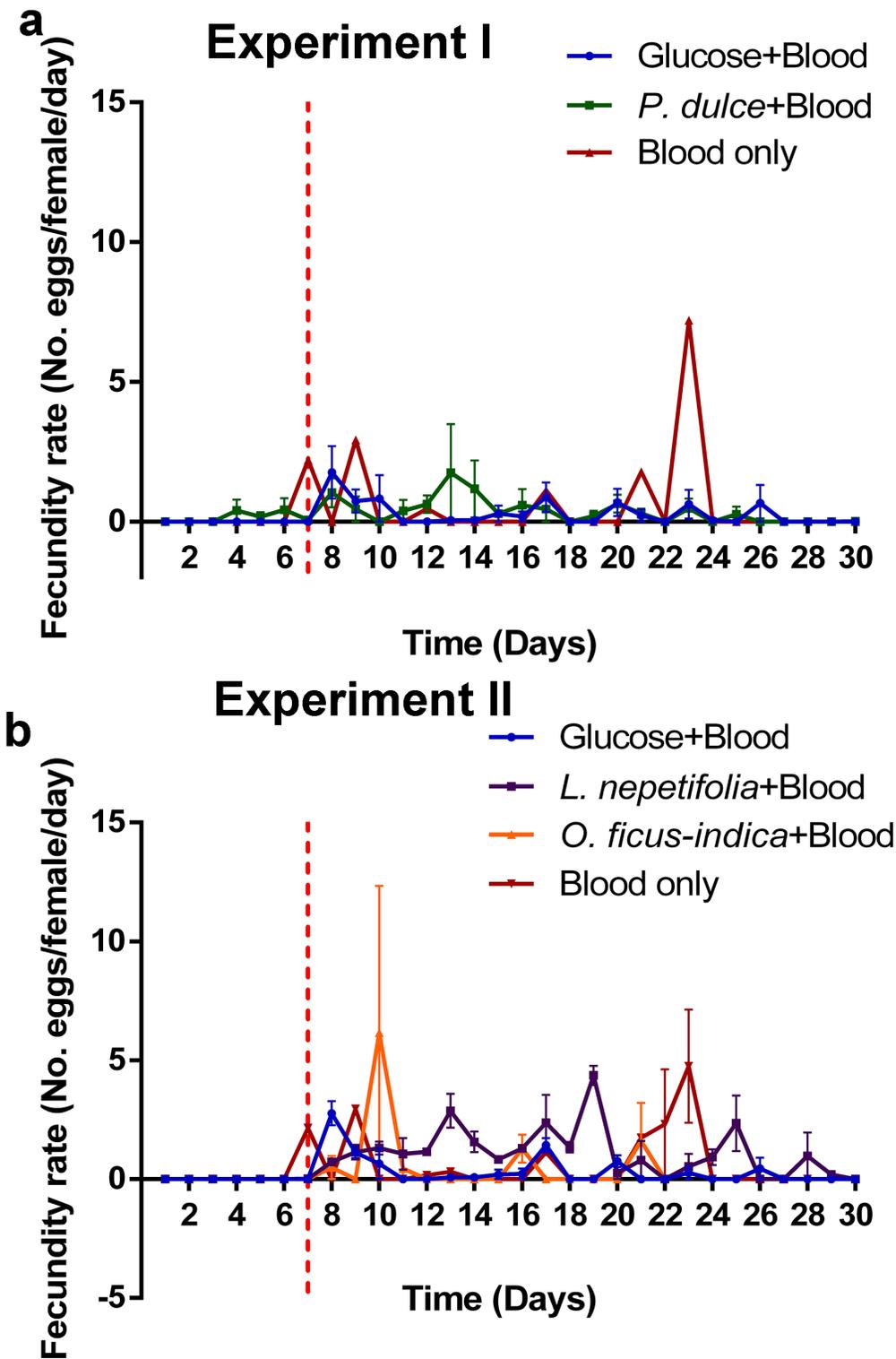
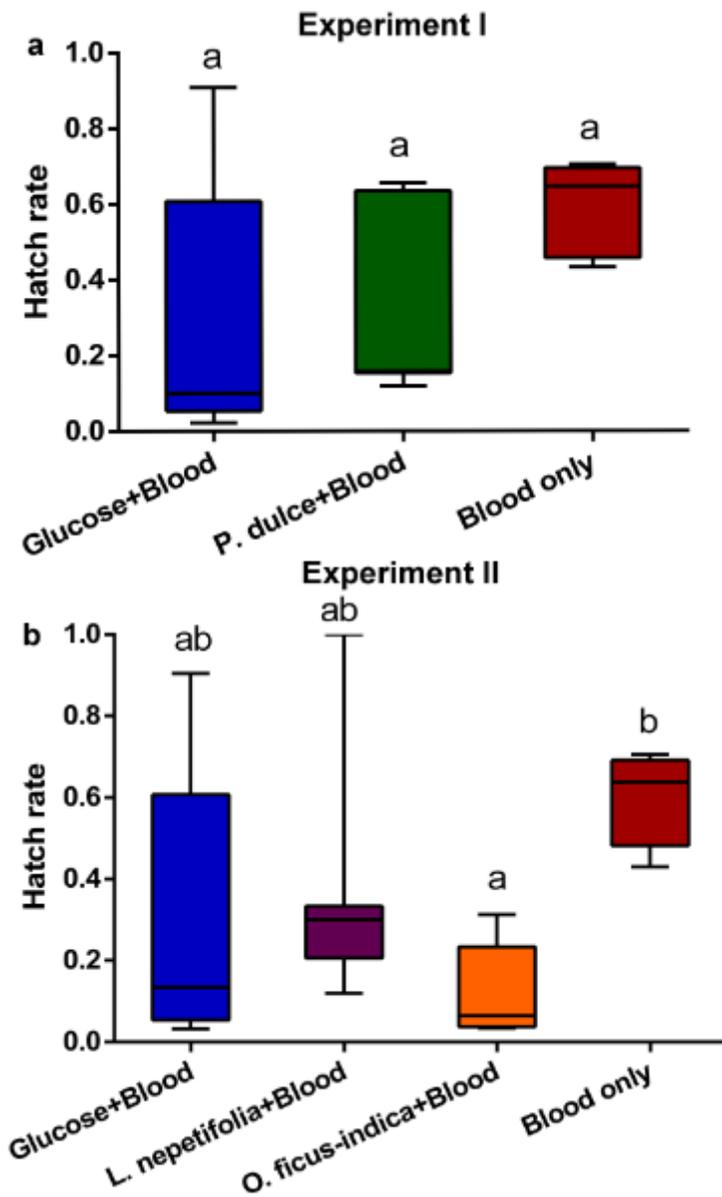


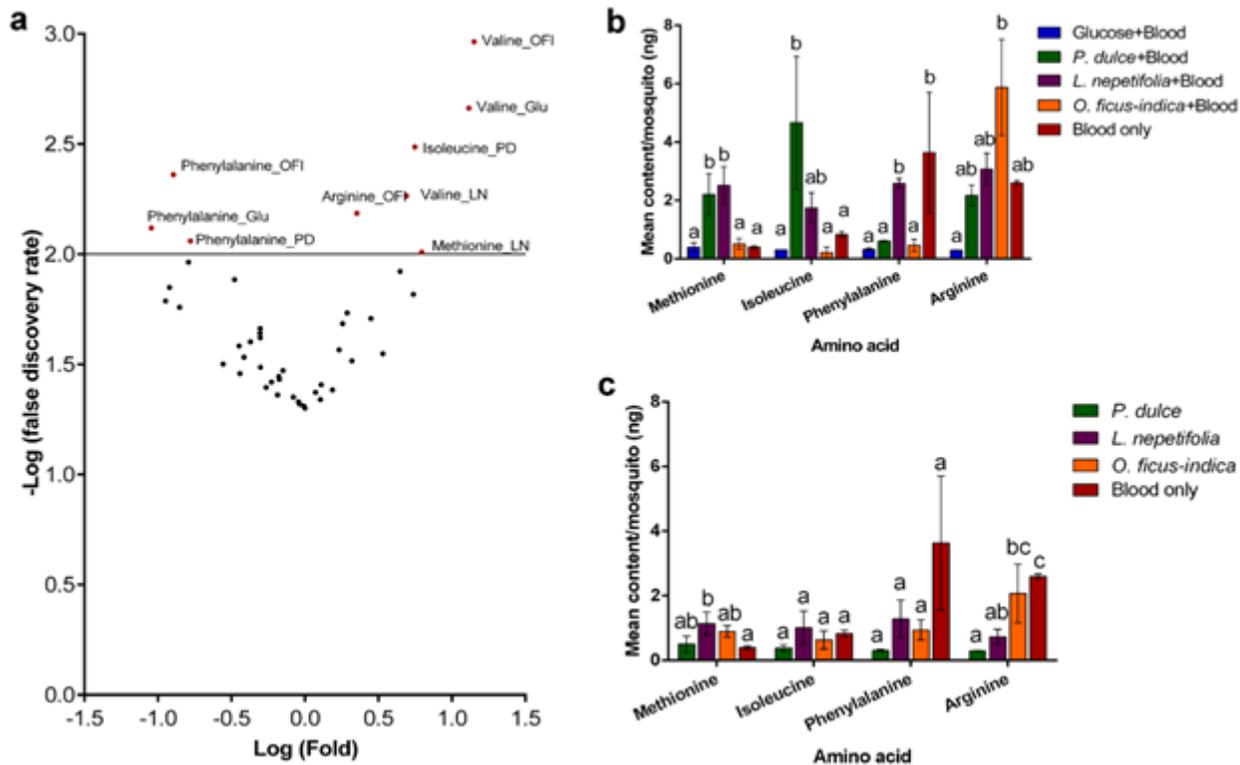
Figure 3

Effect of plant feeding on the fecundity of *Ae. aegypti*. a) Line plots showing the oviposition patterns of mosquitoes fed on *P. dulce*, glucose and exclusive mice bloodmeal. b) Line plots showing the oviposition patterns of mosquitoes fed on *L. nepetifolia*, *O. ficus-indica*, glucose and exclusive mice bloodmeal. The broken red line shows the day when the third bloodmeal was provided.



**Figure 4**

Effect of plant feeding on the viability of *Ae. aegypti* eggs. a) Hatching rates of eggs laid by mosquitoes fed on 6% glucose, *P. dulce* and exclusive bloodmeal. The difference in hatching rates was detected using ANOVA and Turkey Post Hoc test (P-value = 0.0484). b) Hatching rates of eggs laid by mosquitoes fed on 6% glucose, *L. nepetifolia*, *O. ficus-indica* and exclusive bloodmeal. Box plots denoted with different letters are significantly different. The difference in hatching rates was detected using one-way ANOVA and Tukey Post Hoc test.



**Figure 5**

Amino acid content of gut of *Ae. aegypti* fed of different nutrient sources. a) Volcano plot depicting unique amino acids significantly abundant or low in the guts of females fed on *P. dulce*, *L. nepetifolia*, *O. ficus-indica*, and 6% glucose solution compared to those exclusively fed on mice blood. The horizontal line shows where  $p = 0.05$  by F statistics. the significantly high or low abundant amino acids are shown. LN = *L. nepetifolia*, PD = *P. dulce*, OFI = *O. ficus-indica*, Glu = glucose solution. b) Mean amounts of the unique amino acids in the guts of females fed on the five nutrient sources with three initial bloodmeals. The P-values were  $< 0.001$ ,  $< 0.01$ ,  $< 0.05$  and  $< 0.01$  for methionine, isoleucine, phenylalanine, and arginine, respectively. c) Mean amounts of the unique amino acids in the guts of females fed on the three plant nutrients with no initial bloodmeals and those fed exclusively on blood. The P-values were  $< 0.05$ ,  $= 0.25$ ,  $= 0.052$  and  $< 0.01$  for methionine, isoleucine, phenylalanine, and arginine, respectively. The differences in gut amino acid content was detected by one-way ANOVA and Tukey Post Hoc Test. Bars denoted with different letters are significantly different.

## Supplementary Files

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