Supplementary Information for

The influence of feeding behaviour and temperature on the capacity of mosquitoes to transmit malaria

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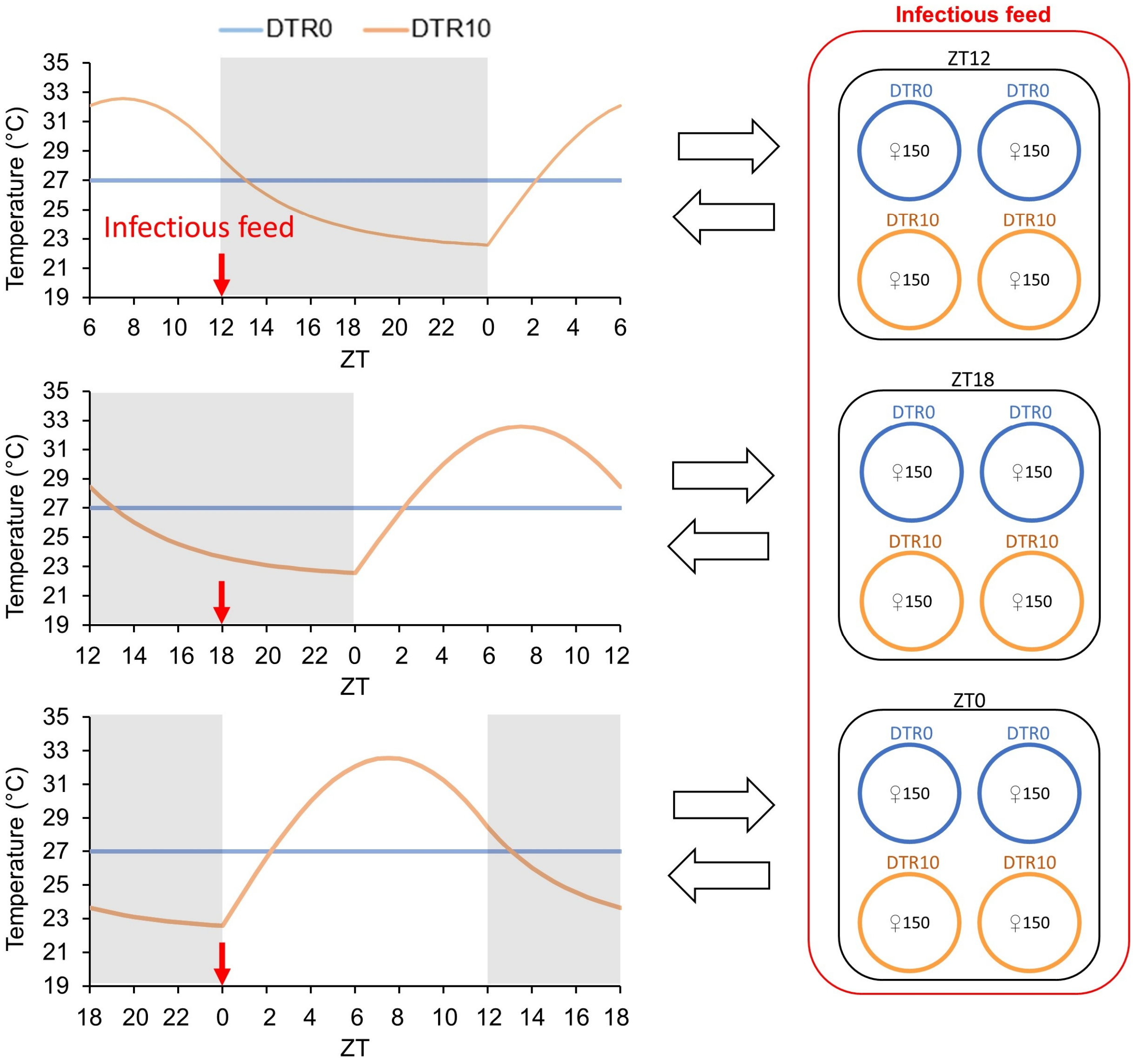
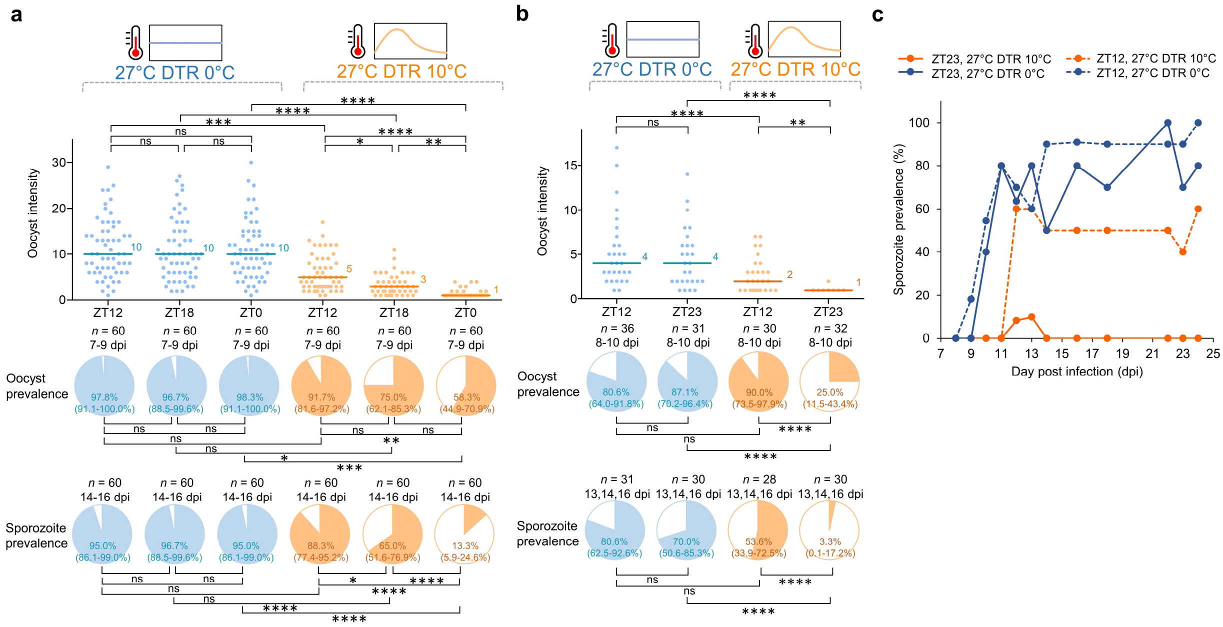
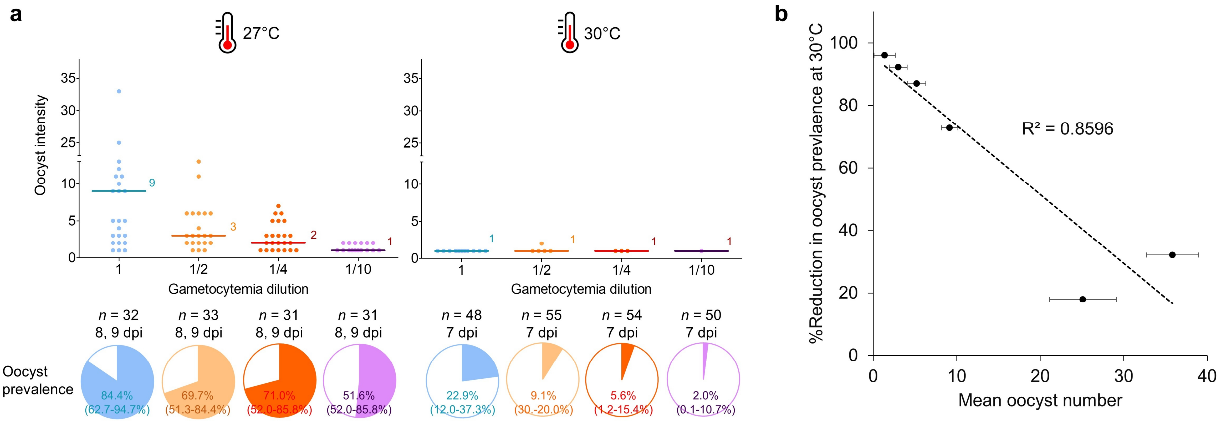


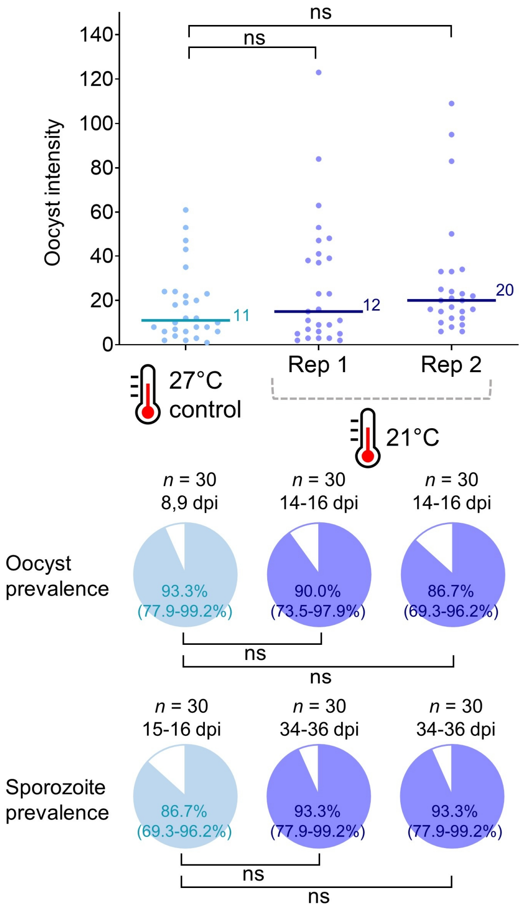
Supplementary Figure 1. Experimental design for infectious feeds. Adult mosquitoes were acclimated in separate incubators set at either constant (i.e. 27°C with a Diurnal Temperature Range [DTR] of 0°C) or fluctuating (i.e. 27°C with a DTR of 10°C) temperature regimes with a timer offset for each time-of-day treatment so that infectious blood feeding took place simultaneously using the same parasite infected blood meals, but the mosquitoes themselves were at different points in their diel cycle (18:00h [ZT12], 00:00h [ZT18], or 06:00h [ZT0]). Feeding took place in an environmental chamber set at 27°C and then blood fed mosquitoes were immediately moved back to their respective incubators. Each treatment group had 300 female mosquitoes in two containers (150 each) unless otherwise specified.



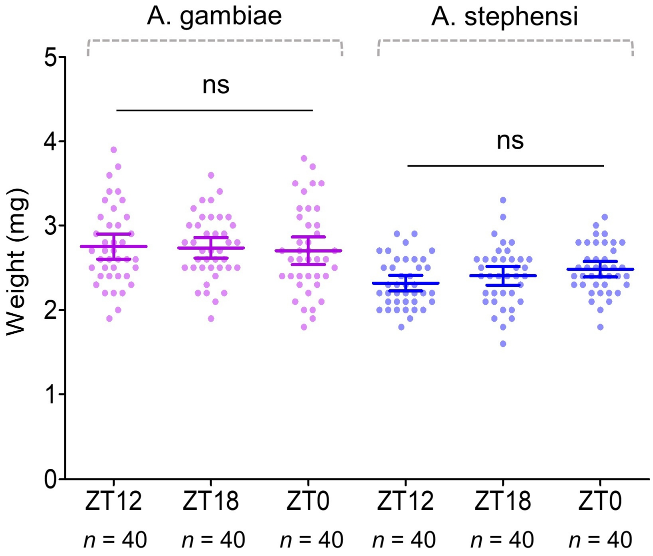
Supplementary Figure 2. Effects of time-of-day of blood meal and fluctuating temperature on vector competence of *A. stephensi* infected with *P. falciparum* and the parasite development rate. **a**, Mosquitoes were offered infected blood meals at a different time-of-day (18:00h [ZT12], 00:00h [ZT18], or 06:00h [ZT0]) and kept under either constant (i.e. 27°C with a Diurnal Temperature Range [DTR] of 0°C) or fluctuating (i.e. 27°C with a DTR of 10°C) temperature regimes. There is no effect of time-of-day of blood feeding under constant temperature regime (i.e. 27°C DTR 0°C) but vector competence (e.g. sporozoite prevalence) is significantly increased for 18:00h (ZT12) or reduced for 06:00h (ZT0) relative to 00:00h (ZT18) under fluctuating temperature regime (i.e. 27°C DTR 10°C). Results of model analyses to examine the effects of time-of-day and temperature regime on oocyst intensity, or oocyst or sporozoite prevalence are reported in Supplementary Table 4. Twenty mosquitoes were sampled daily for dissecting midguts on 7-9 days post infection (dpi) or salivary glands on 14-16 dpi from two replicate containers (i.e. 10 per each). **b**, Simplified version of time-of-day and fluctuating temperature experiment. Mosquitoes were offered infected blood meals at a different time-of-day (18:00h [ZT12] or 05:00h [ZT23]) and kept under constant or fluctuating temperature regimes. There is no effect of time-of-day of blood feeding under constant temperature regime but vector competence (e.g. sporozoite prevalence) is significantly reduced for 05:00h (ZT23) under fluctuating temperature regime. Results of model analyses to examine the effects of time-of-day and temperature regime on oocyst intensity, or oocyst or sporozoite prevalence are reported in Supplementary Table 5. Approximately 10 mosquitoes were sampled daily for dissecting midguts on 8-10 dpi or salivary glands on 13, 14, and 16 dpi. **c**, Daily sporozoite prevalence dynamics. Mosquitoes were offered infected blood meals at a different time-of-day (18:00h [ZT12] or 05:00h [ZT23]) and kept under constant or fluctuating temperature regimes. Extrinsic incubation period is delayed when temperature fluctuates (i.e. 27°C DTR 10°C), independent of biting time. Approximately ten mosquitoes were dissected per day. Partial sporozoite prevalence data were reported in (**b**). For both (**a**) and (**b**)**,** the scatter plots show oocyst intensity, with the data points representing the number of oocysts found in individual mosquitoes, and the horizontal lines the median. The pie charts show oocyst or sporozoite prevalence calculated as the proportion of infected mosquitoes revealed by dissection of midguts and salivary glands, respectively. *n* indicates the number of mosquito sample per treatment group. Numbers in parentheses indicate Clopper-Pearson 95% confidence intervals. Asterisks represent statistically significant difference (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; *P*-values were Bonferroni corrected after pairwise comparisons).



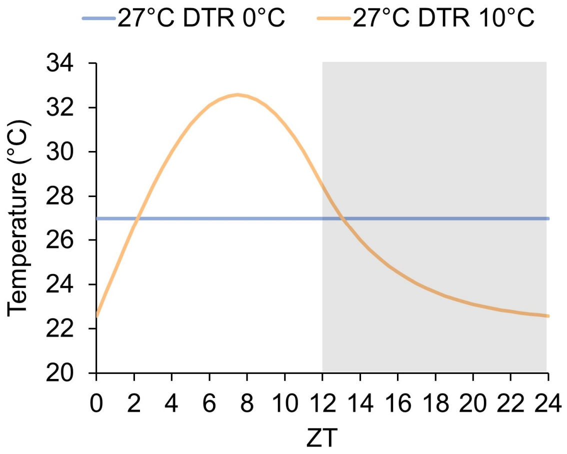
Supplementary Figure 3. Effects of gametocytemia and temperature on vector competence of *A. stephensi* mosquitoes infected with *P. falciparum* malaria. **a**, Mosquitoes were fed on blood meals with serially diluted gametocytemia (1, 1/2, 1/4, or 1/10) and kept at 27°C or 30°C to examine the effects of high temperature interacting with gametocytemia on oocyst infections. Incubation at 30°C reduces oocyst intensity and prevalence across the board, while oocyst intensity and prevalence are also influenced by gametocytemia. Results of model analyses to examine the effects of gametocytemia and temperature treatment on oocyst intensity or oocyst prevalence are reported in Supplementary Table 8. The scatter plots show oocyst intensity, with the data points representing the number of oocysts found in individual mosquitoes, and the horizontal lines the median. The pie charts show oocyst or sporozoite prevalence calculated as the proportion of infected mosquitoes revealed by dissection of midguts and salivary glands, respectively. *n* indicates the number of mosquito sample per treatment group (dpi = days post infection). Numbers in parentheses indicate Clopper-Pearson 95% confidence intervals. **b**, Relationship between per cent reduction in oocyst prevalence due to exposure to 30°C and mean oocyst intensity (error bars = SEM). Per cent reduction represents reduced percentage in oocyst prevalence in the 30°C treatment relative to oocyst prevalence in the 27°C control. Oocyst prevalence and intensity data were derived from experiments reported in Fig. 3a and Supplementary Fig. 3a. The impact of temperature declines as intensity of infection increases. Dashed line indicates linear regression line ($F_{1,\,4} = 24.78$, $P = 0.008$)



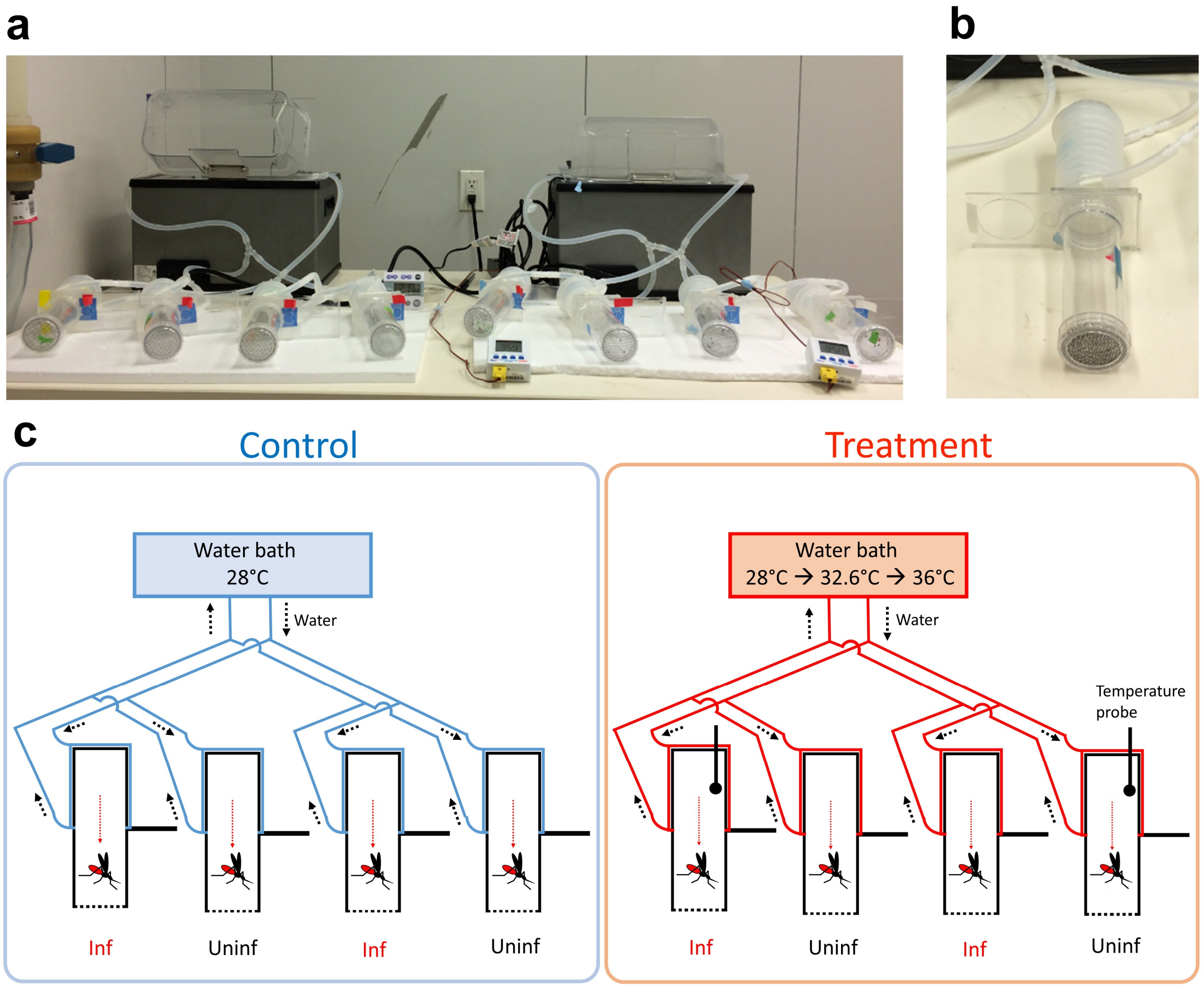
Supplementary Figure 4. Effect of transferring mosquitoes between 21°C and 27°C on vector competence of *A. gambiae* mosquitoes infected with *P. falciparum* malaria. Treatment mosquitoes in two replicate containers were kept at 21°C, blood fed at 27°C, and moved back to 21°C, while control mosquitoes were kept at 27°C throughout and blood fed at 27°C. Transferring mosquitoes between two different temperatures for blood feeding does not affect vector competence. GLM was used to compare control to each replicate container of mosquitoes with pairwise post-hoc contrasts followed by Bonferroni corrections (ns, not significant at $P = 0.05$). The scatter plots show oocyst intensity, with the data points representing the number of oocysts found in individual mosquitoes, and the horizontal lines the median. The pie charts show oocyst or sporozoite prevalence calculated as the proportion of infected mosquitoes revealed by dissection of midguts and salivary glands, respectively. *n* indicates the number of mosquito sample per treatment (dpi = days post infection). Numbers in parentheses indicate Clopper-Pearson 95% confidence intervals.



Supplementary Figure 5. Effects of blood feeding mosquitoes at 27°C transferring from three times-of-day treatments under fluctuating temperature regime (27°C with a DTR of 10°C) on blood meal size of *A. gambiae* and *A. stephensi* mosquitoes. Mosquitoes kept under fluctuating temperature regimes (27°C with a DTR of 10°C) were transferred to 27°C, and offered uninfected blood meals at a different time-of-day (18:00h [ZT12], 00:00h [ZT18], or 06:00h [ZT0]). The whole body weight of blood fed mosquitoes were measured as a proxy for blood meal size. Transferring mosquitoes to 27°C from the prevailing temperature of each time-of-day does not affect blood meal size of mosquitoes. Results of model analyses to examine the effects of species and time-of-day of blood feeding on the body weight are reported in Supplementary Table 11. The scatter plots show body weight of blood fed female mosquitoes. Error bars indicate mean weight with 95% confidence intervals.



Supplementary Figure 6. Plots of temperature treatments used in the current study showing 27°C with a Diurnal Temperature Range (DTR) of 0°C or 10°C. The Parton-Logan model was used for the diurnal fluctuating temperature regime that follows a sinusoidal progression and an exponential decay for the day and night cycle, respectively. Shaded areas indicate scotophase.



Supplementary Figure 7. Experimental setup for thermal avoidance assay. Pictures of (**a**) water linked to multiple tubes and (**b**) individual assay tubes. **c**, A schematic diagram of the experimental setup. Total eight assay tubes were used (four for control and four for treatment group) in an assay run, with a total three rounds of assay. Mosquitoes fed with parasite infected (Inf) or uninfected (Uninf) blood meals were introduced into tubes, and the treatments were rotated between the assay rounds.

Supplementary Table 1. Biting activity profile for *Anopheles* mosquitoes identified to exhibit evening, midnight, or morning biting time in 42 published studies. Biting activities were categorized into 'evening', 'midnight', or 'morning' biting group with peak biting observed before 22:00h, between 22:00 and 05:00h, or after 05:00h, respectively. Studies (i.e. papers reviewed) were grouped into high or low temperature environment (divided by double line in the table).

Year[ref.]	Country	Mosquito species	Description on biting activity[a]	Peak biting time	Temperature (°C)[b]
2017[1]	Cameroon	*A. gambiae* s.l.	Peak between 00:00 - 02:00 (In+Out), Garoua	Midnight[§]	29.5
			Peak between 22:00 - 00:00 (In+Out), Mayo Oulo	Midnight[§]	
			Peak between 00:00 - 02:00 (In+Out), Pitoa	Midnight[§]	
		A. rufipes	Peak between 20:00 - 22:00 (In+Out), Garoua	Evening	
			Peak between 00:00 - 02:00 (In+Out), Mayo Oulo	Midnight	
			Peak between 00:00 - 02:00 (In+Out), Pitoa	Midnight	
2012[2]	Benin	*A. funestus*	Peak between 05:00 - 06:00 (In+Out), Lokohoue, 2011	Morning[§]	28.7
2009[3]	Chad	*A. arabiensis*	Peak between 01:00 - 02:00 (In+Out)	Midnight[§]	27.9
		A. pharoensis	Peak between 21:00 - 22:00 (In+Out)	Evening	
		A. funestus	Peak between 03:00 - 04:00 (In+Out)	Midnight[§]	
		A. ziemanni	Peak between 18:00 - 19:00 (In+Out)	Evening	
2017[4]	Indonesia	*A. vagus*	Peak between 21:00 - 22:00 (In+Out)	Evening	27.8
		A. sundaicus	Peak between 21:00 - 22:00 (In+Out)	Evening	
		A. subpictus	Peak between 23:00 - 00:00 (In+Out)	Midnight	
		A. indefnitus	Peak between 23:00 - 00:00 (In+Out)	Midnight	
		A. peditaeniatus	Peak between 00:00 - 01:00 (In+Out)	Midnight	
		A. nigerrimus	Peak between 20:00 - 21:00 (In+Out)	Evening	
2011[5]	Solomon Islands	*A. farauti*	Peak between 18:00 - 19:00 (In), Pala, Dec 2010	Evening	27.1
			Peak between 19:00 - 21:00 (Out), Pala, Dec 2010	Evening	
2007[6]	Tanzania	*A. gambiae* s.s.	Peak between 01:00 - 02:00 (In)	Midnight[§]	27.0
		A. gambiae s.s.	Peak between 01:00 - 02:00 (Out)	Midnight[§]	
		A. arabiensis	Peak between 20:00 - 21:00 (In)	Evening[§]	
		A. arabiensis	Peak between 22:00 - 23:00 (Out)	Midnight[§]	
2008[7]	French Guiana	*A. darlingi*	Peak between 22:30 - 23:30 (Out), Twenké	Midnight	27.0
			Peak between 22:30 - 23:30 (Out), Taluéne	Midnight	
			Peak between 05:30 - 06:30 (Out), Cayodé	Morning	
2012[8]	Suriname	*A. darlingi*	Peak between 05:00 - 06:00 (In), Drietabiki	Morning	27.0
			Peak between 04:00 - 05:00 (Out), Drietabiki	Midnight	
			Peak between 01:00 - 02:00 (In), Jamaica	Midnight	
			Peak between 01:00 - 02:00 (Out), Jamaica	Midnight	
2014[9]	Senegal	*A. funestus*	Peak between 08:00 - 09:00 (In+Out)	Morning[§]	27.0
2004[10]	Eritrea	*A. gambiae* s.l.	Peak between 02:00 - 03:00 (In), Gash-barka	Midnight[§]	26.8
			Peak between 22:00 - 23:00 (Out), Gash-barka	Midnight[§]	

			Peak between 02:00 - 03:00 (In), Debub	Midnight§	
			Peak between 21:00 - 22:00 (Out), Debub	Evening§	
			Peak between 01:00 - 02:00 (In), Anseba	Midnight§	
			Peak between 21:00 - 22:00 (Out), Anseba	Evening§	
2012[11]	Cameroon	*A. gambiae* s.l.	Peak between 01:00 - 02:00 (Out)	Midnight§	26.8
2017[12]	Papua New Guinea	*A. farauti* 4	peak between 19:00 - 20:00 (Out), Kokofine, 2011	Evening	26.7
			peak between 20:00 - 21:00 (Out), Mauno, 2011	Evening	
2005[13]	Bolivia	*A. darlingi*	Peak between 20:00 - 21:00 (Out)	Evening	26.6
2011[14]	Solomon Islands	*A. farauti*	Peak between 18:00 - 19:00 (In)	Evening	26.5
			Peak between 19:00 - 20:00 (Out)	Evening	
		A. solomonis	Peak between 18:00 - 19:00 (In)	Evening	
			Peak between 18:00 - 19:00 (Out)	Evening	
2017[15]	Tanzania	*A. arabiensis*	Peak between 20:00 - 21:00 (Out)	Evening§	26.5
		A. funestus	Peak between 05:00 - 06:00 (Out)	Morning§	
2008[16]	Ghana	*A. gambiae* s.l.	Peak between 02:00 - 03:00 (Out), Dzorwulu	Midnight§	26.4
			Peak between 03:00 - 04:00 (Out), Kaneshie	Midnight§	
			Peak between 02:00 - 03:00 (Out), Korle Bu	Midnight§	
			Peak between 02:00 - 03:00 (Out), Kotobabi	Midnight§	
			Peak between 02:00 - 03:00 (Out), La	Midnight§	
			Peak between 03:00 - 04:00 (Out), Ushertown	Midnight§	
2013[17]	Uganda	*A. gambiae* s.l.	Peak between 04:00 - 05:00 (In+Out), Bugabula	Midnight§	26.2
			Peak between 02:00 - 03:00 (In+Out), Budiope	Midnight§	
		A. funestus	Peak between 04:00 - 05:00 (In+Out), Budiope	Midnight§	
			Peak between 05:00 - 06:00 (In+Out), Bugabula	Morning§	
2015[18]	Peru	*A. darlingi*	Peak between 21:00 - 22:00 (Out), Riverine	Evening	26.1
			Peak between 22:00 - 23:00 (Out), Highway	Midnight	
2015[19]	Peru	*A. darlingi*	Peak between 18:00 - 19:00 (Out), San José de Lupuna, April 2011	Evening	26.1
			Peak between 23:00 - 00:00 (Out), Villa del Buen Pastor, April 2011	Midnight	
			Peak between 22:00 - 23:00 (Out), Cahuide, May 2012	Midnight	
2009[20]	Colombia	*A. darlingi*	Peak between 18:00 - 19:00 (In)	Evening	26.0
			Peak between 20:00 - 21:00 (Out)	Evening	
		A. oswaldoi	Peak between 18:00 - 19:00 (Out)	Evening	
			Peak between 18:00 - 19:00 (Out)	Evening	
2014[21]	Solomon Islands	*A. farauti*	Peak between 19:00 - 20:00 (In)	Evening	26.0
			Peak between 19:00 - 20:00 (Out)	Evening	
2015[22]	Equatorial Guinea	*Anopheles* spp.	Peak between 03:00 - 04:00 (In)	Midnight	26.0
			Peak between 03:00 - 04:00 (Out)	Midnight	
2016[23]	Solomon Islands	*A. farauti* s.s.	Peak between 18:00 - 19:00 (In)	Evening	26.0
			Peak between 18:00 - 19:00 (Out)	Evening	
2012[24]	Zambia	*A. funestus*	Peak between 04:00 - 05:00 (In), LLINs alone	Midnight§	25.7
			Peak between 05:00 - 06:00 (Out), LLINs alone	Morning§	

			Peak between 04:00 - 05:00 (In), LLINs + IRS	Midnight§	
			Peak between 01:00 - 02:00 (Out), LLINs + IRS	Midnight§	
		A. quadriannulatus	Peak between 20:00 - 21:00 (In), LLINs alone	Evening	
			Peak between 19:00 - 20:00 (Out), LLINs alone	Evening	
			Peak between 20:00 - 21:00 (In), LLINs + IRS	Evening	
			Peak between 21:00 - 22:00 (Out), LLINs + IRS	Evening	
2011[25]	Equatorial Guinea	*A. gambiae* s.s.	Peak between 23:00 - 00:00 (In)	Midnight§	24.6
			Peak between 23:00 - 00:00 (Out)	Midnight§	
2011[26]	Indonesia	*A. aconitus*	Peak between 22:00 - 23:00 (In+Out)	Midnight	24.1
		A. vagus	Peak between 19:00 - 20:00 (In+Out), West Timor	Evening	
		A. barbirostris	Peak between 01:00 - 02:00 (In+Out)	Midnight	
		A. vagus	Peak between 02:00 - 03:00 (In+Out), Java	Midnight	
		A. subpictus	Peak between 22:00 - 23:00 (In+Out), West Timor	Midnight	
2007[27]	Venezuela	*A. darlingi*	Peak between 01:00 - 02:00 (In)	Midnight	24.0
2012[28]	Iran	*A. culcifacies*	Peak between 23:00 - 00:00 (In+Out)	Midnight	23.5
		A. fluviatilis	Peak between 22:00 - 23:00 (In+Out)	Midnight	
		A. stephensi	Peak between 19:00 - 20:00 (In+Out)	Evening	
2011[29]	Tanzania	*A. gambiae* s.l.	Peak between 00:00 - 01:00 (In), 2009	Midnight§	23.3
			Peak between 22:00 - 23:00 (Out), 2009	Midnight§	
		A. funestus	Peak between 20:00 - 21:00 (In), 2009	Evening§	
			Peak between 22:00 - 23:00 (Out), 2009	Midnight§	
2005[30]	India	*A. baimaii*	Peak between 22:00 - 23:00 (In)	Midnight	23.2
2001[31]	Kenya	*A. gambiae* s.l.	Peak between 23:00 - 00:00 (Out), bed net village	Midnight§	23.0
			Peak between 23:00 - 00:00 (Out), control village	Midnight§	
		A. funestus	Peak between 22:00 - 23:00 (Out), bed net village	Midnight§	
			Peak between 23:00 - 00:00 (Out), control village	Midnight§	
2000[32]	Mozambique	*A. arabiensis*	Peak between 01:00 - 02:00 (In)	Midnight§	22.8
			Peak between 23:00 - 00:00 (Out)	Midnight§	
		A. funestus	Peak between 02:00 - 03:00 (In)	Midnight§	
			Peak between 02:00 - 03:00 (Out)	Midnight§	
2015[33]	Uganda	*A. gambiae* s.l.	Peak between 19:00 - 20:00 (In+Out), Engari, rainy season	Evening§	22.3
			Peak between 19:00 - 20:00 (In+Out), Engari, dry season	Evening§	
			Peak between 19:00 - 20:00 (In+Out), Kigorogoro, dry season	Evening§	
2015[34]	Kenya	*A. gambiae* s.l.	Peak between 22:00 - 00:00 (In)	Midnight§	22.1
			Peak between 18:00 - 20:00 (Out)	Evening§	
		A. funestus	Peak between 18:00 - 20:00 (In)	Evening§	
			Peak between 18:00 - 20:00 (Out)	Evening§	
2006[35]	Tanzania	*A. gambiae* s.l.	Peak between 05:00 - 06:00 (In), Lupiro 2004	Morning§	21.8

			Peak between 23:00 - 00:00 (Out), Lupiro 2004	Midnight§	
2014[36]	Kenya	*A. gambiae* s.s.	Peak between 05:00 - 06:00 (In), Asembo 2011	Morning§	21.8
			Peak between 01:00 - 02:00 (Out), Asembo 2011	Midnight§	
		A. arabiensis	Peak between 01:00 - 02:00 (In), Asembo 2011	Midnight§	
			Peak between 03:00 - 04:00 (Out), Asembo 2011	Midnight§	
		A. funestus	Peak between 01:00 - 02:00 (In), Asembo 2011	Midnight§	
			Peak between 01:00 - 02:00 (Out), Asembo 2011	Midnight§	
2015[37]	Madagascar	*A. coustani*	Peak between 22:00 - 23:00 (In)	Midnight	21.3
			Peak between 19:00 - 21:00 (Out)	Evening	
		A. mascarensis	Peak between 01:00 - 02:00 (In)	Midnight	
			Peak between 02:00 - 03:00 (Out)	Midnight	
		A. funestus	Peak between 21:00 - 22:00 (In)	Evening§	
			Peak between 02:00 - 03:00 (Out)	Midnight§	
		A. arabiensis	Peak between 04:00 - 05:00 (In)	Midnight§	
			Peak between 00:00 - 01:00 (Out)	Midnight§	
2016[38]	Ethiopia	*A. arabiensis*	Peak between 00:00 - 01:00 (In)	Midnight§	21.1
			Peak between 21:00 - 22:00 (Out)	Evening§	
		A. pharoensis	Peak between 19:00 - 20:00 (In)	Evening	
			Peak between 19:00 - 20:00 (Out)	Evening	
		A. ziemanni	Peak between 19:00 - 20:00 (In)	Evening	
			Peak between 19:00 - 20:00 (Out)	Evening	
		A. funestus s.l.	Peak between 23:00 - 00:00 (In)	Midnight§	
			Peak between 21:00 - 22:00 (Out)	Evening§	
2010[39]	Ethiopia	*A. arabiensis*	Peak between 19:00 - 20:00 (In)	Evening§	20.0
			Peak between 18:00 - 19:00 (Out)	Evening§	
		A. pharoensis	Peak between 20:00 - 21:00 (In)	Evening	
			Peak between 19:00 - 20:00 (Out)	Evening	
		A. coustani	Peak between 18:00 - 19:00 (In)	Evening	
			Peak between 18:00 - 19:00 (Out)	Evening	
2010[40]	Zambia	*A. arabiensis*	Peak between 24:00 - 01:00 (In)	Midnight§	19.9
			Peak between 01:00 - 02:00 (Out)	Midnight§	
2016[41]	Ethiopia	*A. gambiae* s.l.	Peak between 19:00 - 20:00 (In)	Evening§	18.0
			Peak between 19:00 - 20:00 (Out)	Evening§	
		A. coustani s.l.	Peak between 21:00 - 22:00 (In)	Evening	
			Peak between 19:00 - 20:00 (Out)	Evening	
		A. pharoensis	Peak between 19:00 - 20:00 (In)	Evening	
			Peak between 20:00 - 21:00 (Out)	Evening	
2012[42]	Ethiopia	*A. arabiensis*	Peak between 19:00 - 20:00 (In)	Evening§	17.4
			Peak between 19:00 - 20:00 (Out)	Evening§	

In: peak biting observed for indoor biting.
Out: peak biting observed for outdoor biting.
In+Out: peak biting observed for combined data of indoor and outdoor biting.

[a] If a subset of data showed a shift in biting time in each study, the data set was described for the details such as study sites, year, and/or intervention methods (e.g., long-lasting insecticide-treated bed nets [LLINs], indoor residual spray [IRS], etc.).

[b] Temperature measures represent monthly mean temperature of regional estimates for the study sites and study periods in each paper reviewed, otherwise specified in each paper.

[§] Potential major malaria vectors in Africa (i.e., *A. gambiae* s.l., *A. gambiae* s.s., *A. coluzzii, A. arabiensis,* or *A. funestus*)

Supplementary Table 2. Summary of biting activity profile from Supplementary Table 1

Biting time	No. cases[a] (%) by temperature measured[b]	
	High (25°C or above)	Low (< 25°C)
Evening	33 (21.9)	31 (20.5)
Midnight	40 (26.5)	38 (25.2)
Morning	7 (4.6)	2 (1.3)

[a] A case was determined as a mosquito species or species complex, site, season, and biting location for which biting activity had been determined in a given paper (see Supplementary Table 1).

[b] Temperature measured indicates the representative temperature data for each study reviewed in Supplementary Table 1.

Supplementary Tables 3. GLMMs examining the effects of time-of-day (18:00h [ZT12], 00:00h [ZT18], and 06:00h [ZT0]) and temperature regime (27°C DTR 0°C and 27°C DTR 10°C) on oocyst intensity, or oocyst or sporozoite prevalence in *A. gambiae* (See Fig. 1)

		Oocyst intensity		Oocyst prevalence		Sporozoite prevalence	
Effect	*df*	*F*	*P*	*F*	*P*	*F*	*P*
Time[a]	2	9.91	< 0.0001	13.42	< 0.0001	17.48	< 0.0001
DTR[b]	1	93.02	< 0.0001	74.63	< 0.0001	47.96	< 0.0001
Time × DTR	2	17.36	< 0.0001	18.64	< 0.0001	16.19	< 0.0001
Day[c]	2	0.83	0.436	0.06	0.940	2.51	0.088

$LR\text{-}\chi^2$: Likelihood ratio chi-square value.

[a] Time-of-day.

[b] Diurnal temperature range.

[c] Dissection day (day post infection).

Supplementary Tables 4. Model analyses examining the effects of time-of-day (18:00h [ZT12], 00:00h [ZT18], and 06:00h [ZT0]) and temperature regime (27°C DTR 0°C and 27°C DTR 10°C) on oocyst intensity (GLMM), or oocyst (GLMM) or sporozoite prevalence (GLM) in *A. stephensi* (see Supplementary Fig. 2a)

		Oocyst intensity		Oocyst prevalence		Sporozoite prevalence	
Effect	*df*	*F*	*P*	*F*	*P*	$LR\text{-}\chi^2$	*P*
Time[a]	2	15.07	< 0.0001	1.20	0.318	13.00	0.002
DTR[b]	1	158.25	< 0.0001	18.95	< 0.001	59.59	< 0.0001
Time × DTR	2	13.23	< 0.0001	0.97	0.393	14.08	< 0.001
Day[c]	2	0.21	0.812	0.12	0.890	1.79	0.410

$LR\text{-}\chi^2$: Likelihood ratio chi-square value.

[a] Time-of-day.

[b] Diurnal temperature range.

[c] Dissection day (day post infection).

Supplementary Table 5. GLMs examining the effects of time-of-day (18:00h [ZT12] and 05:00h [ZT23]) and temperature regime (27°C DTR 0°C and 27°C DTR 10°C) on oocyst intensity, or oocyst or sporozoite prevalence in *A. stephensi* (see Supplementary Fig. 2b)

		Oocyst intensity		Oocyst prevalence		Sporozoite prevalence	
Effect	*df*	$LR\text{-}\chi^2$	*P*	$LR\text{-}\chi^2$	*P*	$LR\text{-}\chi^2$	*P*
Time[a]	1	9.31	0.002	8.17	0.004	16.01	< 0.0001
DTR[b]	1	45.64	<0.0001	4.93	0.026	33.29	< 0.0001
Time × DTR	1	4.78	0.029	16.51	< 0.0001	7.38	0.007
Day[c]	2	10.65	0.005	2.35	0.309	0.80	0.672

$LR\text{-}\chi^2$: Likelihood ratio chi-square value.

[a] Time-of-day.

[b] Diurnal temperature range.

[c] Dissection day (day post infection).

Supplementary Table 6. Outputs from a malaria transmission dynamics model illustrating the potential effect of altered or constant vector competence in mosquitoes biting in the evening (EV), at midnight (MD), or in the morning (MN) on malaria prevalence and efficacy of bed nets (LLINs). Post bed net prevalence estimates are taken 3 years after they were introduced at 50% usage and maintained annually to estimate the efficacy of LLINs (See Fig. 2)

Run	Vector competence	Proportion of mosquitoes biting during different periods of the night			Proportion of bites received in bed			Prevalence (%) in 2 – 10-year old children[§]		Estimated efficacy of LLINs (% relative reduction in prevalence)[§]
		EV	MD	MN	EV	MD	MN	Without LLINs	With LLINs	
1	Altered[¶]	0.15	0.7	0.15	0.85[†]	0.85[†]	0.85[†]	59.5 (54.4 – 63.7)	15.6 (11.5 – 19.8)	73.7 (69.0 - 78.9)
2	Altered[¶]	0.7	0.3	0	0.85[†]	0.85[†]	0.85[†]	68.5 (65.6 – 70.8)	25.2 (21.8 – 28.1)	63.3 (66.8 – 60.3)
3	Altered[¶]	0	0.3	0.7	0.85[†]	0.85[†]	0.85[†]	39.0 (30.4 – 48.6)	3.4 (1.4 – 7.6)	91.3 (84.3 – 95.3)
4	Altered[¶]	0.15	0.7	0.15	0.43	0.85[†]	0.43	59.5 (54.4 – 63.7)	22.6 (15.6 – 28.1)	62.0 (56.8 - 67.9)
5	Altered[¶]	0.7	0.3	0	0.43	0.85[†]	0.43	68.5 (65.6 – 70.8)	44.4 (40.4 – 47.6)	35.3 (38.4 – 32.8)
6	Altered[¶]	0	0.3	0.7	0.43	0.85[†]	0.43	39.0 (30.4 – 48.6)	12.1 (6.4 - 20.7)	68.9 (57.4 – 78.9)
7	Constant[ǂ]	0.15	0.7	0.15	0.85[†]	0.85[†]	0.85[†]	58.4 (52.2 – 63.3)	14.7 (9.9 – 19.3)	74.9 (69.5 – 81.1)
8	Constant[ǂ]	0.7	0.3	0	0.85[†]	0.85[†]	0.85[†]	58.4 (52.2 – 63.3)	14.7 (9.9 – 19.3)	74.9 (69.5 – 81.1)
9	Constant[ǂ]	0	0.3	0.7	0.85[†]	0.85[†]	0.85[†]	58.4 (52.2 – 63.3)	14.7 (9.9 – 19.3)	74.9 (81.1 – 69.5)
10	Constant[ǂ]	0.15	0.7	0.15	0.43	0.85[†]	0.43	58.4 (52.2 – 63.3)	21.4 (15.4 – 27.0)	63.3 (57.4 – 70.5)
11	Constant[ǂ]	0.7	0.3	0	0.43	0.85[†]	0.43	58.4 (52.2 – 63.3)	31.4 (24.4 – 37.4)	46.3 (53.3 – 40.9)
12	Constant[ǂ]	0	0.3	0.7	0.43	0.85[†]	0.43	58.4 (52.2 – 63.3)	31.4 (24.4 – 37.4)	46.3 (53.3 – 40.9)

[¶] Vector competence is assumed to be increased, intermediate, or low for mosquitoes biting in the evening, at midnight, or in the morning, respectively.

[ǂ] Vector competence is assumed to be equal with respect to biting time.

[†] See reference *A. gambiae* s.s.[43].

[§] Numbers in parentheses represent 95% confidence intervals.

Supplementary Table 7. GLMs examining the effects of mosquito species (*A. gambiae* and *A. stephensi*) and/or temperature treatment (27°C and 30°C) on oocyst intensity or oocyst prevalence (see Fig. 3a). Oocyst prevalence data were pooled within each temperature treatment group after confirming no difference between two species (Fisher's exact test, two-sided, $P > 0.05$) to ensure model validity[44,45]

		Oocyst intensity		Oocyst prevalence	
Effect	*df*	$LR\text{-}\chi^2$	*P*	$LR\text{-}\chi^2$	*P*
Species	1	0.23	0.632	NA	NA
Temperature	1	78.7	< 0.0001	36.9	< 0.0001
Species × Temperature	1	1.29	0.256	NA	NA

$LR\text{-}\chi^2$: Likelihood ratio chi-square value.

Supplementary Table 8. GLMs examining the effects of gametocytemia dilutions (1, 1/2, 1/4, and 1/10) and temperature treatment (27°C and 30°C) on oocyst intensity or oocyst prevalence in *A. stephensi* (see Supplementary Fig. 3a)

		Oocyst intensity		Oocyst prevalence	
Effect	*df*	$LR\text{-}\chi^2$	*P*	$LR\text{-}\chi^2$	*P*
Gametocytemia	3	2.48	0.479	20.3	< 0.0001
Temperature	1	5.96	0.015	138	< 0.0001
Gametocytemia × Temperature	1	2.72	0.438	1.33	0.724

$LR\text{-}\chi^2$: Likelihood ratio chi-square value.

Supplementary Table 9. Blood feeding compliance of *A. gambiae* mosquitoes fed at either 27°C or 21°C. Mosquitoes were kept at either 27°C DTR 0°C or 21°C DTR 0°C and fed infectious blood meals at a different time-of-day (18:00h [ZT12], 00:00h [ZT18], or 06:00h [ZT0]) at their corresponding temperature (i.e. either 27°C or 21°C). Data for feeding compliance at 27°C were obtained from the infectious feed (2^{nd} feed) reported in Fig. 1 (i.e. 27°C DTR 0°C treatment group), and data for feeding compliance at 21°C were obtained from a separate infectious feed. GLMM examining the effects of temperature and time-of-day on the feeding compliance is reported in Supplementary Table 10

Blood feeding temperature	Time-of-day	No. fed	No. total	% fed
27°C	ZT12	117	118	99.2
		116	118	98.3
	ZT18	113	115	98.3
		114	116	98.3
	ZT0	112	116	96.6
		112	117	95.7
21°C	ZT12	112	119	94.1
		108	118	91.5
	ZT18	115	118	97.5
		108	117	92.3
	ZT0	115	115	100.0
		113	117	96.6

Supplementary Table 10. GLMM examining the effects of blood feeding temperature (27°C and 21°C) and time-of-day (18:00h [ZT12], 00:00h [ZT18], and 06:00h [ZT0]) on feeding compliance (See Supplementary Table 9)

		Feeding compliance	
Effect	*df*	*F*	*P*
Temperature	1	3.05	0.131
Time-of-day	2	0.08	0.926
Temperature × Time-of-day	2	3.98	0.080

Supplementary Table 11. GLMM examining the effects of species (*A. gambiae* and *A. stephensi*) and time-of-day (18:00h [ZT12], 00:00h [ZT18], and 06:00h [ZT0]) on body weight of blood fed mosquitoes (See Supplementary Fig. 5)

		Feeding compliance	
Effect	*df*	*F*	*P*
Species	1	43.09	< 0.0001
Time-of-day	2	0.46	0.635
Species × Time-of-day	2	1.56	0.213

Supplementary Table 12. Summary of experiment design, dissection method, and/or statistical model analysis for empirical studies (additional information are available in the main text)

Reference (description on experiment)	Mosquito dissection	Treatment		Sample size	Dpi[†]	# Replicate container	# Mosquito per container	Model analysis	Dependent variables	Model structure and explanatory variables	Error structure and link for dependent variables
Fig. 1 and Supplementary Table 3 (effects of time-of-day and fluctuating temperature on vector competence in *A. gambiae*)	Midguts	27°C DTR 0°C	ZT12	120	7-9	4	150 or 120[‡]	GLMM	Oocyst intensity, or oocyst or sporozoite prevalence	Time-of-day + Temperature regime + Time-of-day × Temperature regime + Dissection day + Infectious feed[*]	Oocyst intensity - negative binomial distribution with log link; Oocyst and sporozoite prevalence - binomial distribution with logit link
			ZT18	120	7-9	4	150 or 120[‡]				
			ZT0	120	7-9	4	150 or 120[‡]				
		27°C DTR 10°C	ZT12	120	7-9	4	150 or 120[‡]				
			ZT18	120	7-9	4	150 or 120[‡]				
			ZT0	120	7-9	4	150 or 120[‡]				
	Salivary glands	27°C DTR 0°C	ZT12	120	14-16	4	150 or 120[‡]				
			ZT18	120	14-16	4	150 or 120[‡]				
			ZT0	120	14-16	4	150 or 120[‡]				
		27°C DTR 10°C	ZT12	120	14-16	4	150 or 120[‡]				
			ZT18	120	14-16	4	150 or 120[‡]				
			ZT0	120	14-16	4	150 or 120[‡]				
Supplementary Fig. 2a and Supplementary Table 4 (effects of time-of-day and fluctuating temperature on vector competence in *A. stephensi*)	Midguts	27°C DTR 0°C	ZT12	60	7-9	2	120	GLMM	Oocyst intensity or prevalence	Time-of-day + Temperature regime + Time-of-day × Temperature regime + Dissection day + Mosquito container[*]	Oocyst intensity - negative binomial distribution with log link; Oocyst prevalence - binomial distribution with logit link
			ZT18	60	7-9	2	120				
			ZT0	60	7-9	2	120				
		27°C DTR 10°C	ZT12	60	7-9	2	120				
			ZT18	60	7-9	2	120				
			ZT0	60	7-9	2	120				
	Salivary glands	27°C DTR 0°C	ZT12	60	14-16	2	120	GLM	Sporozoite prevalence[¶]	Time-of-day + Temperature regime + Time-of-day × Temperature regime + Dissection day	Sporozoite prevalence - binomial distribution with logit link
			ZT18	60	14-16	2	120				
			ZT0	60	14-16	2	120				
		27°C DTR 10°C	ZT12	60	14-16	2	120				
			ZT18	60	14-16	2	120				
			ZT0	60	14-16	2	120				
Supplementary Fig. 2b, Supplementary Fig. 2c (daily sporozoite prevalence; numbers in parentheses indicate sample size and dpi; statistical analyses are not applied), and Supplementary Table 5 (effects of time-of-day and fluctuating temperature on vector competence and parasite development rate in *A. stephensi*)	Midguts	27°C DTR 0°C	ZT12	36	8-10	1	150	GLM	Oocyst intensity or prevalence	Time-of-day + Temperature regime + Time-of-day × Temperature regime + Dissection day	Oocyst intensity - Poisson distribution[§] with log link; Oocyst prevalence - binomial distribution with logit link
			ZT23	31	8-10	1	150				
		27°C DTR 10°C	ZT12	30	8-10	1	150				
			ZT23	32	8-10	1	150				
	Salivary glands	27°C DTR 0°C	ZT12	31 (appr. 10/day)	13, 14, 16 (8-14, 16, 18, 22-24)	1	150	GLM	Sporozoite prevalence		Sporozoite prevalence - binomial distribution with logit link
			ZT23	30 (appr. 10/day)	13, 14, 16 (8-14, 16, 18, 22-24)	1	150				
		27°C DTR 10°C	ZT12	28 (appr. 10/day)	13, 14, 16 (8-14, 16, 18, 22-24)	1	150				
			ZT23	30 (appr. 10/day)	13, 14, 16 (8-14, 16, 18, 22-24)	1	150				
Fig. 3a and Supplementary Table 7 (effects of high temperatures on parasite establishment)	Midguts	27°C	A. gambiae	40	7-9	1	120	GLM	Oocyst intensity or prevalence[¶]	Oocyst intensity - Species + Temperature treatment + Species × Temperature treatment; Oocyst prevalence - Temperature	Oocyst intensity - negative binomial distribution with log link; Oocyst prevalence - binomial distribution with logit link
			A. stephensi	30	7-9	1	120				
		30°C	A. gambiae	25	6, 7	1	120				
			A. stephensi	28	6, 7	1	120				
		32°C	A. gambiae	29	5, 6	1	120				

			A. stephensi	25	5, 6	1	120				
Fig. 3b (thermal sensitivity of early parasite infection in *A. stephensi*)	Midguts	27°C	Control	37	7-9	1	120	GLM	Oocyst intensity or prevalence	Temperature treatment	Oocyst intensity - Poisson distribution[§] with log link; Oocyst prevalence - binomial distribution with logit link
		30°C	3h	44	5-8	1	120				
			6h	44	5-8	1	120				
			12h	36	6-8	1	120				
			24h	32	6-8	1	120				
			48h	30	6-8	1	120				
Fig.4 (infectious feed for thermal avoidance assay)	Midguts	27°C DTR 10°C prior to blood feeding, and 27°C after blood feeding at 06:00h (ZT0)		60	8	3	100	NA			
Supplementary Fig. 3a and Supplementary Table 8 (effects of gametocytemia dilutions and high temperature on parasite establishment in *A. stephensi*)	Midguts	27°C	1	32	8, 9	1	120	GLM	Oocyst intensity or prevalence	Gametocytemia + Temperature treatment + Gametocytemia × Temperature treatment	Oocyst intensity - negative binomial distribution with log link; Oocyst prevalence - binomial distribution with logit link
			1/2	33	8, 9	1	120				
			1/4	31	8, 9	1	120				
			1/10	31	8, 9	1	120				
		30°C	1	48	7	1	120				
			1/2	55	7	1	120				
			1/4	54	7	1	120				
			1/10	50	7	1	120				
Supplementary Fig. 4 (effect of transferring mosquitoes between different temperatures on vector competence in *A. gambiae*)	Midguts	27°C		30	8,9	1	120	GLM	Oocyst intensity, or oocyst or sporozoite prevalence	Mosquito container	Oocyst intensity - negative binomial distribution with log link; Oocyst and sporozoite prevalence - binomial distribution with logit link
		21°C		60	14-16	2	120				
	Salivary glands	27°C		30	14-16	1	120				
		21°C		60	34-36	2	120				
Supplementary Table 9 and 10 (effect of blood feeding at different temperature on feeding compliance in *A. gambiae*)	NA	27°C DTR 10°C	ZT12	NA	NA	2	120	GLMM	Blood feeding success of individual mosquitoes	Time-of-day + Temperature regime + Time-of-day × Temperature regime + Mosquito container[*]	Blood feeding success - binomial distribution with logit link
			ZT18			2	120				
			ZT0			2	120				
		21°C DTR 10°C	ZT12			2	120				
			ZT18			2	120				
			ZT0			2	120				
Supplementary Fig. 5 and Supplementary Table 11 (effect of transferring mosquitoes between different temperatures on blood meal size in *A. gambiae*)	NA	27°C DTR 10°C	ZT12	20	NA	2	30	GLMM	Mosquito body weight	Species + Time-of-day + Species × Time-of-day + Mosquito container[*]	Mosquito body weight - normal distribution with identity link
			ZT18	20		2	30				
			ZT0	20		2	30				

[†] Dpi: Days post infection

[ǂ] 150 or 120 mosquitoes per container for each of two biological replicate experiments

[*] Included as a random variable in model analysis

[¶] Prevalence data were pooled within each temperature treatment group after confirming no difference between two replicates or species (Fisher's exact test, two-sided, $P > 0.05$) to ensure model validity[44,45]

[§] Poisson distribution was used to ensure best model fit based on AIC value

Supplementary Table 13. Parameter values for the changes in the model used to investigate whether the magnitude of the differences in the human-to-mosquito transmission probability identified experimentally are likely to have a substantial epidemiological impact if the same result was observed in natural settings. Parameter estimates and full model structure are reported previously in Walker et al.[46] which builds on the original model presented in Griffin et al.[47].

Notation	Definition	Value
g_0	A Fourier function is used to generate seasonality that acts by altering the ratio of mosquitoes to humans over the course of a year. $R(t) = g_0 + \sum_{i=1}^{3} g_i \, cos(2\pi t i) + h_i \, sin(2\pi t i)$ This seasonality reflects Western Kenya, Walker et al.[46]	0.2854
g_1		-0.0633
g_2		-0.0902
g_3		0.06
h_1		0.0264
h_2		-0.06
h_3		-0.0453
EIR	Entomological inoculation rate, the number of infectious bites received per person per year	100 bites per person per year, at equilibrium, when $\Upsilon = 1$ and no LLINs are used
Λ_M	The force of infection to mosquitoes	Varies seasonally (0.007 – 0.008)
β	The time-varying emergence rate which is set according to the level of malaria seasonality	Varies seasonally (2.5 – 12.7)
$1/\mu$	The mortality rate, daily hazard of death from external causes	7.6 days
α	The rate at which mosquitoes take a bloodmeal	1 feed every 3 days
ω	The normalizing constant for the biting rate over ages	0.757
Υ	parameter to describe the relative differences in human-to-mosquito transmission probability caused by the time mosquitoes' blood-feed	Changes proportionally with the transmission probability of all infectious people (see Supplementary Table 6)
τ_M	The extrinsic incubation period from blood-feeding until sporozoites are present in the salivary glands	11.5 days
K_0	The maximum carrying capacity of the environment to support mosquito larvae	203.61 (scaled to represent the endemicity of the setting)
$\overline{R}$	The mean rainfall over the year for the setting described here (chosen arbitrarily to match Western Kenya)	0.2854

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