

Appendix S1. Additional methods

Mosquito rearing. *Anopheles stephensi* and *Anopheles gambiae* mosquitoes were reared at 26–27 °C and 75–80% relative humidity with a 12 h light/12 h dark cycle. Eggs were placed in plastic tubs containing approximately 1.5 L of distilled water. Four days after hatching, the larvae were separated into groups of 400 larvae per tub and provided with 10 mg powdered Tetrafin fish food daily. After pupation, pupae were placed into cages for emergence. Adults were allowed to feed on 10% glucose-0.05% PABA solution *ad libitum*, and experiments were performed exclusively on 3–5 day-old females.

Preparation of clay tile substrates. White earthenware clay (Clay King, Spartanburg, SC, USA) was mixed with distilled water and allowed to soak overnight. The next day, the clay-water mixture was homogenized using an electric paint stirrer. The mixture was poured into 150 mm-diameter petri plates and allowed to harden for approximately one week until the interior of the clay was completely dry.

Production and formulation of fungal conidia. Microporous beads coated in fungus were retrieved from long-term storage at -80 °C and incubated at 25 °C on potato dextrose agar (Oxoid, UK) slopes to produce conidia. The conidia were then suspended in sterile 0.05% Tween 80 (Sigma) at a concentration of 10^6 conidia ml^{-1} . One ml of suspension was added to 75 ml of sterile liquid medium (4% d-glucose, 2% yeast extract (Oxoid, UK) in tap water), and the culture was incubated on a shaker at 24 °C and 160 rpm for three days. The culture was then combined with 75 ml of sterile water and used to inoculate mushroom spawn bags (Unicorn, Garland, Texas, USA) containing a sterile mixture of 1 kg barley flakes (Bobs Red Mill, Milwaukie, Oregon, USA) and 600 ml tap water. The bags were sealed and incubated at 24 °C for 10 days. The contents were then dried in paper bags for 4 days to reduce their moisture content below 20%. A Mycoharvester (Acis Manufacturing, Devon, UK) was used to harvest conidia from the barley, and the harvested conidia were dried over silica gel to a moisture level of 5%. The conidia were then sealed in foil sachets and stored at 5 °C.

Dry conidia were suspended in an oil mixture of 80% Ondina:20% Isopar M at concentrations of either 10^7 or 10^9 conidia ml^{-1} . Viability was assessed by plating three samples of the 10^7 conidia ml^{-1} suspension on Sabouraud dextrose agar (Oxoid, UK). The plates were incubated at 25 °C for 20 h. Following incubation, a microscope was used to assess germination visually. Those conidia which had germination tubes at least the length of the conidium were classified as germinated. Approximately 300 conidia were assessed on each plate. All suspensions used in the exposures had mean percent germination rates of $\geq 85\%$.

Parton and Logan temperature model. Parton and Logan (1981) developed a model which described realistic fluctuating daily temperature patterns using a sine function during the day and an exponential decline to a thermal minimum at night. For these experiments, we assumed a 12 h day/12 h night cycle. Temperature was calculated as a function of time, i (expressed as hours past midnight; 6:30 pm = 18.5), according to the following equation:

$$T(i) = \begin{cases} T_{\min} + (T_{\max} - T_{\min}) \sin\left(\frac{\pi(i-6)}{15}\right), & i < 12 \\ T_{\min} + T_s e^{-1} + \frac{(T_s - T_{\min})e^{\frac{T_s-i}{4}}}{1 - e^{-3}}, & i \geq 12 \end{cases} \quad \text{eqn S1}$$

where T_{\max} and T_{\min} are the daily maximum and minimum temperatures, respectively, and T_s is the temperature at sunset. T_{\max} and T_{\min} were calculated as follows:

$$T_{\max} = T_{\text{median}} + \frac{D}{2} \quad \text{eqn S2}$$

$$T_{\min} = T_{\text{median}} - \frac{D}{2} \quad \text{eqn S3}$$

where D is the diurnal temperature range (DTR) and T_{median} is the median daily temperature, estimated based on the mean daily temperature, T_{mean} , according to the following equation:

$$T_{\text{median}} = T_{\text{mean}} + 0.057582D \quad \text{eqn S4}$$

The temperature at sunset, T_s , was calculated as follows:

$$T_s = T_{\min} + (T_{\max} - T_{\min}) \sin\left(\frac{12\pi}{15}\right) \quad \text{eqn S5}$$

Rate summation. We used rate summation (Liu, Zhang & Zhu 1995; Paaijmans, Read & Thomas 2009) to predict the impact of the experimental diurnal temperature range (12 °C) on malarial extrinsic incubation period (EIP, parameter t_E). Corrected EIP (t_{E*}) was estimated as follows:

$$t_{E*} = \left(\sum_{i=0}^{24} r[T(i)] \, di \right)^{-1} \quad \text{eqn S6}$$

where $T(i)$ is mean temperature as a function of time of day (evaluated in hourly increments) as described by Parton and Logan (1981) (see below) and $r(T)$ is parasite development rate as a function of mean temperature, T , as estimated by Mordecai *et al.* (2013):

$$r(T) = T(1.11 \times 10^{-4})(T - 14.7)(34.4 - T)^{1/2} \quad \text{eqn S7}$$

Where daily fluctuation included peak temperatures above the critical maximum temperature for parasite development (i.e. where growth was zero, ~34.4 °C), we constrained parasite growth to the standard curve. This was a conservative assumption that likely underestimated the impact of high temperature fluctuations since it is expected that even transient exposures above the critical maximum temperature could have additional negative effects on growth even when temperatures return to permissive levels (e.g. Paaijmans *et al.* 2013). However, there are currently no data available to enable us to characterize these effects explicitly.

Malaria and fungus infection model. We utilized the susceptible–exposed–infectious (SEI) model developed by Hancock *et al.* (2009) to predict how environmental temperature and fungal coverage would impact the ability of *B. bassiana* to reduce infectious mosquito population densities. Malaria infection dynamics are tracked in susceptible (S), exposed (E , infected with malaria but not able to transmit disease) and infectious (I , able to transmit disease) classes. S_1 , E_1 and I_1 represent mosquitoes which are not infected with fungus, and S_2 , E_2 , and I_2 represent mosquitoes which are infected with fungus. Mosquitoes enter the susceptible class at constant rate, ε , and become infected with malaria at rate abx , where a is the human bite rate, b is malaria transmission efficiency and x is the proportion of humans with transmissible malaria. Mosquitoes move to the infectious class following a fixed delay (t_E , the length of the malarial EIP). Background mortality occurs at rate μ . Mosquitoes become exposed to fungus at rate F and experience additional mortality (M_F) which occurs as a function of time since fungal exposure, u :

$$M_F(u) = \beta \mu_F (\mu_F u)^{\beta-1} \quad \text{eqn S8}$$

where μ_F and β are temperature-dependent constants. The densities of mosquitoes in each class are represented by the following equations:

$$S_1(t) = \int_0^t \varepsilon \theta_{S_1}[t - \tau] d\tau \quad \text{eqn S9}$$

$$\text{where } \theta_{S_1}[t - \tau] = \exp[-(t - \tau)(\mu + abx + F)] \quad \text{eqn S10}$$

$$S_2(t, u) = S_1(t - u)F\theta_{S_2}[u] \quad \text{eqn S11}$$

$$\text{where } \theta_{S_2}[u] = \exp\left[-\int_0^u \mu + M_F(\xi) + abx d\xi\right] \quad \text{eqn S12}$$

$$E_1(t, p) = S_1(t - p)abx\theta_{E_1}[p], \quad 0 \leq p < t_E \quad \text{eqn S13}$$

$$\text{where } \theta_{E_1}[p] = \exp[-p(\mu + F)] \quad \text{eqn S14}$$

$$E_2(t, p, u) = H[u, p]E_1(t - u, p - u)F\theta_{E_2}[u, u] + (1 - H[u, p])S_2(t - p, u - p)abx\theta_{E_2}[p, u], \quad 0 \leq p < t_E \quad \text{eqn S15}$$

$$\text{where } \theta_{E_2}[t_1, t_0] = \exp\left[-\int_{t_0-t_1}^{t_0} \mu + M_F(\xi) d\xi\right] \quad \text{eqn S16}$$

$$\text{and } H[u, p] = \begin{cases} 1, & u \leq p \\ 0, & u > p \end{cases} \quad \text{eqn S17}$$

$$I_1(t) = \int_0^t E_1(\tau, t_E) \theta_{I_1}[t - \tau] d\tau \quad \text{eqn S18}$$

$$\text{where } \theta_{I_1}[t - \tau] = \exp[-(t - \tau)(\mu + F)] \quad \text{eqn S19}$$

$$I_2(t, u) = I_1(t - u)F\theta_{I_2}[u, u] + \int_{t-u}^t E_2(\tau, t_E, u - (t - \tau)) \theta_{I_2}[t - \tau, u] d\tau \quad \text{eqn S20}$$

$$\text{where } \theta_{I_2}[t_1, t_0] = \exp\left[-\int_{t_0-t_1}^{t_0} \mu + M_F(\xi) d\xi\right] \quad \text{eqn S21}$$

where t is overall time and p is time since malaria infection. See Table S1 for additional parameter information.

The equilibrium densities of mosquitoes in each class are calculated based on the equations below. One asterisk (e.g. E_1^*) indicates the equilibrium density of mosquitoes in a given class at time t and two asterisks (e.g. E_1^{**}) indicates the equilibrium total density of mosquitoes in that class.

$$S_1^* = \frac{\varepsilon}{F + abx + \mu} \quad \text{eqn S22}$$

$$E_1^*(p) = abxS_1^* \exp(-p(F + \mu)) \quad \text{eqn S23}$$

$$E_1^{**} = \frac{abxS_1^* (1 - \exp(-t_E(F + \mu)))}{F + \mu} \quad \text{eqn S24}$$

$$I_1^* = \frac{abxS_1^* \exp(-t_E(F + \mu))}{F + \mu} \quad \text{eqn S25}$$

$$S_2^*(u) = S_1^*F\theta_{S_2}[u] \quad \text{eqn S26}$$

$$S_2^{**} = \int_0^\infty S_2^*(u) du \quad \text{eqn S27}$$

$$E_2^*(p, u) = H[u, p]E_1^*(p - u)F\theta_{E_2}[u, u] + (1 - H[u, p])S_2^*(u - p)abx\theta_{E_2}[p, u] \quad \text{eqn S28}$$

$$E_2^{**} = \int_0^\infty \int_0^{T_E} E_2^*(p, u) dp du \quad \text{eqn S29}$$

$$I_2^*(u) = I_1^*F\theta_{I_2}[u, u] + \int_0^u E_2^*(t_E, \tau)\theta_{I_2}[u - \tau, u] d\tau \quad \text{eqn S30}$$

$$I_2^{**} = \int_0^\infty I_2^*(u) du \quad \text{eqn S31}$$

Additional model details may be found in Hancock *et al.* (2009).

Proportional reduction in infectious mosquito density was calculated as follows:

$$\text{Proportional reduction} = \frac{I_0^{**} - (I_1^{**} + I_2^{**})}{I_0^{**}} \quad \text{eqn S32}$$

where I_0^{**} was the predicted total equilibrium infectious mosquito density in the absence of fungus, and $I_1^{**} + I_2^{**}$ was the density in the presence of fungus at a given temperature and application rate.

References

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