Supporting Information

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SI Materials and Methods

Mosquitoes. The first experiment was carried out at the Wadsworth Center Arbovirus Laboratory, Slingerlands, NY. The *Ae. aegypti* examined were collected in Nakhon Ratchasima Province, Thailand in May 2006 and maintained in the laboratory for nine generations before they were used in this study. The second experiment was carried out at the Institut Pasteur in Paris, France. *Ae. aegypti* were collected in Kamphaeng Phet Province, Thailand in December 2009 and the second generation in the laboratory was used in experimental infections. In the first experiment, larvae were reared at a constant temperature of 26 °C, 70% relative humidity, and under 12:12 h light:dark cycle. They were fed a standard diet of 1:1:1 mix of ground Koi food, fortified rabbit chow, and bovine liver powder. Three days before the infectious feed, adults were separated into three Conviron incubators programmed to follow three distinct temperature regimes with the same average temperature of 26 °C: (*i*) temperature maintained constant at 26 °C (DTR = 0 °C), (*ii*) 24-h sinusoidal cycles of 26 ± 5 °C (DTR = 10 °C), and (*iii*) 24-h sinusoidal cycles of 26 ± 10 °C (DTR = 20 °C). Temperature was set to change every hour with a precision of ±1 °C. In the second experiment, larvae were reared at 25 °C, 80% relative humidity, and under 16:8 h light:dark cycle. They were fed a standard diet of yeast. Three days before the infectious feed, adult mosquitoes were separated into two Binder incubators programmed to follow two temperature regimes: (*i*) temperature maintained constant at 26 °C (DTR = 0 °C) and (*ii*) 24-h sinusoidal cycles of 26 ± 10 °C (DTR = 20 °C). The temperature was set to change under a ramping mode with a precision of ±0.5 °C. In the incubators, adult mosquitoes were fed 10% sucrose ad libitum under a 12:12 h light:dark cycle. High relative humidity (>85%) was maintained with water-filled plastic containers and wet cotton pads.

Experimental Infections. In both experiments, experimental infection of mosquitoes took place in a biosafety level-3 facility where they were kept throughout the experiment. In the first experiment, mosquitoes were orally challenged with a DENV-2 strain isolated from a child in 1999 in Kamphaeng Phet, Thailand (1) that had been passaged once in *Toxorhynchites splendens* mosquitoes and three times in *Aedes albopictus* (C6/36) cells before its use in this study. Confluent cultures of C6/36 cells were inoculated at a multiplicity of infection (MOI) of 0.01 and incubated at 28 °C under 5% CO_2 for 8 d before cells and medium were harvested to prepare an infectious blood meal. The blood meal consisted of 47.5% defibrinated rabbit blood, 2.5% sucrose, and 50% virus suspension at a final titer of 1.3×10^8 plaque forming units per milliliter as measured by plaque assay on African green monkey kidney (Vero) cells (2). Five- to 8-day-old adult mosquitoes deprived of sugar for 24 h were exposed to the infectious blood meal for 1 h via a Hemotek membrane feeding apparatus (Discovery Workshops), using desalted porcine intestine as the membrane. After feeding, mosquitoes were briefly sedated with CO_2, and fully engorged females sedated on wet ice were retained. For each temperature treatment, engorged females were transferred into four 0.5-L cartons of ~75 females and returned to their respective incubators. In the second experiment, mosquitoes were orally challenged with a DENV-1 strain isolated in 2009 from a patient in Kamphaeng Phet, Thailand, that had been previously passaged four times in C6/36 cells. Confluent cultures of C6/36 cells were inoculated at an MOI of 0.2 and incubated at 28 °C for 7 d before cells and medium were harvested. The blood meal consisted of one volume of washed rabbit erythrocytes, one volume of virus suspension, and 10 μL/mL ATP at a final titer of 5×10^5 focus forming units per milliliter as measured by fluorescent focus assay in Vero cells (2). Six- to 8-day-old adult mosquitoes deprived of sugar for 24 h were exposed to the infectious blood meal for 40 min through a piece of desalted porcine intestine stretched over a water-jacketed glass feeder maintained at 37 °C. After feeding, mosquitoes were sedated for 20 min at 4 °C, and fully engorged females sedated on wet ice were retained. For each temperature treatment, engorged females were transferred into five 0.5-L cartons of ~35 females each and returned to their respective incubators. In both experiments, mortality was monitored in each carton by removing and counting dead mosquitoes every 1–3 d.

Vector Competence. We used rates of virus dissemination from the midgut as a proxy for transmission potential, as have most previous studies on *Ae. aegypti* vector competence for DENV (e.g., refs. 3–5). There is no documented evidence for salivary gland barriers for *Ae. aegypti* that would prevent DENV invasion of the salivary glands or secretion into the saliva following dissemination from the midgut (6). Viral infection and dissemination were evaluated by detection of infectious DENV in bodies and legs, respectively, at 5, 7, 11, 14, 20, 27, and 32 dpi. In the first experiment, a sample of 25 mosquitoes per treatment was randomly collected at each time point and processed as described in ref. 7. In the second experiment, a sample of 3–28 (mean 16) mosquitoes per treatment was randomly collected at each time point. Legs were removed and placed in 1.0 mL of Gibco Leibovitz's L-15 medium (Invitrogen) containing 10% heat-inactivated FCS. Mosquito bodies were placed into 1.0 mL of Leibovitz's L-15 medium containing 10% heat-inactivated FCS, and all samples were stored at −80 °C before processing. Samples were homogenized in a mixer mill (Qiagen) and clarified by centrifugation. The proportion of infected bodies and legs was determined by plaque assay on Vero cells in the first experiment and by immunofluorescent assay on C6/36 cells in the second experiment (2). The presence of infectious virus in each sample was determined qualitatively; i.e., either positive or negative.

Data Analysis. The proportion of mosquitoes with infected bodies (midgut infection) and legs (disseminated infection) was analyzed with a nominal logistic regression as a function of EIP, DTR, and their interaction. The experiment was included as a covariate in the analysis. Because the number of temperature regimes was not the same in both experiments, DTR was nested within the experiment. EIP was considered an ordinal variable. The minimal model was obtained by stepwise removal of strongly nonsignificant effects ($P > 0.15$). Survival distributions were analyzed using the Kaplan–Meier method for univariate survival with censored data; i.e., mosquitoes collected for virus detection were included in the analysis. Homogeneity of survival functions between temperature treatments was tested with a log-rank test (8). Differences were considered statistically significant at $P < 0.05$. Statistical analyses were performed with the software JMP version 5.1.2.

Temperature Models. The diurnal fluctuation in air temperature (T_a) was approximated using two commonly applied models (Fig. S3). The first model was a simple sine function fitted between daily maximum and minimum temperatures, as was used in the

vector competence assays. The second model was a sinusoidal progression during daytime and a decreasing exponential curve during the night (12:12 h day:night cycle). This slightly more complicated model better captures the slight asymmetries that can occur during the daily heating and cooling phases that are not captured in a simple sine model (9). Under both scenarios, T_a was calculated at 30-min intervals for a wide range of mean temperature and DTR combinations. Mean temperatures varied from 14 to 28 °C and DTR from 0 to 22 °C.

EIP Model. Time required by DENV, and many other mosquito-borne viruses, to complete extrinsic incubation in the vector is temperature sensitive (7, 10–14). This relationship has been previously characterized using an enzyme kinetics model (Fig. S1) (15). We used this established model to estimate DENV growth rate at 30-min intervals across the diurnal cycle according to the two temperature models described above. EIP completion was defined when cumulative growth rate reached one (see ref. 16 for a similar approach applied to the EIP of malaria parasites).

Vector Competence Models. Although EIP describes the time required for pathogen incubation in the vector, it is usually considered separately from vector competence, which describes intrinsic susceptibility and does not have a temporal dimension. Because the effect of temperature on DENV infection and transmission probabilities is not well documented, data from other mosquito–flavivirus systems were used to model the relationship between vector competence and temperature. We modeled both infection and transmission probabilities using empirical reports of the highest proportion of infected and transmitting vectors measured at various constant temperatures. For infection probability, empirical data of the following flaviviruses were used: West Nile virus (7, 17–22), Murray Valley encephalitis virus (23–25), and St. Louis encephalitis virus (26). Infection probability (p_I) was 0 for $T_a < 12.4$ °C, increased linearly with temperature for $12.4\,°C \le T_a \le 26.1\,°C$ ($p_I = 0.0729T_a - 0.9037$) until it reached one, and remained equal to 1 for $T_a > 26.10$ °C (Fig. S4). The regression coefficient was derived from the linear part of the modeled transmission probability function (see below). Empirical data from this set of mosquito–flavivirus systems suggest that infection probability remains equal to 1 even at very high temperatures (Fig. S4). A linear model between lower and upper bounds was chosen as the most parsimonious model on the basis of available evidence, of which outputs match well with the empirical infection data generated in this study. Available data indicate that transmission probability (p_T), defined as the proportion of midgut-infected mosquitoes transmitting virus, drops at high temperatures even when p_I remains stable (7, 26). To capture this effect, the thermodynamic function of Brière et al. (27) was fitted to a set of empirical p_T data for the following flaviviruses: West Nile virus (7, 13, 19), Murray Valley encephalitis virus (24, 25), and St. Louis encephalitis virus (26). The relationship between p_T and temperature was described by a nonmonotonic function (Fig. S4; $R^2 = 0.726$) given by the following equation:

$$p_T = 0.001044T_a(T_a - 12.286)\sqrt{(32.461 - T_a)}.$$

Probabilities of DENV infection and transmission were estimated by averaging probabilities calculated at 30-min intervals across the diurnal cycle according to the two temperature models described above, over a 24-h period.

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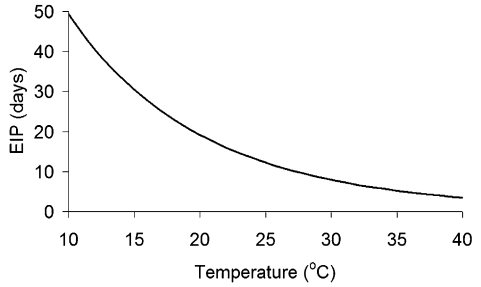

Fig. S1. Model of the effect of ambient temperature on EIP. Duration in days of the extrinsic incubation period of DENV as a function of temperature according to an enzyme kinetics model (details in *SI Materials and Methods*).

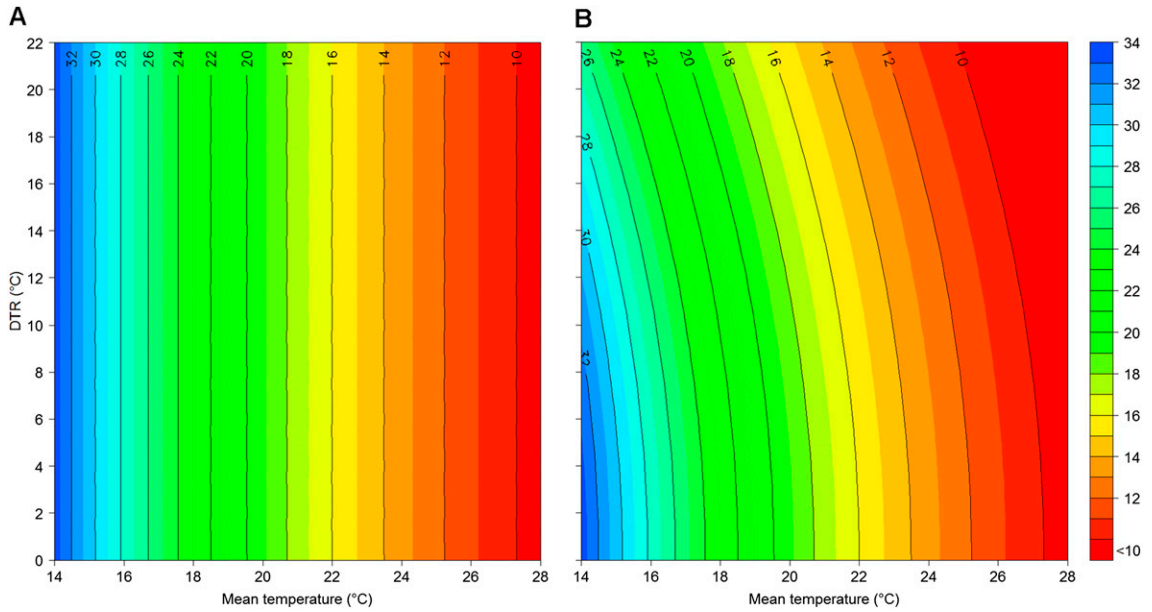

Fig. S2. Theoretical effects of amplitude and pattern of temperature fluctuations on EIP. The duration of the DENV extrinsic incubation period (right-hand bar, in days) is shown by contours of colors as a function of mean temperature (*x* axis) and the range of diurnal temperature fluctuations (*y* axis) for two functions of temperature variation. In *A*, temperature variation is described by a sine function, whereas in *B*, temperature variation consists of combined sine and exponential functions (a sinusoidal progression during daytime and a decreasing exponential curve during the night).

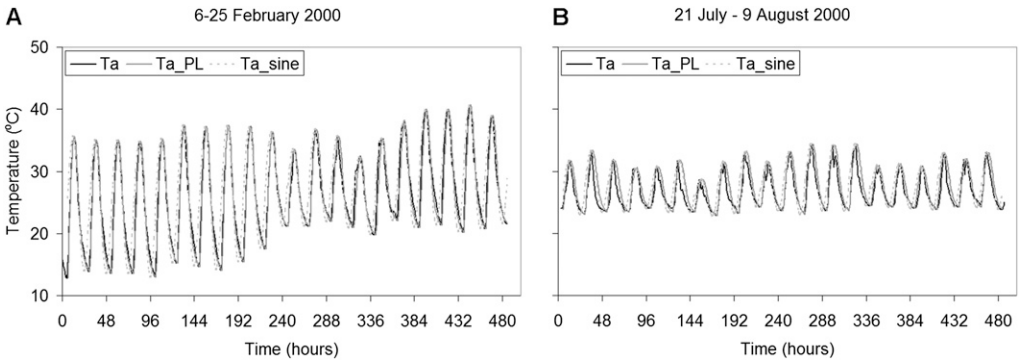

Fig. S3. Temperature models fitted to the temperature profiles shown in Fig. 1. The diurnal fluctuation in air temperature (Ta) is approximated during (*A*) low and (*B*) high DENV transmission seasons using either a simple sine function fitted between daily maximum and minimum temperatures (Ta_sine), or the Parton–Logan function (Ta_PL) that follows a sinusoidal progression during daytime and a decreasing exponential curve during the night.

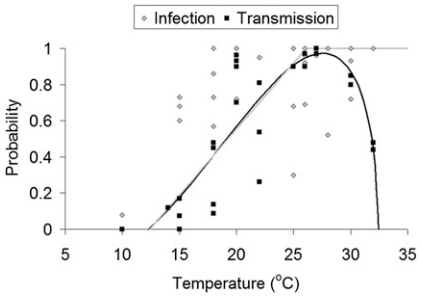

Fig. S4. Model of the effect of ambient temperature on vector competence. Relationship between temperature and the probability that a mosquito becomes infected with a flavivirus (diamonds) and subsequently transmits the virus (squares) according to a thermodynamic function fitted to a set of empirical data from various mosquito–flavivirus systems (details in *SI Materials and Methods*).

Table S1. Theoretical probabilities of DENV infection and transmission under various temperature fluctuation profiles

	Infection		Transmission	
	Sine	Sine + Exp	Sine	Sine + Exp
DTR = 0 °C	0.99	0.99	0.95	0.95
DTR = 10 °C	0.88	0.88	0.83	0.81
DTR = 20 °C	0.76	0.76	0.45	0.46

Temperature fluctuation around a mean temperature of 26 °C is described either by a sine function (Sine) or by combined sine and exponential functions (Sine + Exp).