Supplementary Information

Dengue virus infection modifies mosquito blood-feeding behavior to increase transmission to the host

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1. Supplementary Methods

Mosquitoes

The *Aedes aegypti* colony was established from eggs collected in Singapore in 2010 and reared in the insectary ever since. Colony eggs were hatched in MilliQ water. Larvae, at a density of 2.5-3 larvae per cm^2 of shallow water, were fed a mixture of 1:1 bovine liver powder: brewer's yeast (MP Biomedicals) and fish food flakes (TetraMin Crisps Pro) until pupation. Adults were reared in cages (Bioquip) supplemented with 10% sucrose solution and water. The insectary was maintained at 28˚C with 50% relative humidity on a 12:12h dark: light cycle. Mosquitoes were reared together until females were isolated and separated into two groups, to be used under infection and control conditions.

Mouse

AG129 mice purchased from B&K Universal (UK) and C57BL/6Ntac mice purchased from InVivos (Singapore) were housed in the biosafety level 2 (BSL-2) animal facility at Duke-NUS, Singapore. All animal experiments were carried out as outlined in approved protocols (IACUC permission number: 2016/SHS/1194 and 2016/SHS/1196). Mice were housed with the following conditions: light cycle of 17h light/7h dark, humidity of about 57%, temperature of 23.5°C. No more than 5 mice per cage were fed irradiated rat and mouse Diet (Specialty Feed) *ad libitum*. Eight to 12 week-old male mice were used for behavior and transmission assays. Fur on the mouse belly was shaved one day prior behavioral assay to prevent any influence of shaving-induced inflammation.

Virus

The dengue virus serotype 2 EDEN2 strain was collected in 2005 from a patient at the Singapore General Hospital (sequence ID: EU081177.1) (1) and passaged no more than 7 times before use. EDEN2 was propagated in C6/36 *Aedes albopictus* cells (ATCC CRL-1660) maintained in RPMI (Roswell Park Memorial

Institute Medium) (Gibco) with 2% FBS (Research Instruments Pte), titrated using BHK-21 cells (ATCC) as detailed (2), aliquoted, and stored at -80°C until use.

Mosquito oral infection

Three to five-day-old female mosquitoes were starved for 18h and offered an artificial blood meal containing 40% volume of washed erythrocytes from specific-pathogen-free (SPF) pig's blood (PWG Genetics), 5% 10 mM ATP (ThermoScientific), 5% human serum (Sigma) and 50% virus solution in RPMI (Gibco). Titer for infectious blood was 5×10^7 pfu / ml and was validated by plaque assay on BHK-21 cells. Control mosquitoes were offered non-infectious blood with 50% RPMI instead of virus solution. Mosquitoes were left to feed for 1.5 h using Hemotek membrane feeding system (Discovery Workshops) covered with porcine membrane (sausage casing). Fully engorged females following infectious or non-infectious blood feeding were selected and maintained with constant access to 10% sugar solution at 28°C with 50% relative humidity on a 12:12 h dark: light cycle for ten days before analysis.

Absolute quantification of DENV load

RNA was extracted from 20 randomly-selected mosquitoes 10 days after oral infection or non-infectious blood feeding. Total RNA was extracted using E.Z.N.A total RNA kit I (OMEGA Bio-Tek) and used for one step RT-qPCR with *iTaq Universal Probes One-Step* (Bio-Rad) targeting gRNA with primers 5' CAG GTT ATG GCA CTG TCA CGA T 3' and 5' CCA TCT GCA GCA ACA CCA TCT C 3' and Hex/bhq-1 probe 5' CTC TCC GAG AAC AGG CCT CGA CTT CAA 3'. gRNA was quantified on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) with a thermal profile of 50°C for 10 min, 95°C for 1 min and 40 cycles of 95°C for 10 sec and 60°C for 15 sec. RNA fragments encompassing the qPCR target were produced and used to generate an absolute standard curve as described (2).

Design of the behavioral assay device

The device was custom designed with clear acrylic and consisted of an upper and a lower platform (Fig. 1B and C). The upper platform was designed to secure an anesthetized mouse and was perforated to allow mosquito access to the mouse belly through four observation chambers. The upper platform could be lowered onto the lower platform to position the observation chambers in contact with the mouse belly. The lower platform housed four observation chambers and acted as alignment guide to position the chambers below the mouse belly. The observation chambers were 7.5 × 10 × 30 mm (W × L × H) and housed a single mosquito that was locked by a cotton plug. Clear acrylic was used except for the back, which was made of white acrylic to provide a contrasting background. A fine mesh was sealed on top of the chamber, allowing the mosquito to pass its proboscis through. Internal walls except the front were etched with steps and the surface was roughened to facilitate mosquito movement.

A Blackfly 0.3 MP camera (FLIR Integrated Imaging Solutions) was mounted onto a base plate board (Thor Labs). An LED light was installed underneath the camera on the platform. The entire platform setup was placed in an incubation chamber to maintain the temperature at 28°C.

Behavior video recording

At 10 days post infection, a single cold-anesthetized mosquitoes was loaded and starved in each observation chamber at 28°C for 18–24 h prior to behavior recording. Each mosquito was used for only one recording. Four chambers were mounted side-by-side on the lower platform and contained two control and two infected mosquitoes in alternate positions (Fig. 1 D and E; Fig. S1B). One AG129 mouse was anesthetized by intraperitoneal injection of 10 µl per mg of mouse of a solution containing 5 mg/ml of ketamine and 1 mg/ml of xylazine. Mice were then placed onto the upper platform. To normalize the starting position for the mosquitoes, chambers were gently tapped to drop them at the bottom before lowering the upper platform. Video recording was started when the upper platform was lowered onto the chambers and lasted for 30 min. Mice were changed every four recordings to limit the influence of previous bites. Behavior recording was conducted at 28°C across several days from 4–9 pm to synchronize with the mosquito circadian rhythm.

Behavior annotation

Videos recorded at 60 frames per second were saved in mp4 file format, converted to individual frames as photos in JPEG file format with VLC (videolan.org) and annotated manually. An in-house software translated the frame number into duration and allowed exportation of the annotated behavior in excel format (Annotation software; https://drive.google.com/file/d/1r-GPTsmUuWE4y1WeAeYD7S8Lr1nbcvvy/view). The annotation software allowed distance calculation and was used to measure proboscis length and girth. Registered activities included (i) locomotor activities: walking and immobile, (ii) positions: mid-range, short-range, in contact with the mouse, (iii) proboscis activities: insertion into the skin, inserted length, downward and upward motions within the skin, grooming (defined as leg-aided rubbing like *Drosophila* (3)), and wriggling (defined as short proboscis movements outside the skin), (iv) blood-feeding-related activities: blood ingestion, abdomen swelling, (v) body maintenance activities: wing grooming (defined as leg-aided rubbing). The conditions of the mosquitoes (infected vs. control) were blinded during the annotation. The annotation outputs were computed into 80 behavioral parameters (S1 Table) using a Visual Basic for Applications (VBA) macro (S1 Text). To allow for multivariate statistical analysis, which removes a repeat with missing values, we ascribed conservative values to parameters when the related behavior did not occur (S2 Table).

Multivariate statistical analysis

Factor analysis based on correlation matrix was applied to the 44 host-seeking behavioral parameters for all DENV and control mosquitoes and separately to the 36 biting behavioral parameters for DENV and control mosquitoes that probed. To improve interpretability, factor analysis was conducted with varimax rotation, which minimizes the number of parameters with high loadings within each factor, thereby generating factors with fewer highly influential parameters. The factor analysis grouped correlated parameters into factors and assigned a loading score to each parameter, indicating the parameter influence on the factor value.

Factor loadings were extracted to biologically interpret factors based on the parameter ordination for the highest loadings (> 0.6). Each factor explained a percentage of total variance that represented its contribution to the overall mosquito behavior. Only factors that explained more than 5% of the total variance were analyzed. Differences in scores for the orthogonal factors were tested using a one-sided unpaired T-test, assuming that the impact of DENV either increased or decreased the factor scores. To minimize the known large variance in animal behavior (4, 5), which lowers statistical power, we set the significance threshold at 0.055. Statistical analyses were performed with SYSTAT 13.0 (Systat Software).

Biological interpretation of the factors

Host-seeking behavior – data in S4-S5 Table and Fig. 2B

Factor 1: Five of the parameters measured durations and numbers of walking events both at short-range and in contact. The factor also positively correlated with two parameters that described the number of immobile events, as this correlates with walking initiation and arrest. The factor was associated with high locomotor activity and thus interpreted as 'restlessness'.

Factor 2: Loading signs indicated an inverse correlation between 'duration at mid-range distance' (positive sign) and 'duration in contact' (negative sign). Duration at mid-range distance shows lack of host attraction, whereas duration in contact represents attraction. Together with a positive loading for 'duration at mid-range distance after in contact without probing', we inferred that factor 2 represented a lack of attraction to the host even after sensing the surface chemistry of the mouse skin. 'Averaged and total duration of immobilization' also negatively correlated with factor 2, suggesting that lack of host attraction is associated with shorter immobilization periods. Moreover, the positive correlation of 'time to 1st proboscis wriggling' shows that delayed proboscis wriggling was associated with a lack of host attraction and shed new light on this poorly characterized behavior. Altogether, we interpreted factor 2 as 'lack of host attraction'.

Factor 3: All the associated parameters measured durations before mosquitoes enter in contact with the mouse or initiate probing. As the factor loadings were negative, factor 3 indicated the speed with which the mosquitoes progressed towards the host. We interpreted factor 3 as 'attraction to host'.

Factor 4 and 5: Factor 4 was associated with all parameters related to proboscis grooming and interpreted as 'proboscis grooming', while factor 5 was associated with all parameters related to wing grooming and was interpreted as 'wing grooming'.

Factor 6: The factor was inversely related to 'duration at short-range' and 'duration at short-range before 1st probing', indicating an association with host attraction from short-range distance. The additional negative correlations with 'duration and average duration of immobilization at short-range distance' confirmed that the factor represented active engagement towards the mouse. We interpreted factor 6 as 'attraction to host from short-range distance'.

Factor 7: All the associated parameters measured duration and average durations of walking. During walking, mosquitoes search for cues. As the highest loading was for 'average duration of walking event in contact', we interpreted factor 7 as 'search for skin cues'.

Biting behavior – data in S7-S8 Table and Fig. 3B

Factor 1: 'Number of probes' and 'duration of probing' were negatively correlated with the factor, indicating that factor 1 was associated with less probing. 'Number of proboscis motions' and 'duration of proboscis motions' were also negatively correlated with the factor, as they occur during probing.

Importantly, factor 1 was negatively associated with three parameters measuring bite failure (i.e. 'number of unsuccessful bites', 'number of proboscis motions during unsuccessful bites' and 'duration of proboscis motions during unsuccessful bites'), indicating an association with successful bites, resulting in blood ingestion. Furthermore, 'number of probes' and 'total duration of probing before 1st blood ingestion' were inversely related with the factor, indicating an association with lower number of probes to reach blood. Finally, factor 1 was inversely correlated with 'duration of biting', which combines durations of probing and blood ingestion. As 'blood ingestion duration' (P58) was not clustered in factor 1 (S8 Table), this implies that probing duration was the main determinant of duration of biting. Altogether, we interpreted factor 1 as 'probing efficiency'.

Factor 2: Negative correlations with 'time from start to 1st blood ingestion', 'time from 1st time in contact to 1st blood ingestion' and 'duration in contact before 1st blood ingestion' indicated an association with swift mosquito progression towards blood ingestion. Furthermore, negative correlations with 'time from start to 1st successful bite' and 'time from 1st time in contact to 1st probing' indicated an association with eagerness to bite. Interestingly, factor 2 positively correlated with 'number of successful bites' and 'number of blood ingestion events'. These positive correlations suggest that swift progress towards blood ingestion was associated with higher frequency of blood ingestion. Altogether, factor 2 was interpreted as 'blood appetite'. Further supporting this interpretation, 'total abdomen swelling after blood-feeding' (P61) was moderately associated with the factor (S8 Table). Two other parameters apparently unrelated to blood appetite were also clustered. 'Duration in contact after blood ingestion' was positively associated and may indicate that larger blood meals immobilize mosquitoes after repletion. A positive correlation with 'length of inserted proboscis during blood ingestion' suggested that higher blood appetite forces the proboscis deeper into the skin.

Factor 3: Six of the correlated parameters measured number, duration and average duration of proboscis motions, clearly associating the factor with proboscis activity. Among these six parameters, two were related to any probes and four to successful bites. 'Average duration of probe' was also positively correlated as it provides more time for proboscis motions. We interpreted factor 3 as 'proboscis activity during successful bites'.

Factor 4: The factor positively correlated with two parameters associated with proboscis motions during unsuccessful bites and 'average duration of probe before 1st blood ingestion'. We interpreted factor 4 as 'proboscis activity during unsuccessful bites'.

Factor 5: 'Duration of blood ingestion' and 'average duration of blood ingestion' were positively correlated with the factor, indicating an association with blood ingestion capacity. Supporting this interpretation, factor 5 was moderately correlated with the quantity of ingested blood as measured by 'total abdomen swelling after blood ingestion' (P61) and 'average abdomen swelling per blood ingestion' (P62) (S8 Table). We interpreted factor 5 as 'blood ingestion capacity'.

Univariate statistical analysis

The differences in each behavioral parameter were analyzed with a one-sided Mann-Whitney test with a significance threshold set at 0.05. The differences in biting and blood ingestion rates were tested with χ^2. Mann-Whitney and χ^2 analyses were done with SYSTAT 13.0 (Systat Software). The differences in viral loads between successive bites were analyzed with one-way repeated-measures ANOVA on log-transformed values with Prism (v 8.0.2, GraphPad).

Large cage host-seeking behavioral assay

At 10 days post-infection, 10 mosquitoes starved for 24h were placed in a 30 x 30 x 30 cm mesh cage (BugDorm). Non-infected mosquitoes were used as controls. One anesthetized C57BL/6Ntac mouse was positioned on the top of the cage over a mesh that would allow the mosquitoes to bite the mouse. Mosquito behavior in the cage was video-recorded with a smartphone for 30 min. For each condition (infected vs. control), three cages with different mosquitoes and different mice were done. The time from the start to the first contact with the mouse was recorded. The differences between infected and non-infected mosquitoes were analyzed using survival analysis (Prism v 8.0.2, GraphPad).

Calculation of biting and blood ingestion rates

The biting rates were calculated by dividing the number of biting mosquitoes over the number of observed mosquitoes. Blood ingestion rates were calculated by dividing the number of blood-fed mosquitoes over the number of biting mosquitoes.

Successive bite assay

Enclosed in the observation chamber, the same mosquito was allowed to bite three different shaved anesthetized C57BL/6Ntac mice in a row, with an interval of 10 min between mice. Mosquitoes were used 10 days post-oral infection. At 20 sec. after initiating a bite, the mosquito was disturbed to prevent blood-feeding. After 10 min of rest, the same mosquito was offered a different mouse and allowed to bite. In total, eight different mosquitoes were studied, each with three different mice. The experiment was conducted over two days with four mosquitoes studied each day.

Twenty-four hours after the biting, the mice were euthanized and approximately 1 cm^2 of belly skin and the inguinal lymph nodes were harvested. Total RNA was extracted using the RNeasy mini kit (Qiagen) according to the manufacturer's instructions. DENV load was quantified by quantitative real-time PCR using the SuperScript™ III Platinum™ One-step RT-qPCR system (Invitrogen) in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad) with primer pair C14A 5'-AATATGCTGAAACGCGAGAGAAACCGCG -3' and C69B 5'-CCCATCTCITCAIIATCCCTGCTGTTGG -3', and probe VICD2C38B 5'- AGCATTCCAAGTGAGAATCTCTTTGTCAGCTGT -3' (6). Absolute quantification was done using a standard curve generated from a serial dilution of DNA plasmid containing the sequence of interest.

Mathematical modelling of DENV epidemiology

A mathematical model relying on the simple SIR (Susceptible, Infectious, Recovered) compartmental model was used to determine the impact of blood feeding changes on DENV transmission potential, as inferred through the basic reproduction ratio R0. SIR models are routinely applied to model dengue epidemiology (7–10). The model is detailed below as a system of ordinary differential equations:

$$\frac{dS_m}{dt} = \mu(S_m + I_m) - a_s b_m S_m \left(\frac{I}{S + I + R}\right) - \mu S_m$$

$$\frac{dI_m}{dt} = a_s b_m S_m \left(\frac{I}{S + I + R}\right) - \mu I_m$$

$$\frac{dS}{dt} = -a_i b_h I_m \left(\frac{S}{S + I + R}\right)$$

$$\frac{dI}{dt} = a_i b_h I_m \left(\frac{S}{S + I + R}\right) - \sigma I$$

$$\frac{dR}{dt} = \sigma I$$

The populations are compartmentalized according to their infectious status (*S*, *I* and *R* for Susceptible, Infectious and Recovered human populations, respectively; *Sm* and *Im* for Susceptible and Infectious mosquitoes, respectively – mosquitoes do not recover from infection). At the beginning of the simulation, all human individuals are susceptible and become infected according to the biting rate of infectious mosquitoes (a_i, which changed according to the behavioral scenario considered, see below), the number of infectious mosquitoes (*Im*, which dynamically fluctuated) and the probability of infection when exposed to infectious mosquitoes (b_h, set at 0.5). When infected, humans recovered following the recovery rate σ, set at 5 days^{-1}, and could not be infected again. Mosquito infection depends on the biting rate of susceptible mosquitoes (a_s, set at 4 days^{-1}), the number of infectious humans (*I*, which dynamically fluctuated) and probability of mosquito infection upon exposure to infected human (b_m, set at 0.5). While we did not consider human demography because we aimed to investigate the exponential growth of epidemics (e.g., the R0), we set mosquito demography, μ, at 21 days^{-1}. In this model, we considered that both human and mosquito population sizes were not affected by changes in blood feeding behavior. The values set for the various parameters are classical for arbovirus transmission models (11). We then ran simulations for 180 days starting with one human as infectious and all mosquitoes as susceptible. We quantified the R0 on the produced dynamics using two different R0 packages (https://cran.r-project.org/web/packages/R0/index.html and https://cran.r-project.org/web/packages/EpiEstim/index.html).

The variations in blood-feeding behavior between the infected and non-infected mosquitoes were included in the modelling parameter a_i by incorporating changes in P19 ('Duration at mid-range distance before 1st time in contact') and P39 ('Number of unsuccessful bites') means upon infection (Datasets S2 and S3). P19 and P39 were significantly affected by infection (see main text) and indicated changes in host-seeking and biting behaviors, respectively. R0 was calculated in different conditions: (i) Uninfected-mosquito behavior with a a_i equals to a_s; (ii) Host-seeking alterations: original a_i was changed proportionally to the impact of infection on P19 mean, i.e. multiplied by 1.51 (Dataset S2); (iii) Biting behavior alterations: original a_i was changed proportionally to the impact of infection on P39 mean, i.e. multiplied by 1.99 (Dataset S3); (iv) Host-seeking and biting alterations: original a_i was changed proportionally to the sum of the impact of infection on P19 and P39 means, i.e. multiplied by 3.5.

Supplementary References

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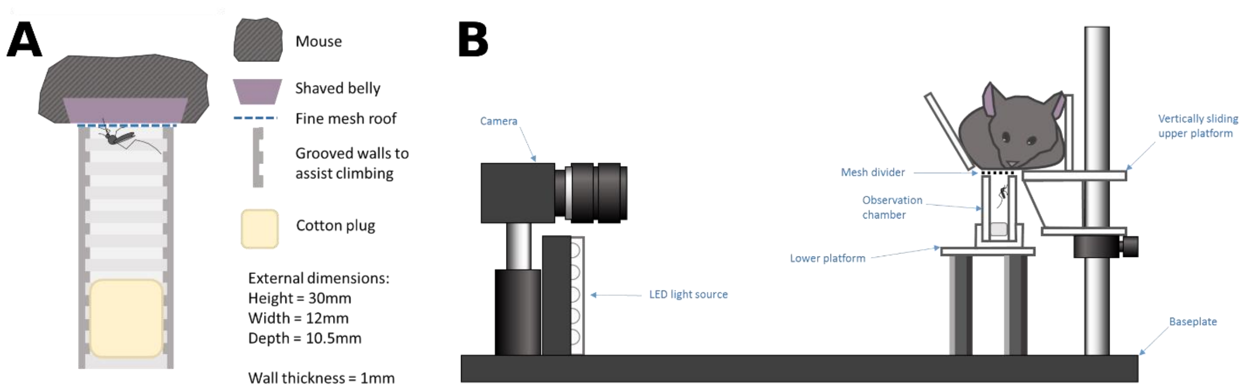
2. Supplementary Movies

S1 Movie. One example of recorded video.

S2 Movie. Example of annotation of mosquito behavior.

3. Supplementary Figures



S1 Fig. Design of the behavior assay device

A. Dimensions of the observation chamber. **B**. Whole device including camera, LED light source, baseplate, lower platform, upper platform, observation chamber.

4. Supplementary Datasets

Dataset S1. Annotations of the mosquito behaviors.

Dataset S2. Values for the host-seeking behavioral parameters.

The 44 parameters associated with host-seeking were calculated for all control (CTRL) and DENV-infected (DENV) mosquitoes. Parameters are identified by their number as in Table 1. Mann-Whitney (MW) test p-values between both conditions are detailed. P-values lower than 0.05 are indicated in bold. Medians and arithmetic means for each parameter are detailed.

Dataset S3. Values for biting behavioral parameters.

The 36 parameters associated with biting were calculated only for control (CTRL) and DENV-infected (DENV) mosquitoes that probed. Parameters are identified by their number as in Table 1. Mann-Whitney (MW) test p-values between both conditions are detailed for all samples, for mosquitoes without zero (measured activity did not occur) and for mosquitoes that bloodfed. P-values lower than 0.05 are indicated in bold. Medians for all samples, for samples without zero, for mosquitoes that bloodfed only and arithmetic means for all samples are detailed.

Dataset S4. VBA code to calculate the behavior parameters from the behavioral annotations.

5. Supplementary Tables

S1 Table. Definition of behavioral parameters

Parameters were categorized according to their position in the different feeding behavior sequences and stages (host-seeking or biting behavior). Biological interpretation of the parameters was attributed based on the understanding of the associated behavior.

Nb.	Behavior phase	Biological interpretation	Behavioral parameter definition	Host-seeking or Biting behavior
1	Mid-range	Sensing cues	Total duration at mid-range distance	Host-seeking
2	Short-range	Attraction to host	Time from start to 1st time at short-range distance	Host-seeking
3			Time from 1st time at short-range distance to 1st probing	Host-seeking
4			Time from 1st time at short-range distance to 1st blood ingestion	Host-seeking
5			Duration at mid-range distance before 1st time at short-range distance	Host-seeking
6			Duration at short-range distance before 1st probing	Host-seeking
7		Sensing cues	Total duration at short-range distance	Host-seeking
8			Number of immobile events at short-range distance	Host-seeking
9			Duration immobile at short-range distance	Host-seeking
10			Average duration of immobile event at short-range distance	Host-seeking
11			Number of walking events at short-range distance	Host-seeking
12			Duration of walking at short-range distance	Host-seeking
13			Average duration of walking event at short-range distance	Host-seeking
14			Duration at short-range distance after blood ingestion	Biting
15			Duration at short-range distance after unsuccessful bite	Biting
16	Contact	Attraction to host	Time from start to 1st time in contact	Host-seeking
17			Time from 1st time in contact to 1st probing	Biting
18			Time from 1st time in contact to 1st blood ingestion	Biting
19			Duration at mid-range distance before 1st time in contact	Host-seeking
20			Duration at short-range distance before 1st time in contact	Host-seeking
21			Duration in contact before 1st probing	Host-seeking
22			Duration in contact before 1st blood ingestion	Biting
23			Duration in contact after blood ingestion	Biting
24		Sensing cues	Duration at mid-range distance after in contact without probing	Host-seeking
25			Total duration in contact	Host-seeking
26			Number of walking events in contact	Host-seeking
27			Duration of walking in contact	Host-seeking
28			Average duration of walking event in contact	Host-seeking

29			Number of proboscis wriggling before probing	Host-seeking
30			Duration of proboscis wriggling before probing	Host-seeking
31			Average duration of proboscis wriggling before probing	Host-seeking
32	Probing	Attraction to host	Time from start to 1st probing	Host-seeking
33			Time from 1st probing to 1st blood ingestion	Biting
34		Efficiency	Time from start to 1st successful bite	Biting
35			Number of probes	Biting
36			Duration of probing	Biting
37			Average duration per probe	Biting
38			Number of successful bites	Biting
39			Number of unsuccessful bites	Biting
40			Number of probes before 1st blood ingestion	Biting
41			Total duration of probing before 1st blood ingestion	Biting
42			Average duration of probing before 1st blood ingestion	Biting
43			Number of proboscis motions per probe	Biting
44			Total number of proboscis motions	Biting
45			Duration of proboscis in motion	Biting
46			Average duration of proboscis in motion per probe	Biting
47			Average number of proboscis motions per successful bite	Biting
48			Number of proboscis motions during successful bites	Biting
49			Duration of proboscis in motion during successful bites	Biting
50			Average duration of proboscis in motion per successful bite	Biting
51			Average number of proboscis motions per unsuccessful bite	Biting
52			Number of proboscis motions during unsuccessful bites	Biting
53			Duration of proboscis in motion during unsuccessful bites	Biting
54			Average duration of proboscis in motion per unsuccessful bite	Biting
55			Length of inserted proboscis during blood ingestion	Biting
56	Blood ingestion	Attraction to host	Time from start to 1st blood ingestion	Biting
57		Efficiency	Number of blood ingestion events	Biting
58			Duration of blood ingestion	Biting
59			Average duration of blood ingestion event	Biting
60			Duration of biting	Biting
61			Total abdomen swelling after blood feeding	Biting
62			Average abdomen swelling per blood ingestion event	Biting
63	Body maintenance	Proboscis	Time from start to 1st proboscis grooming	Host-seeking
64			Number of proboscis grooming events	Host-seeking
65			Duration of proboscis grooming	Host-seeking
66			Average duration of proboscis grooming event	Host-seeking
67			Time from start to 1st proboscis wriggling	Host-seeking
68			Number of proboscis wriggling events	Host-seeking
69			Duration of proboscis wriggling	Host-seeking

70			Average duration of proboscis wriggling event	Host-seeking
71		Wings	Time from start to 1st wing grooming	Host-seeking
72			Number of wing grooming events	Host-seeking
73			Duration of wing grooming	Host-seeking
74			Average duration of wing grooming event	Host-seeking
75	Locomotor activity	Immobility	Number of immobile events	Host-seeking
76			Duration immobile	Host-seeking
77			Average duration of immobile event	Host-seeking
78		Walking	Number of walking events	Host-seeking
79			Duration of walking	Host-seeking
80			Average duration of walking event	Host-seeking

S2 Table. Unit of each behavioral parameter and value if the behavior does not occur.

Nb.	Behavior parameters	Unit	Value given if the behavior does not occur
1	Total duration at mid-range distance	Sec.	If no mid-range = 0
2	Time from start to 1st time at short-range distance	Sec.	If no short-range = recording duration
3	Time from 1st time at short-range distance to 1st probing	Sec.	If no probing = recording duration left after 1st short-range
4	Time from 1st time at short-range distance to 1st blood ingestion	Sec.	If no blood ingestion = recording duration left after 1st short-range
5	Duration at mid-range distance before 1st time at short-range distance	Sec.	If no short-range = recording duration
6	Duration at short-range distance before 1st probing	Sec.	If no short-range = recording duration; If no probing = short-range duration
7	Total duration at short-range distance	Sec.	If no short-range = 0
8	Number of immobile events at short-range distance	Count	If no immobile event = 0
9	Duration immobile at short-range distance	Sec.	If no immobile event = 0
10	Average duration of immobile event at short-range distance	Sec.	If no immobile event = 0
11	Number of walking events at short-range distance	Count	If no walking event = 0
12	Duration of walking at short-range distance	Sec.	If no walking event = 0
13	Average duration of walking event at short-range distance	Sec.	If no walking event = 0
14	Duration at short-range distance after blood ingestion	Sec.	If no blood ingestion = 0
15	Duration at short-range distance after unsuccessful bite	Sec.	If no unsuccessful bite = 0
16	Time from start to 1st time in contact	Sec.	If no contact = recording duration
17	Time from 1st time in contact to 1st probing	Sec.	If no probing = recording duration left after 1st contact; If no contact = recording duration
18	Time from 1st time in contact to 1st blood ingestion	Sec.	If no blood ingestion = recording duration left after 1st contact; If no contact = recording duration
19	Duration at mid-range distance before 1st time in contact	Sec.	If no contact = total duration at mid-range
20	Duration at short-range distance before 1st time in contact	Sec.	If no contact = total duration at short-range
21	Duration in contact before 1st probing	Sec.	If no probing = total duration in contact
22	Duration in contact before 1st blood ingestion	Sec.	If no blood ingestion = total duration in contact
23	Duration in contact after blood ingestion	Sec.	If no blood ingestion = 0
24	Duration at mid-range distance after in contact without probing	Sec.	If no contact = 0
25	Total duration in contact	Sec.	If no contact = 0
26	Number of walking events in contact	Count	If no walking = 0
27	Duration of walking in contact	Sec.	If no walking = 0

28	Average duration of walking event in contact	Sec.	If no walking = 0
29	Number of proboscis wriggling before probing	Count	If no proboscis wriggling = 0
30	Duration of proboscis wriggling before probing	Sec.	If no proboscis wriggling = 0
31	Average duration of proboscis wriggling before probing	Sec.	If no proboscis wriggling = 0
32	Time from start to 1st probing	Sec.	If no probing = recording duration
33	Time from 1st probing to 1st blood ingestion	Sec.	If no blood ingestion = recording duration left after 1st probing; If no probing = recording duration
34	Time from start to 1st successful bite	Sec.	If no successful bite = recording duration
35	Number of probes	Count	If no probe = 0
36	Duration of probing	Sec.	If no probe = 0
37	Average duration per probe	Sec.	If no probe = 0
38	Number of successful bites	Count	If no successful probe = 0
39	Number of unsuccessful bites	Count	If no unsuccessful probe = 0
40	Number of probes before 1st blood ingestion	Count	If no blood ingestion = total number of probes
41	Duration of probing before 1st blood ingestion	Sec.	If no blood ingestion = total duration of probing
42	Average duration of probing before 1st blood ingestion	Sec.	If no blood ingestion = average duration of probe
43	Number of proboscis motions per probe	Count	If no motion = 0
44	Total number of proboscis motions	Count	If no motion = 0
45	Duration of proboscis in motion	Sec.	If no motion = 0
46	Average duration of proboscis in motion per probe	Sec.	If no motion = 0
47	Average number of proboscis motions per successful bite	Count	If no motion or successful bite = 0
48	Number of proboscis motions during successful bites	Count	If no motion or successful bite = 0
49	Duration of proboscis in motion during successful bites	Sec.	If no motion or successful bite = 0
50	Average duration of proboscis in motion per successful bite	Sec.	If no motion or successful bite = 0
51	Average number of proboscis motions per unsuccessful bite	Count	If no motion or unsuccessful bite = 0
52	Number of proboscis motions during unsuccessful bites	Count	If no motion or unsuccessful bite = 0
53	Duration of proboscis in motion during unsuccessful bites	Sec.	If no motion or unsuccessful bite = 0
54	Average duration of proboscis in motion per unsuccessful bite	Sec.	If no motion or unsuccessful bite = 0
55	Length of inserted proboscis during blood ingestion	mm	If no blood ingestion = 0
56	Time from start to 1st blood ingestion	Sec.	If no blood ingestion = recording duration
57	Number of blood ingestion events	Count	If no blood ingestion = 0
58	Duration of blood ingestion	Sec.	If no blood ingestion = 0
59	Average duration of blood ingestion event	Sec.	If no blood ingestion = 0
60	Duration of biting	Sec.	If no probing and blood ingestion = 0

61	Total abdomen swelling when blood feeding	mm	If no blood ingestion = 0
62	Average abdomen swelling per blood ingestion event	mm	If no blood ingestion = 0
63	Time from start to 1st proboscis grooming	Sec.	If no proboscis grooming = 0
64	Number of proboscis grooming events	Count	If no proboscis grooming = 0
65	Duration of proboscis grooming	Sec.	If no proboscis grooming = 0
66	Average duration of proboscis grooming event	Sec.	If no proboscis grooming = 0
67	Time from start to 1st proboscis wriggling	Sec.	If no proboscis wriggling = recording duration
68	Number of proboscis wriggling events	Count	If no proboscis wriggling = 0
69	Duration of proboscis wriggling	Sec.	If no proboscis wriggling = 0
70	Average duration of proboscis wriggling event	Sec.	If no proboscis wriggling = 0
71	Time from start to 1st wing grooming	Sec.	If no wing grooming = recording duration
72	Number of wing grooming events	Count	If no wing grooming = 0
73	Duration of wing grooming	Sec.	If no wing grooming = 0
74	Average duration of wing grooming event	Sec.	If no wing grooming = 0
75	Number of immobile events	Count	If no immobile event = 0
76	Duration immobile	Sec.	If no immobile event = 0
77	Average duration of immobile event	Sec.	If no immobile event = 0
78	Number of walking events	Count	If no walking event = 0
79	Duration of walking	Sec.	If no walking event = 0
80	Average duration of walking event	Sec.	If no walking event = 0

S3 Table. Total variance explained by factors in the host-seeking behavior analysis and their statistical significance. P-values lower than 0.055 are identified in bold.

Factor Nb.	1	2	3	4	5	6	7	8	9	10	11
Variance explained, %	14.8	10.6	8.9	8.0	7.9	7.6	7.5	7.4	6.6	3.8	3.3
p-value[1]	0.44	0.35	**0.047**	0.29	0.16	**0.054**	0.27	0.11	0.29	0.18	0.27

S4 Table. **Composition of host-seeking behavioral factors**

Only parameters with loadings > 0.6 were shown. Parameter numbers are as in S1 Table.

	Parameter Nb.	Definition	Loading
Factor 1	11	Number of walking events at short-range distance	0.886
	78	Number of walking events	0.886
	12	Duration of walking at short-range distance	0.860
	26	Number of walking events in contact	0.825
	75	Number of immobile events	0.786
	8	Number of immobile events at short-range distance	0.763
	79	Duration of walking	0.743
Factor 2	1	Total duration at mid-range distance	0.858
	76	Duration immobile	-0.829
	25	Total duration in contact	-0.749
	77	Average duration of immobile event	-0.68
	24	Duration at mid-range distance after in contact without probing	0.668
	67	Time from start to 1st proboscis wriggling	0.608
Factor 3	16	Time from start to 1st time in contact	-0.827
	2	Time from start to 1st time at short-range distance	-0.775
	19	Duration at mid-range distance before 1st time in contact	-0.754
	21	Duration at short-range distance before 1st time in contact	-0.731
	20	Duration in contact before 1st probing	-0.651
	5	Duration at mid-range distance before 1st time at short-range distance	-0.619
Factor 4	64	Number of proboscis grooming events	0.965
	65	Duration of proboscis grooming	0.962
	66	Average duration of proboscis grooming event	0.842
	63	Time from start to 1st proboscis grooming	-0.838
Factor 5	73	Duration of wing grooming	0.925
	72	Number of wing grooming events	0.878
	74	Average duration of wing grooming event	0.838
	71	Time from start to 1st wing grooming	-0.811
Factor 6	9	Duration immobile at short-range distance	-0.953
	7	Total duration at short-range distance	-0.841
	10	Average duration of immobile event at short-range distance	-0.823
	6	Duration at short-range before 1st probing	-0.605
Factor 7	28	Average duration of walking event in contact	0.973
	80	Average duration of walking event	0.971
	27	Duration of walking in contact	0.936
Factor 8	29	Number of proboscis wriggling before probing	0.984
	30	Duration of proboscis wriggling before probing	0.984
	31	Average duration of proboscis wriggling before probing	0.984
Factor 9	69	Duration of proboscis wriggling	0.804
	70	Average duration of proboscis wriggling event	0.804

S5 Table. Parameter loadings in each of the factors for the host-seeking behavior multivariate analysis.

Parameter numbers are as in S1 Table.

Highly defining parameters; loading > 0.6

Moderately defining parameters; 0.3 < loading < 0.6

Parameter	Factor number										
Nb.	1	2	3	4	5	6	7	8	9	10	11
1	0.063	0.858	-0.253	-0.109	-0.076	0.148	-0.087	0.059	-0.232	0.211	-0.054
76	-0.164	-0.829	0.076	-0.208	-0.018	-0.237	-0.138	-0.058	-0.027	0.268	0.016
25	-0.282	-0.749	0.202	0.121	-0.097	0.384	0.088	-0.047	0.255	-0.196	-0.039
77	-0.321	-0.68	0.044	-0.143	-0.055	-0.098	-0.08	0.011	0.091	0.347	-0.281
24	0.204	0.668	0.475	-0.107	-0.062	0.07	-0.04	0.098	-0.252	0.256	-0.133
67	0.353	0.608	-0.231	-0.112	0.199	-0.175	-0.065	-0.068	-0.378	0.261	0.174
32	0.464	0.534	-0.311	-0.12	0.173	-0.214	-0.07	0.046	-0.388	0.193	0.194
11	0.886	0.219	0.111	-0.033	0.103	0.018	0.025	0.256	-0.055	0.034	-0.154
78	0.886	0.196	0.126	-0.02	0.113	0.042	0.039	0.263	-0.047	-0.019	-0.183
12	0.86	0.255	0.13	-0.039	0.084	-0.038	0.065	0.145	-0.079	0.096	0.074
26	0.825	0.123	0.156	0.016	0.133	0.103	0.073	0.263	-0.02	-0.161	-0.25
75	0.786	-0.031	0.129	-0.065	-0.008	-0.193	-0.07	-0.186	-0.191	0.002	0.239
8	0.763	0.067	0.082	-0.034	-0.019	-0.29	-0.054	-0.183	-0.185	0.06	0.222
79	0.743	0.178	0.13	-0.037	0.088	0.01	0.579	0.107	-0.065	-0.012	0.034
9	0.097	-0.055	-0.036	-0.064	0.086	-0.953	-0.038	-0.062	0.009	0.019	0.096
7	0.378	0.054	0.013	-0.051	0.266	-0.841	-0.026	-0.004	-0.102	0.035	0.139
10	-0.192	-0.053	-0.045	-0.033	0.023	-0.823	0.021	0.01	0.27	0.075	-0.241
6	0.434	-0.073	-0.096	-0.076	0.281	-0.605	-0.025	0.003	-0.438	0.106	0.191
64	-0.048	-0.004	0.03	0.965	-0.019	0.035	-0.018	-0.002	-0.006	-0.005	0.021
65	-0.038	-0.02	0.032	0.962	-0.02	0.031	-0.01	-0.002	-0.013	-0.026	0.026
66	-0.02	-0.026	0.031	0.842	-0.028	0.038	0.001	-0.01	-0.003	-0.11	-0.011
63	0.045	-0.014	-0.029	-0.838	0.024	-0.043	0.026	0.008	-0.015	0.046	0.027
28	-0.056	-0.048	0.025	-0.017	-0.038	0.005	0.973	-0.016	0.001	-0.003	0.081
80	-0.062	-0.021	0.017	-0.016	-0.028	-0.006	0.971	-0.018	0.021	0.02	0.182
27	0.247	-0.002	0.069	-0.018	0.052	0.064	0.936	0.009	-0.017	-0.139	-0.032
29	0.123	0.036	0.017	-0.008	-0.029	0.017	-0.003	0.984	-0.017	0.003	-0.009
30	0.123	0.036	0.017	-0.008	-0.029	0.017	-0.003	0.984	-0.017	0.003	-0.009
31	0.123	0.036	0.017	-0.008	-0.029	0.017	-0.003	0.984	-0.017	0.003	-0.009
73	-0.009	0.031	-0.067	-0.019	0.925	-0.121	-0.001	0.014	-0.087	-0.005	0.076
72	-0.016	-0.002	-0.068	-0.025	0.878	-0.126	-0.042	0.008	-0.125	-0.013	0.159
74	0.159	0.116	0.045	-0.021	0.838	0.006	0.065	-0.042	0.014	0.054	-0.173
71	-0.232	0.018	-0.069	0.031	-0.811	0.099	0.019	0.064	0.084	-0.037	0.039
16	-0.169	0.291	-0.827	-0.037	0.043	-0.141	-0.051	-0.016	-0.138	-0.04	0.145
2	-0.122	0.241	-0.775	-0.008	-0.083	0.078	-0.034	-0.025	0.019	-0.042	-0.135
19	-0.141	-0.132	-0.754	-0.034	-0.091	0.048	-0.052	-0.027	0.123	0.399	-0.177
21	0	0.242	-0.731	-0.067	0.182	0.068	-0.043	0.001	-0.177	-0.011	0.184
20	-0.126	-0.089	-0.651	-0.048	0.043	-0.311	-0.008	0.011	-0.343	-0.042	-0.067
5	-0.117	-0.303	-0.619	-0.022	-0.123	0.008	-0.024	-0.02	0.165	0.416	-0.325
69	-0.19	-0.252	0.101	-0.02	-0.117	-0.041	0.001	-0.027	0.804	-0.228	0.126
70	-0.147	-0.286	0.046	-0.081	-0.094	-0.167	-0.053	-0.017	0.804	0.138	0.089
4	0.445	0.47	-0.1	-0.071	0.172	-0.148	-0.058	0.036	-0.505	-0.034	0.08
68	-0.093	-0.15	0.112	0.196	-0.106	0.134	0.085	-0.011	0.142	-0.801	-0.152
13	-0.016	0.1	0.097	0.011	0.013	-0.039	0.309	-0.023	0.141	0.099	0.722
3	0.499	0.416	-0.2	-0.129	0.182	-0.231	-0.064	0.056	-0.478	0.218	0.162

S6 Table. Total variance explained by factors in the biting behavior analysis and their statistical significance. P-values lower than 0.055 are identified in bold.

Factor Nb.	1	2	3	4	5	6	7	8
Variance explained, %	25.3	19.2	16.4	10.8	6.5	4.6	4.8	3.5
p-value[1]	**0.052**	0.31	0.39	0.22	0.40	0.34	0.31	0.25

[1]p-value as determined by T-test.

S7 Table. **Composition of biting behavioral factors**

Only parameters with loadings > 0.6 were shown. Parameter numbers are as in S1 Table.

	Parameter Nb.	**Definition**	**Loading**
Factor 1	35	Number of probes	-0.931
	39	Number of unsuccessful bites	-0.917
	52	Number of proboscis motions during unsuccessful bites	-0.916
	53	Duration of proboscis in motion during unsuccessful bites	-0.915
	36	Duration of probing	-0.91
	45	Duration of proboscis in motion	-0.901
	44	Total number of proboscis motions	-0.899
	60	Duration of biting	-0.848
	40	Number of probes before 1st blood ingestion	-0.775
	41	Total duration of probing before 1st blood ingestion	-0.764
Factor 2	56	Time from start to 1st blood ingestion	-0.903
	34	Time from start to 1st successful bite	-0.9
	23	Duration in contact after blood ingestion	0.861
	18	Time from 1st time in contact to 1st blood ingestion	-0.837
	17	Time from 1st time in contact to 1st probing	-0.729
	55	Length of inserted proboscis during blood ingestion	0.715
	38	Number of successful bites	0.658
	22	Duration in contact before 1st blood ingestion	-0.627
	57	Number of blood ingestion events	0.615
Factor 3	50	Average duration of proboscis in motion per successful bite	0.911
	47	Average number of proboscis motions per successful bite	0.859
	49	Duration of proboscis in motion during successful bites	0.836
	46	Average duration of proboscis in motion per probe	0.814
	37	Average duration per probe	0.811
	48	Number of proboscis motions during successful bites	0.802
	43	Number of proboscis motions per probe	0.717
Factor 4	54	Average duration of proboscis in motion per unsuccessful bite	0.924
	51	Average number of proboscis motions per unsuccessful bite	0.924
	42	Average duration of probing before 1st blood ingestion	0.910
Factor 5	58	Duration of blood ingestion	0.903
	59	Average duration of blood ingestion event	0.897

S8 Table. Parameter loadings in each of the factors of the biting behavior multivariate analysis. Parameter numbers are as in S1 Table.

Highly defining parameters; loading > 0.6
Moderately defining parameters; 0.3 < loading < 0.6

Parameter Nb.	Factor number							
	1	2	3	4	5	6	7	8
35	-0.931	-0.06	-0.16	-0.141	-0.082	0.021	-0.03	0.006
39	-0.917	-0.142	-0.191	-0.125	-0.109	0.007	0.039	0.024
52	-0.916	-0.139	-0.05	0.277	-0.101	0.02	0.009	0.005
53	-0.915	-0.194	-0.046	0.231	-0.12	-0.098	0.03	0.02
36	-0.91	-0.085	0.282	0.17	-0.094	-0.083	-0.126	0.013
45	-0.901	-0.093	0.294	0.17	-0.098	-0.085	-0.129	0.01
44	-0.899	-0.046	0.258	0.203	-0.072	0.047	-0.144	-0.023
60	-0.848	0.019	0.319	0.151	0.277	-0.082	-0.222	0.017
40	-0.775	-0.311	-0.202	-0.182	-0.057	-0.126	0.193	0.086
41	-0.764	-0.365	0.063	0.292	-0.026	-0.231	0.201	0.077
22	-0.582	-0.627	-0.038	0.278	-0.074	-0.133	0.082	0.105
33	-0.571	-0.473	-0.06	0.188	-0.078	-0.608	0.111	0.065
56	-0.195	-0.903	0.025	0.098	-0.071	-0.105	0.071	0.131
34	-0.199	-0.9	-0.029	0.104	-0.096	-0.108	0.051	0.128
23	0.145	0.861	0.002	-0.032	0.054	0.095	-0.07	0.4
18	-0.325	-0.837	-0.089	0.261	-0.078	-0.176	0.016	0.071
17	0.219	-0.729	-0.065	0.175	-0.023	0.505	-0.118	0.03
55	0.196	0.715	0.337	-0.056	0.203	0.16	0.03	-0.003
38	0.027	0.658	0.275	-0.108	0.227	0.107	-0.547	-0.145
57	0.021	0.615	0.274	-0.14	0.178	0.079	-0.612	0.002
61	0.32	0.591	0.015	-0.04	0.465	0.142	0.172	-0.14
50	-0.025	0.234	0.911	-0.086	0.09	0.046	-0.018	-0.004
47	-0.06	0.197	0.859	-0.151	0.106	0.123	-0.052	-0.094
49	-0.095	0.223	0.836	-0.118	0.037	0.018	-0.391	-0.023
46	0.004	-0.152	0.814	0.399	-0.025	-0.077	0.136	0.138
37	0.021	-0.123	0.811	0.4	-0.021	-0.076	0.155	0.145
48	-0.138	0.217	0.802	-0.139	0.055	0.074	-0.402	-0.074
43	-0.019	-0.131	0.717	0.528	0.084	0.101	0.136	0.045
54	-0.227	-0.19	0.009	0.924	-0.077	-0.04	0.028	0.062
51	-0.162	-0.101	0.031	0.924	0.02	0.076	0.037	0.023
42	-0.168	-0.173	0.086	0.91	0.009	-0.057	0.01	0.05
58	0.055	0.246	0.122	-0.029	0.903	-0.008	-0.25	0.01
59	0.17	0.138	0.077	0.006	0.897	0.06	0.126	-0.01
15	-0.08	-0.358	-0.139	-0.027	-0.078	-0.824	-0.046	0.008
62	0.372	0.4	0.038	0.035	0.406	0.179	0.564	-0.144
14	0.097	0.111	-0.05	-0.121	0.033	0.022	-0.003	-0.955

S9 Table. Duration before proboscis insertion for the transmission assay.

Mosquito number	Successive bite number	Duration before proboscis insertion[1]
1	1	< 1 min
	2	< 1 min
	3	< 1 min
2	1	< 1 min
	2	< 1 min
	3	< 2 min
3	1	< 1 min
	2	< 1.5 min
	3	< 1 min
4	1	<1 min
	2	< 2 min
	3	< 3 min
5	1	< 1 min
	2	< 3 min
	3	< 1 min
6	1	< 1 min
	2	< 3 min
	3	< 1 min
7	1	< 1 min
	2	< 2 min
	3	< 2 min
8	1	< 1 min
	2	< 2 min
	3	< 1 min

[1] Proboscis insertion was monitored visually.