Supporting Information

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SI Methods

Mosquito Rearing and Blood Feeding. *Anopheles gambiae* sensu stricto (SUA 2La/2La), an M-form isolate originating from Suakoko, Liberia, were reared in the Vanderbilt Insectary Facility as described. Four- to 6-d-old adult mosquitoes were reared together and divided into two cohorts, one of which received 45 min of access to "humanized" mice [anesthetized mice, draped in a well-worn and unwashed athletic sock to provide human-derived foot odor, following the guidelines set forth by the Division of Animal Care and approved by the Vanderbilt Institutional Animal Care and Use Committee (protocol M/08/069)] in the middle of the dark phase of the light cycle (~ZT18). Both cohorts had constant access to 10% (wt/vol) sucrose solution.

Mosquito Rearing for Oviposition Assay. Larvae were reared under low density (>1 cm^2 water surface per larva) in deionized water to ensure large size adults. Larval food was provided ad libitum as fine ground powder mix of yeast and fish food (Kaytee Products) at 1:2 ratio. Pupae were hand collected and allowed to eclose in a small cage overnight. Uneclosed pupae were removed the following morning to synchronize the age of adults within the same cage. Females and males were reared together at a density of ~200 adults per cage and were given free access to 10% sucrose solution. After 5 d, females were provided with human blood (Bioreclamation) by using Hemotek membrane feeding system (Hemotek). Human foot odor (derived from a well-worn and unwashed athletic sock that draped the feeder) and 5% CO_2 were provided to promote blood feeding. Human blood was stored at 4 °C and not used more than 6 d after being collected from donor.

RNA Isolation and RNA Sequencing. Over the 2 d after blood feeding, ~200 female mosquitoes were collected from each cohort at each of the five sequential, post–blood-feeding time points (1, 12, 24, 36, and 48 h). Blood-fed females were regularly and repeatedly assessed for egg development, and we confirmed that more than 95% of females assessed as being "fully blood fed" were gravid. In all, 10 tissue samples were collected. For each collection, tissues were hand-resected into TRIzol, and total RNA was isolated. mRNA isolation and cDNA library preparation were carried out by using the Illumina mRNA sequencing kit (Illumina). Libraries were barcoded and sequenced in 100-bp, paired-end fashion, on a single lane of an Illumina HiSeq2000. Approximately 30 million reads were generated for each sample.

Data Processing and Abundance Profiling. Individual Illumina read files (fastq) were trimmed and filtered by using Trimmomatic, a software package specifically designed for trimming NGS reads. Paired-end Trimmomatic parameters used were as follows: LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36. FastQC was used for dataset quality checking. Bowtie2 was used to build an index of the soft RepeatMasked assembly of the *An. gambiae* genome (P3) merged with the *An. gambiae* mitochondrial genome. Reads were then aligned to the index using Bowtie2. Transcripts abundances were calculated by extracting the uniquely mapping reads from the Bowtie2 alignments using a Python script (HTSeq) in conjunction with the version 3.6 gene annotation file of the genome. For the purposes of mapping and quantitation, all alternate transcript isoforms for a given gene were collapsed under that gene's respective, *An. gambiae* gene annotation (AGAP) designation. Abundance levels of each AGAP transcript in each sample was expressed in terms of reads per kilobase per million reads mapped as calculated from the numbers of uniquely mapping reads (1).

Differential Transcript Abundance Calculation. Statistical significance along with fold change was determined by pairwise comparison of the previously generated blood-fed (Bf) and non–blood-fed (nBf) mapped read sets, at each of the five time points by using GFOLD (2) configured for a 95% confidence interval. The result was a set of GFOLD values (GFOLD "reliable" log2 fold change) for each AGAP at each time point; GFOLD values other than zero are considered as significantly, differentially expressed. DEsEq. (3) normalization factors were then extracted from the GFOLD output and used to scale the read count of each sample pair at each time point. Transcripts that displayed at least 40 DEseq normalized reads (equivalent to ~2 RPKM) in at least one of the 10 samples were classified as "detectable."

Overrepresentation Analysis. Hypergeometric probabilities were calculated (4) based on positive, negative or neutral GFOLD values and are all relative to the Bf sample. Overrepresented gene families were those with a calculated $P > 0.95$. Underrepresented gene families were those with a calculated $P < 0.05$.

Cluster Analysis. K-means clustering analysis was conducted on the GFOLD values of differential expression across the five time points. Every transcript that displayed a GFOLD value of greater than +1 or less than −1 at any of the time points was included in the analysis. Of the 13,320 annotated genes in the *An. gambiae* genome, 1,235 met the criteria for inclusion in the cluster analysis.

Odorant Receptivity Changes. Relative differences in odorant receptivity between the Bf and the nBf cohorts was calculated from physiologic, odorant-response data from previously published functional deorphinization of *An. gambiae* odorant receptors. The SSR data were first filtered to remove any AgOrs or chemicals that did not elicit a 50-spikes-per-second increase over baseline in at least one assay. Odor-induced decreases in spiking frequency were treated as indeterminate and treated as zero, resulting in response data of 31 AgOrs to 69 odorants. The response of each AgOr (spikes per second increase) to each odorant was then weighted by the abundance level (number of DEseq normalized, mapped reads) of that AgOr. Odorant responses in weighted spikes per second were then summed for each odorant. This weighting was repeated for each cohort at each time point. Finally, the post-blood feeding "receptivity change" of the antenna to each odorant was calculated per odorant by dividing the summed weighted spikes per second for each chemical in the Bf group by the summed weighted spikes per second for each chemical in the nBf group. The raw results are reported in Dataset S1.

Dual Choice Oviposition Assay. To prepare gravid females for the bioassay, 5- to 6-d-old females were allowed to blood feed, and only fully engorged females were transferred into a separate cage with access to 10% sucrose solution. Gravid mosquitoes were reared in the same environmental chamber (27 °C, 80% relative humidity, light:dark = 12:12 h) in which the bioassay was conducted and were assayed 2 d after blood feeding. Ten gravid females were transferred into the "releasing chamber" for each "assay" (Fig. 4). The releasing chamber has a screen on top, and the females were only allowed to enter assay cage (14.9 × 15.2 × 13 cm, polypropylene) through a center pathway (diameter = 6 cm) after dark cycle began. Seven milliliters of test water [10^{-4} M solution, 0.1% DMSO in double distilled water (ddH2O)] and control water

(0.1% DMSO in ddH2O) were contained in PET cup (top diameter = 4.5 cm, height = 4.1 cm, bottom diameter = 2.9 cm) at 26-cm distance in the middle of cage. Location of the test water cup was rotated between cages to minimize any potential positional effects, and assay cages were randomly placed within in a larger acrylic enclosure (acrylic, 86 × 120 × 86 cm) to minimize external effects on oviposition behavior. Assay cages were cleaned by using 70% EtOH and dried > 24 h in chamber before assay. Collected eggs were counted to calculate an oviposition preference ratio between test and control water cups. Proportion data were square root arcsine transformed to meet normality, and paired *t* test (one-tailed) was used to examine oviposition preference to test water over control water. Three different cohorts of mosquitoes were tested in each of three different sets of assays.

1. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature methods* 5(7):621–628.
2. Feng J, et al. (2012) GFOLD: a generalized fold change for ranking differentially expressed genes from RNA-seq data. *Bioinformatics* 28(21):2782–2788.
3. Anders S, Huber W (2010) Differential expression analysis for sequence count data. *Genome biology* 11(10):R106.
4. Zhang B, Kirov S, Snoddy J (2005) WebGestalt: an integrated system for exploring gene sets in various biological contexts. *Nucl Acids Res* 33(suppl 2):W741–W748.

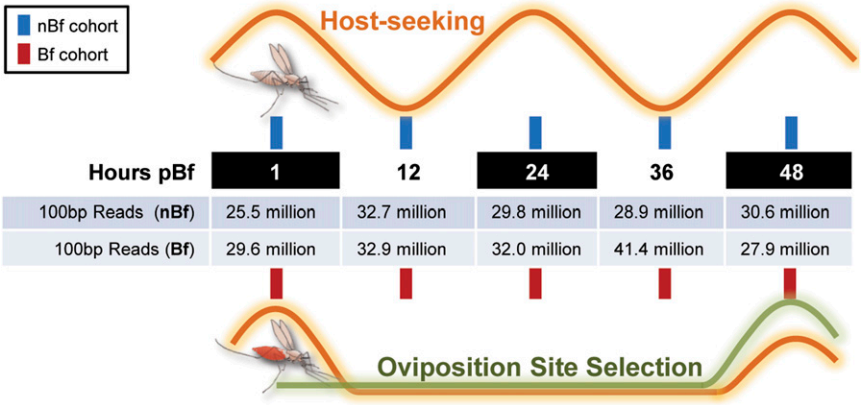

Fig. S1. Experimental rational, design, and Illumina read counts. (*Upper*) Cartoon of the diel nature of adult female *An. gambiae* host seeking behaviors (orange) as it varies with the light-dark cycle (white-black bars). (*Lower*) Cartoon of the blood meal-dependent, temporary suspension of host seeking behaviors and the appearance of behaviors predicated on ovipositon site selection. The present study divided a population of 4- to 6-d-old adult mosquitoes into two cohorts, a non–blood-fed (nBf) cohort maintained on a sugar meal, and a blood-fed cohort (Bf) that received a blood meal in the middle of the dark phase of the first night. Tissue collections were made at time points (1, 12, 24, 36, and 48 h) following the blood feeding from the nBf cohort (blue) and the Bf cohort (red). RNA from each time point was Illumina sequenced, and the read count for each sample is displayed adjacent to its respective time point and cohort.

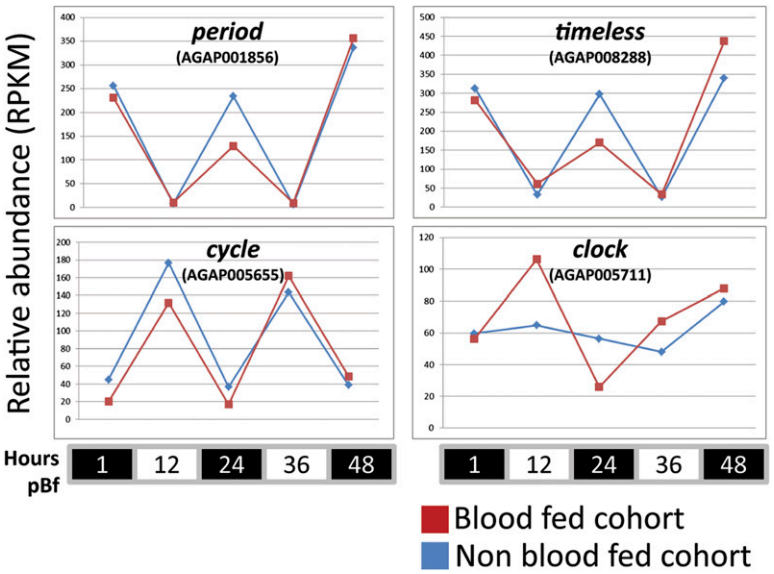

Fig. S2. Time series patterns of abundance of the four genes of the circadian molecular clock.

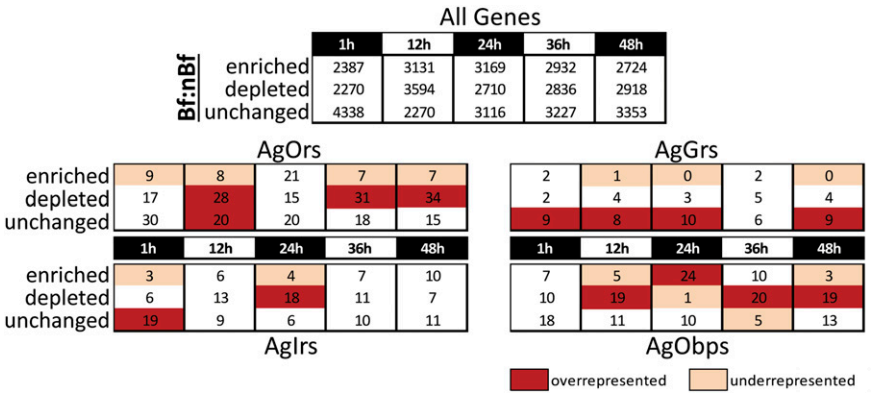

Fig. S3. Chemosensory transcript enrichments or depletions in antennae following a blood meal. Enriched, number of annotated genes (AGAPs) that showed significantly higher (GFOLD value > 0) abundances in the post–blood-fed (Bf) sample relative to the non–blood-fed (nBf) sample. Depleted, number of AGAPs that showed significantly lower (GFOLD value < 0) abundances in the Bf sample. Unchanged, number of AGAPs that showed no difference in transcript abundance (GFOLD value = 0) in either sample. (*Upper*) Differential expression status of all, detectable AGAPs (8,995 AGAPS in total) at each of the five time points. (*Lower*) Differential expression status of all detectable members of each of the four chemosensory gene families. Colored cells indicate a differential expression status for that gene family at that time point that is either greater than expected at random (overrepresented) or less than expected at random (underrepresented).

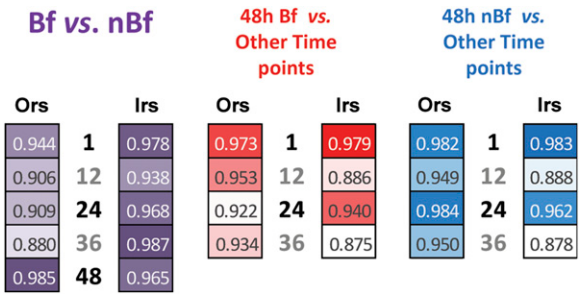

Fig. S4. Spearman coefficients for transcript-abundance-based rank order comparisons of *An. gambiae* odorants (AgOrs) and *An. gambiae* ionotropic glutamates (AgIrs). (*Left*) Per time point Spearman coefficients of AgOr and AgIr rank orders within blood-fed (Bf) and non–blood-fed (nBf) antenna. (*Center*) Spearman coefficients of AgOr and AgIr rank orders between the 48-h time point and each of the other four time points in the Bf sample. (*Right*) Spearman coefficients of AgOr and AgIr rank orders between the 48-h time point and each of the other four time points in the nBf sample.

Dataset S1. RNAseq read count data

Dataset S1

Worksheet 1: Listing of AGAPs, AGAP transcript length, nBf time point read counts and Bf time point read counts. Worksheet 2: GFOLD (0.05) values for each time point comparison Bf:nBf. Worksheet 3: AGAP clustering output from K-means cluster analysis along with fold change values. Worksheet 4: SSR physiology data weighted by DESeq-normalized, AgOr read counts, and summed per odorant.