Cite as: S. N. Emami *et al.*, *Science* 10.1126/science.aah4563 (2017).

A key malaria metabolite modulates vector blood seeking, feeding, and susceptibility to infection

S. Noushin Emami,[1] Bo G. Lindberg,[1] Susanna Hua,[1] Sharon Hill,[2] Raimondas Mozuraitis,[3,4] Philipp Lehmann,[5] Göran Birgersson,[2] Anna-Karin Borg-Karlson,[3] Rickard Ignell,[2] Ingrid Faye[1]*

[1]Department of Molecular Biosciences, Wenner-Gren Institute, Stockholm University, SE 106 91 Stockholm, Sweden. [2]Unit of Chemical Ecology, Department of Plant Protection Biology, SLU, SE 230 53 Alnarp, Sweden. [3]Department of Chemistry, Organic Chemistry, Royal Institute of Technology, SE 100 44 Stockholm, Sweden. [4]Laboratory of Chemical and Behavioral Ecology, Institute of Ecology, Nature Research Centre, LT-08412 Vilnius, Lithuania. [5]Department of Zoology, Stockholm University, SE 106 91 Stockholm, Sweden.

***Corresponding author. Email: ingrid.faye@su.se**

Malaria infection renders humans more attractive to *Anopheles gambiae* sensu lato mosquitoes than uninfected people. The mechanisms remain unknown. Here, we show that an isoprenoid precursor produced by *Plasmodium falciparum*, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), affects *A. gambiae* s.l. blood meal seeking and feeding behaviors, as well as susceptibility to infection. HMBPP acts indirectly by triggering human red blood cells to increase the release of CO_2, aldehydes, and monoterpenes, which together enhance vector attraction, and stimulate vector feeding. When offered in a blood meal, HMBPP modulates neural, antimalarial, and oogenic gene transcription without affecting mosquito survival or fecundity, while in a *P. falciparum* infected blood meal, sporogony is increased.

Earlier studies have shown that mosquito vectors are more attracted to hosts infected with malaria than to healthy hosts, including humans, mice and birds (*1*–*4*). The *Plasmodium* parasites infecting these animals differ, and the vector species attracted and transmitting the disease include *Anopheles* and *Culex* mosquitoes. The increase in attraction coincides, at least in part, with changes in odor profiles of the respective hosts carrying malaria (*5*–*7*). Behavioral alterations are also conferred by other Apicomplexan parasites such as *Toxoplasma gondii*, which increases its transmission between infected prey and predator hosts (*8*, *9*). The molecular mechanisms remain unknown.

HMBPP is a precursor in the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway for the synthesis of isopentenyl pyrophosphate (IPP) and its isoform, dimethylallyl pyrophosphate (DMAPP), are building blocks for isoprenoids. All higher eukaryotes, including humans and mosquitoes, use the mevalonate pathway for IPP and DMAPP synthesis. By contrast, most eubacteria and apicomplexan parasites, including *Plasmodium falciparum* (*10*) use the alternative MEP pathway. HMBPP is an activator of human Vγ9Vδ2 T-cells and triggers innate immune responses in *Anopheles gambiae* s.l. (*10*, *11*).

We observed a propensity of female mosquitoes to land and feed on membrane feeders containing HMBPP-supplemented red blood cells (hmbRBCs) compared with control red blood cells (RBCs) (Fig. 1A). In a dual choice attraction bioassay, 95% of the host-seeking mosquitoes chose hmbRBCs over RBCs, indicating the involvement of volatile factors derived from hmbRBCs. HMBPP-supplemented serum or glucose solution (5%) containing para-aminobenzoic acid (PABA 0.05%) did not, however, increase mosquito attraction, which suggests that the attraction is an RBC dependent effect (Fig. 1A). The diol, (2*E*)-2-methylbut-2-ene-1,4-diol, a putative volatile form of HMBPP, had no effect on the attraction of mosquitoes to RBCs, indicating that the phosphate groups are required for the activity of HMBPP (fig. S1).

We compared RBC feeding rates with hmbRBCs, *P. falciparum* asexual trophozoite-, and gametocyte-infected RBCs (tiRBCs and giRBCs, respectively). The proportion of females that fed more than doubled when hmbRBCs, tiRBCs or giRBCs were provided (Fig. 1B). We next investigated whether the amount of HMBPP released in the medium of giRBCs was sufficient to stimulate mosquito blood feeding. The proportion of mosquitoes feeding on RBCs supplemented with HMBPP or supernatants from giRBCs, respectively, were compared over a wide range of concentrations (Fig. 1, C and D). This confirmed that 10 μM HMBPP, used in Fig. 1, A and B, corresponded to the concentration present in the undiluted supernatant from giRBCs, and also that substantially lower doses were sufficient to trigger enhanced mosquito feeding (Fig. 1C). Moreover, treatment of tiRBCs with a fosmidomycin derivative to block HMBPP synthesis (*12*) reduced feeding to control levels, despite supplementation with the downstream metabolite IPP (Fig. 1D, triangle).

Thus, HMBPP released from parasite-infected RBCs is a key metabolite for triggering mosquito feeding stimulation.

To further decipher the phagostimulatory action of HMBPP, we provided cell-free meals to mosquitoes and examined the percentage of females that landed and initiated probing and feeding (referred to as percent persistency within 5 min) (Fig. 1, E and F). Approximately 80% of the mosquitoes displayed behavioral persistence when provided HMBPP-supplemented serum compared with 20% of those provided with serum alone (Fig. 1E). Supplementation of a physiological salt solution with HMBPP generated a similar effect (Fig. 1F). Hence, HMBPP acts as a phagostimulant that is neither dependent on factors from RBCs, nor from the serum. IPP displayed no phagostimulatory effect, suggesting a direct activity of HMBPP (Fig. 1F).

Using a larger Y-tube olfactometer, we confirmed that hmbRBCs could mimic the attraction of mosquitoes to giRBCs, while IPP supplemented RBCs (ippRBCs) did not (Fig. 2A). We concluded that HMBPP indirectly stimulates attraction via the release of volatiles from RBCs, and acts directly as a feeding stimulant, whereas the structurally similar downstream IPP confers neither of these effects (*13*, *14*). These findings all point to parasite-derived HMBPP modulatory effects within the malaria-infected human host affecting the blood seeking and feeding behaviors of its vector, the anopheline mosquito.

To further study the RBC-dependent effects of HMBPP on mosquito attraction, we investigated the volatiles released from hmbRBC, giRBC and RBCs. Since carbon dioxide (CO_2), emitted from vertebrates through skin and breath, is a key activator and attractant for host-seeking anopheline mosquitoes (*15*, *16*), we first quantified CO_2 emission. Through combined gas chromatography-mass spectrometry (GC-MS) (*15*) and quantitative respirometry we estimated CO_2 release. The amount of CO_2 released into the gas headspace above hmbRBC suspensions increased by 16% compared with RBCs alone (Fig. 2B and fig. S2, A and B), an increase that is sufficient to promote mosquito attraction (*16*, *17*). Carbon dioxide supplementation of RBCs was, however, not sufficient to fully reproduce mosquito attraction to hmbRBCs (Fig. 2A), indicating the involvement of additional volatiles. To identify these volatiles, we collected the headspace above hmbRBC and giRBC suspensions and assayed the behavioral response of mosquitoes in the presence or absence of CO_2 (Fig. 2A). The response to headspace extracts of hmbRBCs and giRBCs, in the presence, but not in the absence of CO_2, fully reproduced that of hmbRBCs (Fig. 2A). Solid-phase microextraction (SPME) and GC-MS analyses of the headspace from hmbRBCs identified an increase in aldehydes (C8-C10:al; 1.7-to-5.2 fold) and monoterpenes (α- and ß-pinene, limonene; 1.2-to-1.6 fold) compared with that of the headspace of RBCs (Fig. 2, C and D, and fig. S3). A synthetic blend of these volatiles with CO_2, at their enhanced natural emission rates and ratios, was able to reproduce the behavioral attraction of *A. gambiae* to hmbRBCs in a dose dependent manner (Fig. 2E). The synthetic blend also attracted females more strongly than that of RBCs alone, both with and without CO_2 (Fig. 2E). This indicates that the HMBPP-modulating effects on host-seeking of anophelines depend on an odor blend in the right context. In conclusion, via HMBPP, we found that *P. falciparum* enhanced the emission of RBC-derived attractants, which act together to increase the likelihood of parasite transmission to the mosquito vector.

HMBPP significantly increased the size of mosquito blood meals (Fig. 3C), independently of mosquito body size (fig. S4A). A previous laboratory study indicated that the parasite can cause an increase in the amount of blood imbibed by the mosquito, which could potentially increase nutrient gain and enhance reproductive capacity of the vector (*18*). However, we found that neither mosquito fecundity, nor survival was affected by HMBPP, despite larger blood meals (Fig. 3, A and B).

HMBPP is released into the blood by *P. falciparum*, and has the potential to regulate parasite sporogonic success in the mosquito. Since inhibition of the *Plasmodium* MEP pathway is lethal without continuous IPP addition (*12*), we tested this by feeding mosquitoes with giRBCs with or without additional HMBPP and subsequently monitored the burden of infection in the mosquito during the parasite sporogonic period. We used equal gametocyte density in both treatments (3%). Oocyst prevalence (proportion of oocyst-carrying mosquitoes) was not significantly different between treatments (close to 100% in both groups; fig. S4B), whereas oocyst intensity (number of oocyst per midgut) was higher in mosquitoes fed on HMBPP-supplemented giRBCs (Fig. 3D). The addition of HMBPP to blood meals also resulted in significantly higher sporozoite prevalence (proportion of sporozoite-carrying mosquitoes) and intensity (number of sporozoites per salivary gland) (Fig. 3E and fig. S4C). Taken together, these findings show that HMBPP per se has no obvious deleterious effects on vector fitness, but increases the mosquito susceptibility to *Plasmodium* infection in terms of parasite prevalence (proportion of parasite-transmissible mosquitoes) and intensity (parasite loads).

To gain an overall view of the initial effects of HMBPP on vector mosquito transcription, Illumina sequencing was performed on whole body RNA extracts from fully engorged females, 1, 3, 6 and 24 hours post-ingestion (hpi), fed on RBCs or hmbRBCs (Table 1 and table S1). Pathway analysis and ontology revealed a set of synaptic genes upregulated at 3 hpi that play roles in multiple neural pathways. Homologs of neuronal synaptobrevin (AGAP005507) and synaptotagmin 1 (AGAP007942) (*19*, *20*) are involved in vesicle traffick-

ing in *Drosophila*, while N-methyl-D-aspartate receptor 2 (AGAP012429) (*21*) and dopamine β-monooxygenase (AGAP010485) (*22*) are involved in modulating the activity of neural synapses. These findings indicated that the hmbRBC meal affects gene expression of the modulatory machinery at neural synapses. Moreover, axon guidance protein gene (AGAP008943), homologous to *turtle* in *Drosophila* (CG16857), as well as dopamine β-monooxygenase (AGAP010485), are known to be indirectly involved in oogenesis (*23*).

Among the major biological processes affected by the presence of HMBPP, are several endopeptidase-encoding genes, such as trypsins, chymotrypsins, serine proteases and an aminopeptidase (tables S1 and S2). At 1 and 3 hpi these transcripts increase, possibly as an effect of the increased blood meal size (*24*), when females are provided hmbRBC (Fig. 3C). The majority of these enzyme encoding genes are downregulated at 6 and 24 hpi (table S2).

The temporal changes of several immune gene transcripts observed are in agreement with those previously reported (*11*). Notably, the antimicrobial peptides *cecropin 1* and *lysozyme C1* were generally upregulated at early time points but were downregulated by 6 hpi, and increased again at 24 hpi, which coincides with the time when ookinetes traverse the midgut in malaria-infected *Anopheles*.

Additionally, antiplasmodial factors involved in the complement-like thioester-containing protein 1 (TEP1) complex known to bind to malaria ookinetes and oocysts were differentially regulated (*25*). These include TEP1, leucine-rich immune proteins (LLRs), including APL1A, APL1C, LRIM1, LRIM17, as well as CLIP serine proteases (*26*, *27*). The LLRs were downregulated at 3 hpi, while their counterpart TEP1 was upregulated at 24 hpi together with APL1C (Table 1), indicating that the TEP1 complex response was affected in hmbRBC fed mosquitoes.

A general downregulation of mosquito oogenesis genes, such as the lipid transporting vitellogenins (AGAP000420*3*, AGAP001826 and AGAP008369), haem peroxidase HPX8 (AGAP004038) as well as the *Drosophila fused* ortholog (AGAAGAP012519) was observed (Table 1). The gene, *fused* encodes a serine/threonine kinase that regulates *hedgehog* signaling and is crucial for the embryonic development of the fertilized egg (*28*, *29*). A previous laboratory study indicated that *Plasmodium* parasites can cause an increase in the amount of blood imbibed by the mosquito that could potentially increase nutrient gain and enhance reproductive capacity of the vector (*18*). However, neither mosquito fecundity, nor survival was affected by HMBPP despite the stimulated larger blood meals (Fig. 3, A and B). The reduction observed in transcription of oogenesis genes may reflect a balancing effect, maintaining normal fecundity despite the increased hmbRBC blood-volume imbibed (Fig. 3, A and B).

Notably, at 24 hpi, 80% of the differentially expressed genes are downregulated and 10% of these encode components of the ribosomal complex as well as other genes involved in mRNA translation (Table 1). HMBPP thus appears to suppress translation, in turn suppressing synthesis of protein and peptide mediators of the vector innate immune response and oogenesis (*30*). Therefore, a decrease in transcription (and translation) would consequently increase the number of parasites, which is in line with our results (Fig. 3, D and E). In an attempt to interpret the final outcome of this seemingly detrimental effect, we estimated the protein levels in HMBPP-treated and control mosquitoes at 24, 48 and 72 hpi. No significant difference in the total levels of proteins was detected, which again indicates the ability of the mosquito to maintain homeostasis, despite the manipulating effects of HMBPP (fig. S4D).

In summary, the transcriptional regulation of synaptic genes may affect behavioral modulation observed in the mosquito, while the regulation of immune genes indicates that the vector can still recognize and respond to HMBPP as a foreign entity. However, while the translational machinery is hampered by the downregulation of numerous ribosomal genes at 24 hpi, mosquito survival remains normal in hmbRBC-fed mosquitoes (Fig. 3A), which is crucial for parasite transmission. (*30*). Likewise, the lower levels of oogenesis gene transcription maintain fecundity at control levels despite the increased amount of blood imbibed (Fig. 3). Thus, we hypothesize that the extra blood imbibed is digested by the vector and diverted to the parasite to increase proliferation and thereby amplified transmission.

We have shown that HMBPP triggered an enhanced release of attractants from infected blood, increasing the likelihood that an infected human host gets bitten by the vector. Moreover, HMBPP directly stimulated mosquito probing and increased blood meal size, leading to an increased probability of parasite transmission. HMBPP also manipulated mosquito physiology to enhance receptivity to infection, without negatively impacting the fitness of the mosquito host. Transcriptome analyses confirmed that parasite-derived HMBPP modulated the expression of behavioral, physiological and immune response genes in its anopheline host (fig. S5).

REFERENCES AND NOTES

1. J. C. Koella, F. L. Sørensen, R. A. Anderson, The malaria parasite, *Plasmodium falciparum*, increases the frequency of multiple feeding of its mosquito vector, *Anopheles gambiae*. *Proc. R. Soc. B* **265**, 763–768 (1998). doi:10.1098/rspb.1998.0358 Medline
2. R. Lacroix, W. R. Mukabana, L. C. Gouagna, J. C. Koella, Malaria infection increases attractiveness of humans to mosquitoes. *PLOS Biol.* **3**, e298 (2005). doi:10.1371/journal.pbio.0030298 Medline
3. E. P. Batista, E. F. Costa, A. A. Silva, *Anopheles darlingi* (Diptera: Culicidae) displays increased attractiveness to infected individuals with *Plasmodium vivax*

gametocytes. *Parasit. Vectors* **7**, 251 (2014). doi:10.1186/1756-3305-7-251 Medline
4. S. Cornet, A. Nicot, A. Rivero, S. Gandon, Malaria infection increases bird attractiveness to uninfected mosquitoes. *Ecol. Lett.* **16**, 323–329 (2013). doi:10.1111/ele.12041 Medline
5. C. M. De Moraes, N. M. Stanczyk, H. S. Betz, H. Pulido, D. G. Sim, A. F. Read, M. C. Mescher, Malaria-induced changes in host odors enhance mosquito attraction. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 11079–11084 (2014). doi:10.1073/pnas.1405617111 Medline
6. M. Kelly, C.-Y. Su, C. Schaber, J. R. Crowley, F.-F. Hsu, J. R. Carlson, A. R. Odom, Malaria parasites produce volatile mosquito attractants. *MBio* **6**, e00235-15 (2015). doi:10.1128/mBio.00235-15 Medline
7. A. Z. Berna, J. S. McCarthy, R. X. Wang, K. J. Saliba, F. G. Bravo, J. Cassells, B. Padovan, S. C. Trowell, Analysis of breath specimens for biomarkers of *Plasmodium falciparum* infection. *J. Infect. Dis.* **212**, 1120–1128 (2015). doi:10.1093/infdis/jiv176 Medline
8. A. Vasudevan, V. Kumar, Y. N. Chiang, J. Y. Yew, S. Cheemadan, A. Vyas, α2μ-globulins mediate manipulation of host attractiveness in *Toxoplasma gondii-Rattus norvegicus* association. *ISME J.* **9**, 2112–2115 (2015). doi:10.1038/ismej.2015.33 Medline
9. M. A. Hakimi, A. Bougdour, *Toxoplasma*'s ways of manipulating the host transcriptome via secreted effectors. *Curr. Opin. Microbiol.* **26**, 24–31 (2015). doi:10.1016/j.mib.2015.04.003 Medline
10. C. T. Morita, C. Jin, G. Sarikonda, H. Wang, Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vγ2Vδ2 T cells: Discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. *Immunol. Rev.* **215**, 59–76 (2007). doi:10.1111/j.1600-065X.2006.00479.x Medline
11. B. G. Lindberg, E. A. Merritt, M. Rayl, C. Liu, I. Parmryd, B. Olofsson, I. Faye, Immunogenic and antioxidant effects of a pathogen-associated prenyl pyrophosphate in *Anopheles gambiae*. *PLOS ONE* **8**, e73868 (2013). doi:10.1371/journal.pone.0073868 Medline
12. E. Yeh, J. L. DeRisi, Chemical rescue of malaria parasites lacking an apicoplast defines organelle function in blood-stage *Plasmodium falciparum*. *PLOS Biol.* **9**, e1001138 (2011). doi:10.1371/journal.pbio.1001138 Medline
13. M. E. Smalley, J. Brown, N. M. Bassett, The rate of production of *Plasmodium falciparum* gametocytes during natural infections. *Trans. R. Soc. Trop. Med. Hyg.* **75**, 318–319 (1981). doi:10.1016/0035-9203(81)90349-7 Medline
14. A. L. Ouédraogo, S. J. de Vlas, I. Nébié, E. Ilboudo-Sanogo, J. T. Bousema, A. S. Ouattara, J. P. Verhave, N. Cuzin-Ouattara, R. W. Sauerwein, Seasonal patterns of *Plasmodium falciparum* gametocyte prevalence and density in a rural population of Burkina Faso. *Acta Trop.* **105**, 28–34 (2008). doi:10.1016/j.actatropica.2007.09.003 Medline
15. G. W. Frame, W. G. Strauss, H. I. Maibach, Carbon dioxide emission of the human arm and hand. *J. Invest. Dermatol.* **59**, 155–159 (1972). doi:10.1111/1523-1747.ep12625939 Medline
16. B. A. Omondi, S. Majeed, R. Ignell, Functional development of carbon dioxide detection in the maxillary palp of *Anopheles gambiae*. *J. Exp. Biol.* **218**, 2482–2488 (2015). doi:10.1242/jeb.116798 Medline
17. T. Dekker, M. Geier, R. T. Cardé, Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odours. *J. Exp. Biol.* **208**, 2963–2972 (2005). doi:10.1242/jeb.01736 Medline
18. H. Hurd, Manipulation of medically important insect vectors by their parasites. *Annu. Rev. Entomol.* **48**, 141–161 (2003). doi:10.1146/annurev.ento.48.091801.112722 Medline
19. D. Park, P. Li, A. Dani, P. H. Taghert, Peptidergic cell-specific synaptotagmins in *Drosophila*: Localization to dense-core granules and regulation by the bHLH protein DIMMED. *J. Neurosci.* **34**, 13195–13207 (2014). doi:10.1523/JNEUROSCI.2075-14.2014 Medline
20. Y. Kidokoro, Roles of SNARE proteins and synaptotagmin I in synaptic transmission: Studies at the *Drosophila* neuromuscular synapse. *Neurosignals* **12**, 13–30 (2003). doi:10.1159/000068912 Medline
21. F. Li, J. Z. Tsien, Memory and the NMDA receptors. *N. Engl. J. Med.* **361**, 302–303 (2009). doi:10.1056/NEJMcibr0902052 Medline
22. R. Branicky, W. R. Schafer, Tyramine: A new receptor and a new role at the synapse. *Neuron* **62**, 458–460 (2009). doi:10.1016/j.neuron.2009.05.005 Medline
23. G. Newquist, J. Hogan, K. Walker, M. Lamanuzzi, M. Bowser, T. Kidd, Control of male and female fertility by the netrin axon guidance genes. *PLOS ONE* **8**, e72524 (2013). doi:10.1371/journal.pone.0072524 Medline
24. H. M. Müller, F. Catteruccia, J. Vizioli, A. della Torre, A. Crisanti, Constitutive and blood meal-induced trypsin genes in *Anopheles gambiae*. *Exp. Parasitol.* **81**, 371–385 (1995). doi:10.1006/expr.1995.1128 Medline
25. D. J. Obbard, D. M. Callister, F. M. Jiggins, D. C. Soares, G. Yan, T. J. Little, The evolution of TEP1, an exceptionally polymorphic immunity gene in *Anopheles gambiae*. *BMC Evol. Biol.* **8**, 274 (2008). doi:10.1186/1471-2148-8-274 Medline
26. M. Williams, B. J. Summers, R. H. Baxter, Biophysical analysis of *Anopheles gambiae* leucine-rich repeat proteins APL1A1, APL1B and APL1C and their interaction with LRIM1. *PLOS ONE* **10**, e0118911 (2015). doi:10.1371/journal.pone.0118911 Medline
27. A. S. Raikhel, R. Jurenka, Eds., *Progress in Mosquito Research*, vol. 51 of *Advances in Insect Physiology* (Academic Press, 2016).
28. R. T. Lee, Z. Zhao, P. W. Ingham, Hedgehog signalling. *Development* **143**, 367–372 (2016). doi:10.1242/dev.120154 Medline
29. K. Narbonne-Reveau, F. Besse, C. Lamour-Isnard, D. Busson, A. M. Pret, *fused* regulates germline cyst mitosis and differentiation during *Drosophila* oogenesis. *Mech. Dev.* **123**, 197–209 (2006). doi:10.1016/j.mod.2006.01.001 Medline
30. I. Mohr, N. Sonenberg, Host translation at the nexus of infection and immunity. *Cell Host Microbe* **12**, 470–483 (2012). doi:10.1016/j.chom.2012.09.006 Medline
31. H. Hurd, P. J. Taylor, D. Adams, A. Underhill, P. Eggleston, Evaluating the costs of mosquito resistance to malaria parasites. *Evolution* **59**, 2560–2572 (2005). doi:10.1111/j.0014-3820.2005.tb00969.x Medline
32. T. Ponnudurai, A. D. Leeuwenberg, J. H. Meuwissen, Chloroquine sensitivity of isolates of *Plasmodium falciparum* adapted to *in vitro* culture. *Trop. Geogr. Med.* **33**, 50–54 (1981). Medline
33. D. Walliker, paper presented at the UCLA Symposia on Molecular and Cellular Biology, New Series 42 (1987).
34. W. Trager, J. B. Jensen, Human malaria parasites in continuous culture. *Science* **193**, 673–675 (1976). doi:10.1126/science.781840 Medline
35. R. Carter, L. Ranford-Cartwright, P. Alano, The culture and preparation of gametocytes of *Plasmodium falciparum* for immunochemical, molecular, and mosquito infectivity studies. *Methods Mol. Biol.* **21**, 67–88 (1993). Medline
36. H. Briegel, Determination of uric acid and hematin in a single sample of excreta from blood-fed insects. *Experientia* **36**, 1428 (1980). doi:10.1007/BF01960142
37. H. Briegel, Fecundity, metabolism, and body size in *Anopheles* (Diptera: Culicidae), vectors of malaria. *J. Med. Entomol.* **27**, 839–850 (1990). doi:10.1093/jmedent/27.5.839 Medline
38. R. S. Nasci, The size of emerging and host-seeking *Aedes aegypti* and the relation of size to blood-feeding success in the field. *J. Am. Mosq. Control Assoc.* **2**, 61–62 (1986). Medline
39. D. D. Chadee, J. C. Beier, Blood-digestion kinetics of four *Anopheles* species from Trinidad, West Indies. *Ann. Trop. Med. Parasitol.* **89**, 531–540 (1995). doi:10.1080/00034983.1995.11812986 Medline
40. J. Pawliszyn, M. J. Yang, M. L. Orton, Quantitative determination of caffeine in beverages using a combined SPME-GC/MS method. *J. Chem. Educ.* **74**, 1130–1132 (1997). doi:10.1021/ed074p1130
41. A. K. Borg-Karlson, R. Mozuraitis, Solid phase micro extraction technique used for collecting semiochemicals. Identification of volatiles released by individual signalling *Phyllonorycter sylvella* moths. *Z. Naturforsch. C* **51**, 599–602 (1996). doi:10.1515/znc-1996-7-820
42. C. Trapnell, L. Pachter, S. L. Salzberg, TopHat: Discovering splice junctions with RNA-Seq. *Bioinformatics* **25**, 1105–1111 (2009). doi:10.1093/bioinformatics/btp120 Medline
43. C. Trapnell, A. Roberts, L. Goff, G. Pertea, D. Kim, D. R. Kelley, H. Pimentel, S. L. Salzberg, J. L. Rinn, L. Pachter, Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protoc.* **7**, 562–578 (2012). doi:10.1038/nprot.2012.016 Medline
44. W. Huang, B. T. Sherman, R. A. Lempicki, Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* **37**, 1–13 (2009). doi:10.1093/nar/gkn923 Medline

45. M. Crawley, *The R Book* (Wiley, 2007).
46. E. O. Lyimo, W. Takken, Effects of adult body size on fecundity and the pre-gravid rate of *Anopheles gambiae* females in Tanzania. *Med. Vet. Entomol.* **7**, 328–332 (1993). doi:10.1111/j.1365-2915.1993.tb00700.x Medline
47. B. M. Bolker, *Ecological Models and Data in R* (Princeton Univ. Press, 2008).

ACKNOWLEDGMENTS

We thank INFRAVEC for the main funding to IF and for Illumina sequencing and Bioinformatics Access Facility to BL and SNE. We thank Sven and Lilly Lawski foundation for the Postdoctoral Scholarship to SNE. This work was supported by the Linnaeus Initiative "Insect chemical ecology, ethology and evolution, IC-E3 Formas, SLU", and the Knut and Alice Wallenberg Foundation (KAW 2012.0058). We would like to extend our thanks to Thomas Svensson for his valuable assistance and knowledge transfer in the two-choice olfactometer technique. We thank SI for a scholarship to Alexei Cravcenco, KTH, Department of Chemistry, who synthesized (2*E*)-2-methylbut-2-ene-1,4-diol. We are grateful to Lisa Ranford-Cartwright for comments and proofreading of the manuscript. Materials are made available by a Materials Transfer Agreement from Stockholm University. All data to understand and assess the conclusions of this research are available in the main text, supplementary materials and via the following repositories: The data presented in this publication have been deposited in National Centre for Biotechnology's Gene Expression Omnibus and are accessible through GEO Series accession number GSE94316. S. Noushin Emami, Ingrid Faye, Rickard Ignell, Sharon Hill, and Göran Birgersson are inventors on a patent application (GB patent application no. 1701743.5) submitted by the main applicant, S. Noushin Emami (Potter Clarkson LLP- Stockholm University) that covers the phagostimulatory effects of HMBPP and the synthetic attractant odor blend. Author contributions: IF and SNE conceived the study. The initial dual choice assay was designed by SNE, IF, and RM; and performed by SNE and RM. RI, SRH designed, and SNE performed the two-choice olfactometer assay. RM, A-K B-K and GB designed, performed the GC-MS and analyzed the results. GB also designed the SPME collection of volatiles and designed the release of volatiles for bioassay. RM, PL and SNE designed and performed the respirometry experiments. SNE designed, and SNE and SH performed the mosquito fitness study. SNE performed parasite culturing, mosquito infections as well as the feeding proportion study and the statistical analyses. BL designed the temporal transcriptome study and prepared RNA for Illumina sequencing. SNE, BL, and SRH performed bioinformatics analyses of the sequencing data. BL performed the mosquito total protein assay. BL, SNE constructed figures and tables. IF, SNE and SH designed and SH created the artwork. IF, BL, RI, SRH and SNE wrote the manuscript. All authors proofread and commented on the final draft of the manuscript. The authors declare no competing financial interests.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/cgi/content/full/science.aah4563/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S3
References (*31*–*47*)

29 June 2016; resubmitted 15 November 2016
Accepted 31 January 2017
Published online 9 February 2017
10.1126/science.aah4563

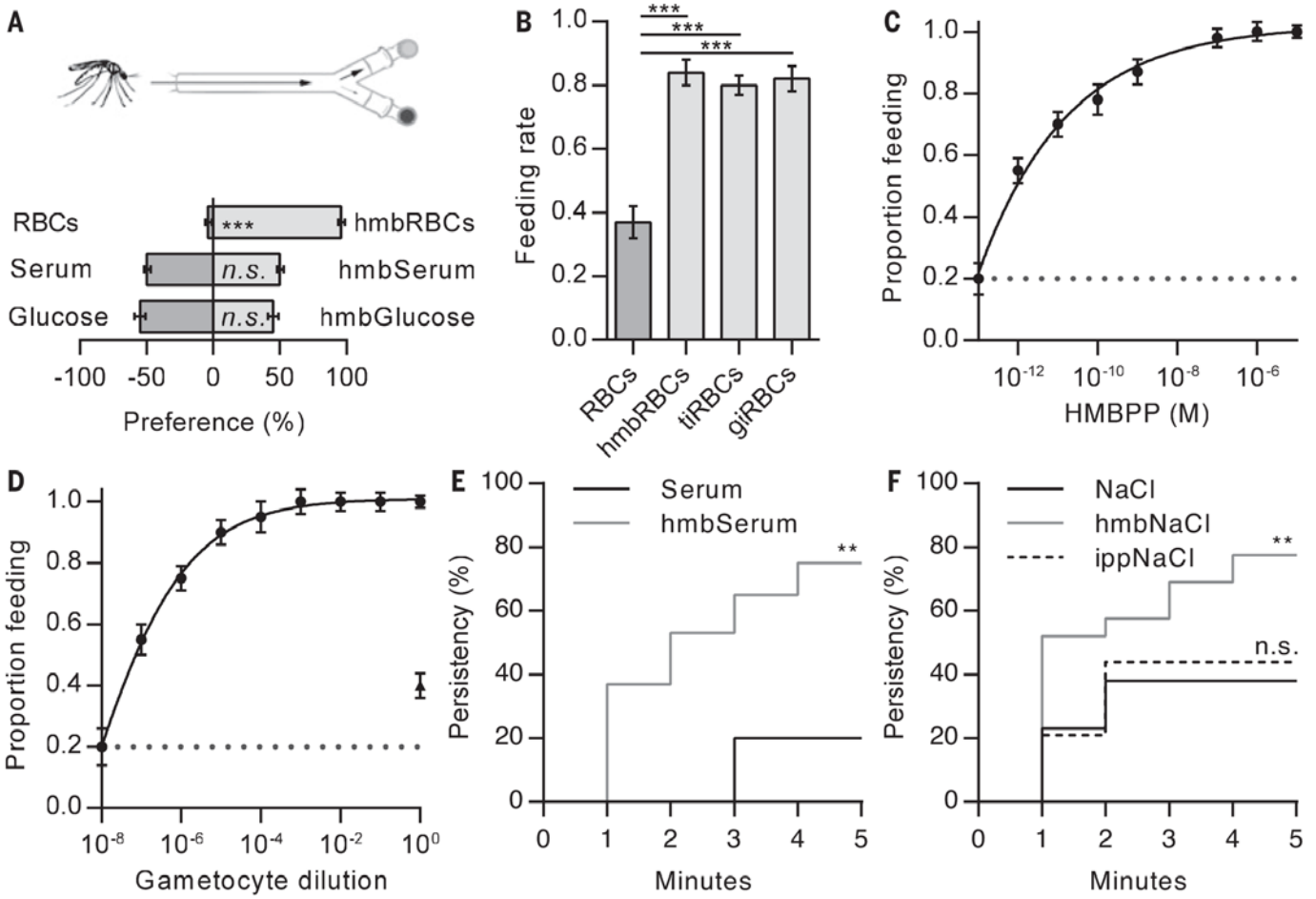

Fig. 1. Direct and indirect effects of HMBPP on mosquito attraction and feeding. (**A**) Mosquito preference (lower panel) for red blood cells (RBCs), serum and glucose with PABA in the presence or absence of HMBPP (hmb) in a dual choice attraction assay (upper panel) [RBCs: $\chi^2_1 = 29.11$, $p < 0.001$; Serum: $\chi^2_1 = 0.26$, $p = 0.60$; Glucose with PABA: $\chi^2_1 = 0.34$, $p = 0.56$]. (**B**) Feeding proportions of mosquitoes allowed to feed for 90 s on RBCs, HMBPP-supplemented RBCs (hmbRBCs), asexual trophozoite-, or gametocyte-infected RBCs (tiRBCs and giRBCs, respectively) [hmbRBCs: $z = 4.25$, $p < 0.001$; tiRBCs: $z = 4.59$, $p < 0.001$; giRBCs: $z = 4.60$, $p < 0.001$]; and on (**C**) RBCs supplemented with different concentrations of HMBPP, (**D**) dilutions in RBCs of gametocyte culture supernatants (control RBCs,—) and FR-900098-treated tRBCs supplemented with IPP, ▲; $\chi^2_1 = 1.21$, $p = 0.42$). (**E** and **F**) Percentage of mosquitoes landing, probing and initiating feeding [Persistency (%)] within five minutes on (E) serum, with and without HMBPP, or (F), physiological salt solution, with or without HMBPP (hmbNaCl), as well as IPP-supplemented NaCl (ippNaCl) [hmbSerum: $\chi^2_1 = 10.62$, $p = 0.001$; hmbNaCl: $\chi^2_1 = 7.93$, $p = 0.005$; ippNaCl: $\chi^2_1 = 1.01$, $p = 0.55$]. Error bars, ± SE; asterisks denote significant differences. Each experiment was repeated six times with in total 180 mosquitoes per treatment (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; n.s., nonsignificant).

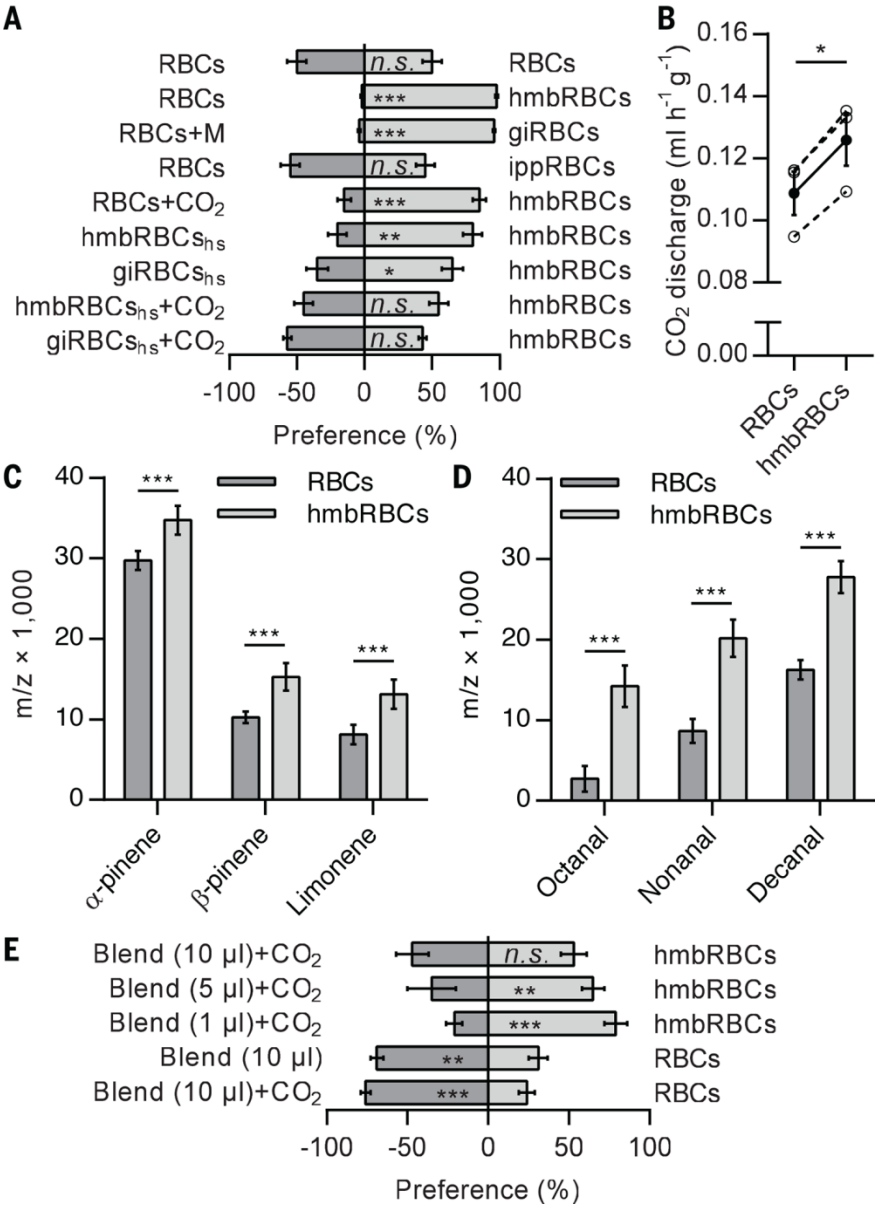

Fig. 2. Volatiles released by red blood cells attract mosquitoes. (**A**) Attraction of mosquitoes to red blood cells (RBCs), RBCs plus media (M) or HMBPP-supplemented RBCs (hmbRBCs) was assessed in comparison to RBCs, hmbRBCs, gametocyte culture supernatant-supplemented RBCs (giRBCs) and isopentenyl pyrophosphate-supplemented RBCs (ippRBCs) and their headspace extracts, with or without CO_2 in the wind tunnel assay, as indicated [RBCs/RBCs: $\chi^2_1 = 0.25$, $p = 0.62$; RBCs/hmbRBCs: $\chi^2_1 = 79.14$, $p < 0.001$; RBCs+M/giRBCs: $\chi^2_1 = 17.53$, $p < 0.001$; RBCs/ippRBCs: $\chi^2_1 = 0.49$, $p = 0.48$; RBCs+CO_2/hmbRBCs: $\chi^2_1 = 41.82$, $p < 0.001$; $hmbRBCs_{hs}$/hmbRBCs: $\chi^2_1 = 6.79$, $p = 0.009$; $giRBCs_{hs}$/hmbRBCs: $\chi^2_1 = 14.70$, $p = 0.001$; $hmbRBCs_{hs}$+CO_2/hmbRBCs: $\chi^2_1 = 0.39$, $p = 0.53$; $giRBCs_{hs}$+CO_2/hmbRBCs: $\chi^2_1 = 1.93$, $p = 0.16$]. (**B**) CO_2 discharge from RBC samples with or without HMBPP [$t = 11.28$, df = 1, $p = 0.008$]. (**C** and **D**) GC-MS analyses of headspace extracts from RBCs and hmbRBCs [$\chi^2_1 = 19.96$, $p < 0.001$; $\chi^2_1 = 27.12$, $p < 0.001$]. (**E**) A synthetic volatile blend (Blend), consisting of the compounds identified as enhanced within the headspace of hmbRBCs, was tested against both hmbRBCs and RBCs, with or without CO_2. [Blend (10 µl) + CO_2/hmbRBCs: $\chi^2_1 = 0.26$, $p = 0.28$; Blend (5 µl) + CO_2/hmbRBCs: $\chi^2_1 = 7.28$, $p = 0.007$; Blend (1 µl) + CO_2/hmbRBCs: $\chi^2_1 = 16.56$, $p < 0.001$; Blend (10 µl)/RBCs: $\chi^2_1 = 9.87$, $p = 0.002$; Blend (10 µl) + CO_2/RBCs: $\chi^2_1 = 17.39$, $p < 0.001$]. Error bars, ± SE; asterisks denote significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; *n.s.*, non-significant; $n = 90$).

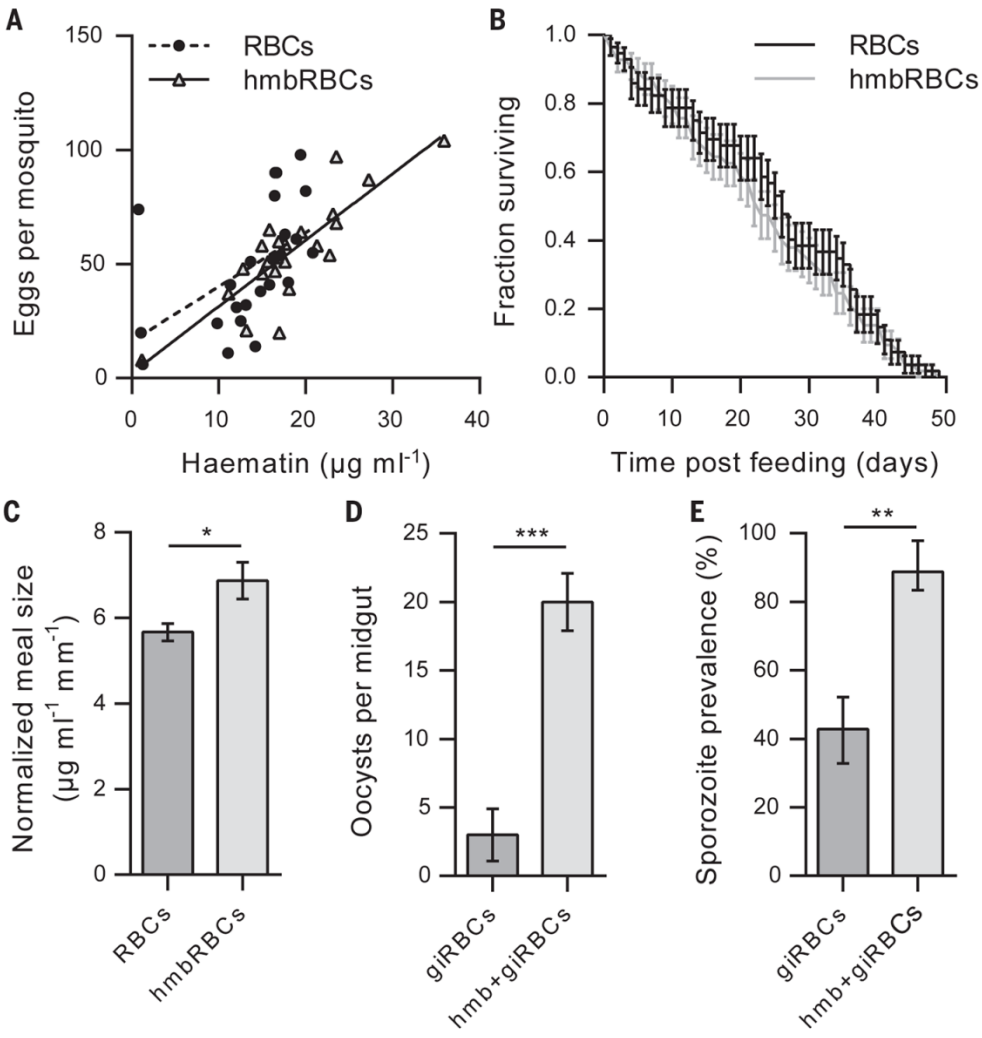

Fig. 3. Effects of HMBPP on mosquito fitness and parasite transmission success and schematic model. Females were fed on red blood cells (RBCs) or HMBPP-supplemented RBCs (hmbRBCs). (**A**) The relationship between fecundity (number of eggs per mosquito) and blood meal size (haematin) [treatment*haematin: χ^2_1 = 6.27, p = 0.01; treatment: χ^2_1 = 0.19, p = 0.66] of individual mosquitoes (▲hmbRBCs ●RBCs). (**B**) Survival of mosquitoes was monitored daily post-feeding until natural death [Cox hazard proportional model, χ^2_1 = 0.71, p = 0.39]. This experiment was performed in triplicate (random effect, in each replicate n = 210). (**C**) Average meal size was determined by the amount of haematin excreted and normalised individually to wing length [χ^2_1 = 5.67, p = 0.02]. (**D**) Average number of oocysts per midgut at 10 dpi [deviance = 33.13, df = 1, p < 0.001]. (**E**) Sporozoite infection (% prevalence in salivary glands of infected mosquitoes) at 14 dpi [χ^2_1 = 8.84, p = 0.002]. Error bars, ± SE; asterisks denote significant differences (*p < 0.05, **p < 0.01, ***p < 0.001).

Table 1. Effects of HMBPP on temporal gene regulation in *A. gambiae* s.l. The whole body *A. gambiae* transcriptome following the ingestion of red blood cells (RBCs) was compared to that of females having ingested RBCs with HMBPP (hmbRBCs) at 1, 3, 6 and 24 hours post-ingestion (hpi). Differential expression levels of genes including those involved in, neural synapses, TEP1 complex, oogenesis, and translation, are listed (displayed as $\log_2$ fold changes over RBC-fed control, $p < 0.01$). The accession numbers and acronyms of annotated genes are shown.

Process	Name	Function	Fold change			
			1 hpi	3 hpi	6 hpi	24 hpi
Synapse	AGAP000732	Synaptic vesicle protein	−1.98			−2.9
	AGAP005507	Neuronal synaptobrevin		2.5		
	AGAP007721	Synaptobrevin				−2.17
	AGAP007942	Synaptotagmin-1		3.89		
	AGAP008943	Protein turtle		3.2		
	AGAP010059	Neurotransmitter-gated ion channel ligand binding	2.81			
	AGAP010485	Dopamine β-monooxygenase		2.77		
	AGAP012433	Ca^{2+} channel protein α1 subunit D		2.22		
	AGAP012581	Neurotransmitter-gated ion channel				−6.25
	NMDAR2	Ionotropic receptor NMDAR2		2.94		
TEP1 complex	APL1C	Anopheles Plasmodium-responsive LRR1C			−1.87	4.03
	CLIPA2	CLIP-domain serine protease			−2.82	
	CLIPA7	CLIP-domain serine protease	−1.92			
	CLIPB15	CLIP-domain serine protease	3.21		−2.68	
	CLIPB17	CLIP-domain serine protease	11.15		−13.13	
	CLIPB4	CLIP-domain serine protease	−2.02			
	CTL4	C-type lectin (CTL)			−1.79	
	LRIM1	Leucine-rich immune protein (long)			−2.08	
	TEP1	Thioester-containing protein 1				2.36
Oogenesis	AGAP003763	Juvenile hormone-inducible protein	2.19			
	AGAP004993	Laminin subunit α				−3.09
	AGAP008369	Lipid transport/vitellinogen	−2.79			
	AGAP008578	ATP-dependent RNA helicase DDX4				−1.83
	AGAP012056	Cofilin		−2.54		
	AGAP012519	Fused				−1.73
	HPX8	Heme peroxidase 8				−4.72
	Lp	Lipophorin	−2.18			−1.95
	MMP1	Matrix metallopeptidase 1				−2.18
	spn-E	ATP-dependent RNA helicase spindle-E	−2.75			
	Vg	Vitellogenin			−1.73	
Translation	AGAP002134	Aminoacyl-tRNA synthetase, class I			−1.69	
	AGAP008738	EIF4E transporter				2.36
	AGAP010613	Elongation factor 1-β			−2.01	
	AGAP012078	Peptide chain release factor 1				−22.34
	RpL10-1	60S ribosomal protein L10-1		−3.65		−4.31
	RpL10-2	60S ribosomal protein L10-2				−11.74
	RpL10a	60S ribosomal protein L10a				−3.16
	RpL12	60S ribosomal protein L12				−4.88
	RpL15	60S ribosomal protein L15				−2.63
	RpL26	60S ribosomal protein L26				−4.77
	RpL28	60S ribosomal protein L28				−3.74
	RpL35a	60S ribosomal protein L35a				−3.06
	RpL5	60S ribosomal protein L5				−2.81
	RpL6	60S ribosomal protein L6				−3.71
	RpS12	40S ribosomal protein S12				−4.24
	RpS14b	40S ribosomal protein S14b				−4.86
	RpS15	40S ribosomal protein S15				−2.44
	RpS18	40S ribosomal protein S18	−2.12			
	RpS24	40S ribosomal protein S24				−2.38
	RpS4	40S ribosomal protein S4				−2.35
	RpS6	40S ribosomal protein S6				−3.32
	RpsA	40S ribosomal protein SA				−8.69

A key malaria metabolite modulates vector blood seeking, feeding, and susceptibility to infection

S. Noushin Emami, Bo G. Lindberg, Susanna Hua, Sharon Hill, Raimondas Mozuraitis, Philipp Lehmann, Göran Birgersson, Anna-Karin Borg-Karlson, Rickard Ignell and Ingrid Faye (February 9, 2017)
published online February 9, 2017

Editor's Summary

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.