Understanding the link between malaria risk and climate

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The incubation period for malaria parasites within the mosquito is exquisitely temperature-sensitive, so that temperature is a major determinant of malaria risk. Epidemiological models are increasingly used to guide allocation of disease control resources and to assess the likely impact of climate change on global malaria burdens. Temperature-based malaria transmission is generally incorporated into these models using mean monthly temperatures, yet temperatures fluctuate throughout the diurnal cycle. Here we use a thermodynamic malaria development model to demonstrate that temperature fluctuation can substantially alter the incubation period of the parasite, and hence malaria transmission rates. We find that, in general, temperature fluctuation reduces the impact of increases in mean temperature. Diurnal temperature fluctuation around means >21°C slows parasite development compared with constant temperatures, whereas fluctuation around <21°C speeds development. Consequently, models which ignore diurnal variation overestimate malaria risk in warmer environments and underestimate risk in cooler environments. To illustrate the implications further, we explore the influence of diurnal temperature fluctuation on malaria transmission at a site in the Kenyan Highlands. Based on local meteorological data, we find that the annual epidemics of malaria at this site cannot be explained without invoking the influence of diurnal temperature fluctuation. Moreover, while temperature fluctuation reduces the relative influence of a subtle warming trend apparent over the last 20 years, it nonetheless makes the effects biologically more significant. Such effects of short-term temperature fluctuations have not previously been considered but are central to understanding current malaria transmission and the consequences of climate change.

basic reproductive rate | climate change | diurnal temperature fluctuations | extrinsic incubation period | *Plasmodium falciparum*

The dynamics and distribution of malaria are strongly determined by climatic factors (1). However, the exact influence of climate and likely consequences of climate change are unclear. In part this is because transmission of disease is determined by a suite of other socioeconomic, environmental and behavioral factors that can exacerbate or negate climatic influences (2–4). But even leaving these nonclimatic issues aside, the effect of climate itself on the intrinsic probability of transmission remains controversial (1, 3, 5).

The influence of climate on vector-borne disease can be determined by the basic reproductive number (R_0), which defines the number of cases of a disease that arise from one case of the disease introduced into a population of susceptible hosts (1). R_0 is determined by a range of entomological and epidemiological parameters. Among these, the extrinsic incubation period (EIP) of the parasite within the mosquito, also referred to for malaria as the period of sporogony, is one of the most critical as this influences R_0 in an exponential fashion (6). Hence, even small changes in the EIP can have a large effect on R_0; this is because it greatly influences the number of infected mosquitoes that live long enough to become infectious. During the EIP, malaria parasites go through various developmental stages and very many replication cycles before migrating to the salivary glands where they can be transmitted to humans. The speed of this development depends on host, parasite and environmental factors, but estimates are on the order of 10–14 days in areas of high malaria transmission (7, 8). In those same areas, 90% of the female mosquitoes die within 12 days (7) and are therefore unlikely to contribute to malaria transmission.

The extrinsic incubation period is extremely temperature sensitive (9, 10). For *Plasmodium falciparum*, the major malaria species throughout much of Africa, the relationship between ambient temperature (T) and the EIP is approximated by EIP = 111/(T-16), describing the iconic Detinova curve (11, 12). Use of this equation is ubiquitous, with the vast majority of studies deriving EIP using measures of average monthly temperature to predict current malaria risk, and hence identify priority areas for allocation of resources for disease control and to assess the impact of climate change on global malaria burdens (8, 13–19). However, mosquitoes and the developing malaria parasites do not experience 'average temperatures,' but are exposed to temperatures that fluctuate throughout the day. In this paper, we highlight how diurnal temperature fluctuation has the potential to dramatically alter the rate of parasite development and hence malaria transmission.

Our approach utilizes a common thermodynamic model (20), which characterizes the nonlinear influence of temperature on biological processes such as growth or development (see *Methods* and Fig. S1). This model enables us to determine cumulative growth of the malaria parasite inside the mosquito over set time intervals (e.g., every 30 min) for different fluctuating temperature regimes, including temperatures approaching the thresholds for malaria development. We determine air temperature using a minimum-maximum temperature model in which temperature follows a sinusoidal progression during daytime and a decreasing exponential curve during the night (21, 22). This model produces realistic diurnal temperature patterns for different maxima and minima (see *Methods* and Fig. S2), and can be used to explore the influence of day length, so enabling us to consider effects such as latitude and seasonality. We find that temperature fluctuations have important impacts on the time until mosquitoes will become infectious and hence on R_0. Proper understanding of the influence of environment on disease risk both now and under future climate change scenarios requires that we incorporate temperature fluctuations into climate-based models.

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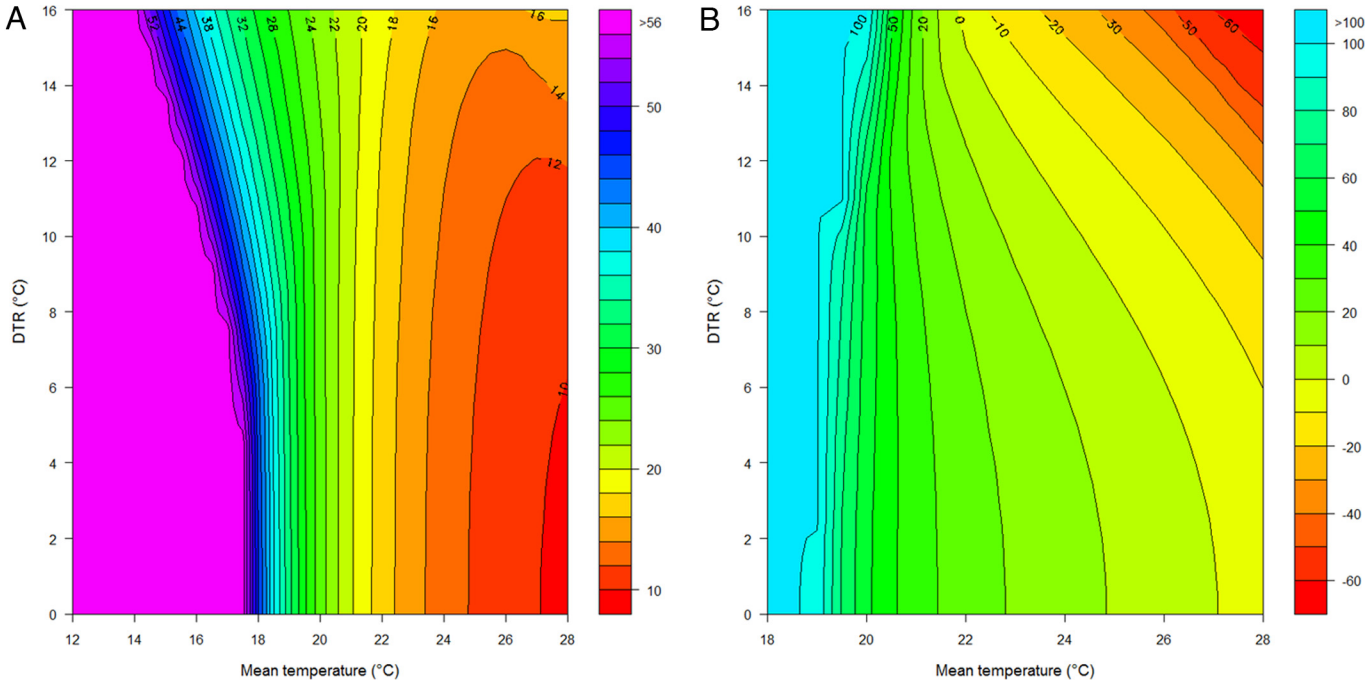

Fig. 1. Changes in the extrinsic incubation period of malaria parasites and in the basic reproductive number as a consequence of temperature fluctuation. (*A*) Duration of the extrinsic incubation period (days, right hand bar) of *Plasmodium falciparum* parasites across a range of mean temperatures (12–28°C) and diurnal temperature ranges (0–16°C). (*B*) The relative change in R_0 (%, right hand bar) across a range of mean temperatures (18–28°C) and diurnal temperature ranges (0–16°C), comparing R_0 estimates derived from the length of EIP shown in Fig. 1*A* with estimates as predicted by the iconic equation of Detinova (12). Models are run with 12:12 day:night cycle.

Results

The predicted effect of diurnal temperature range (DTR) on the extrinsic incubation period of *P. falciparum* for a 12:12 day:night cycle is shown in Fig. 1; the effects predicted under 10:14 and 14:10 day:night cycles are shown in Fig. S3. Temperature fluctuation alters the length of the EIP compared with estimates based on the equivalent means, with both magnitude of DTR and day length shaping the relationship. In areas with means of 20–22°C, the effects of fluctuation are generally small. However, in areas with means below 20°C, the effect of fluctuation is very much larger, with extrinsic incubation period reduced by many days as DTR increases. For instance, at a constant 18°C, EIP is completed within 46 days, but under a diurnal fluctuation of ± 7°C, parasites become infectious nearly a fortnight earlier. The reason is that exposure to warmer temperatures for at least part of the day provides something of a rescue effect, increasing development rate relative to the cooler mean temperatures. This mechanism can even result in parasites being able to develop at average temperatures below the currently assumed minimum threshold temperature. In contrast, at mean temperatures above c.22°C, and especially above 24°C, DTR has the reverse effect leading to a relative increase in the incubation period. Here, periodic exposure to cooler temperatures, and/or hot temperatures that exceed the optimum, leads to longer incubation periods than expected. Day length exacerbates these influences as expected, so that in transmission seasons with longer day lengths, for example, around a low mean temperature the rescue effects of high temperature occur for longer each day, further speeding development. All these conclusions are qualitatively robust to changes in high and low threshold temperatures for parasite development (Fig. S4).

To estimate the consequences of these changes in extrinsic incubation period on absolute R_0 requires data on a suite of entomological parameters. However, it is possible to assess the expected relative change if we hold all parameters constant and vary EIP only (see *Methods*). Importantly, adult mosquito survival is largely insensitive to temperature across much of the transmission range, with mortality only increasing markedly as mean temperature exceeds 35–36°C (23). Here we follow others and assume a median daily survivorship of 0.860 (24) and a maximum mosquito lifespan of 56 days (13). The result is that small changes in EIP due to temperature fluctuation can have a large relative effect on R_0 with, again, the extent of DTR and day length influencing the magnitude and the sign of the response (Fig. 1*B* and Fig. S3 *C* and *D*). When we compare our model with Detinova's equation (12) we see that at mean temperatures less than or equal to 20°C, the incorporation of diurnal temperature fluctuation can more than double the resulting estimate of R_0. This means that risk models based on the Detinova curve may be very significantly under-estimating transmission intensity under cooler conditions. At mean temperatures greater than or equal to 22°C, on the other hand, incorporating fluctuation can more than halve estimates of R_0, suggesting that current risk models are significantly over-estimating R_0 at warmer temperatures. Use of relative R_0 does not in itself quantify absolute disease risk and as pointed out by Rogers & Randolph (1); a 100% increase in R_0 from a value such as 0.2 would still leave R_0 below the threshold for establishment. However, there will be cases where a doubling will result in R_0 exceeding 1, and in already endemic areas, any changes are important in terms of quantifying actual transmission intensity. A doubling of R_0 from 50 to 100, for instance, can severely affect assessments of the likely impact of a particular control measure and/or allocation decisions about the resources necessary to control or even locally eradicate malaria (25).

In addition to revealing the importance of temperature fluctuation for estimating transmission intensity under existing conditions, Fig. 1*A* also enables us to explore likely effects of climate change. In general, increasing mean temperature results in a shortening of the incubation period (although at high mean temperatures and large DTR the reverse is possible). However, as DTR increases, the sensitivity of EIP to warming declines (the contours in Fig. 1*A* get further apart as DTR increases). Thus, the relative effects of increases in mean temperature are likely

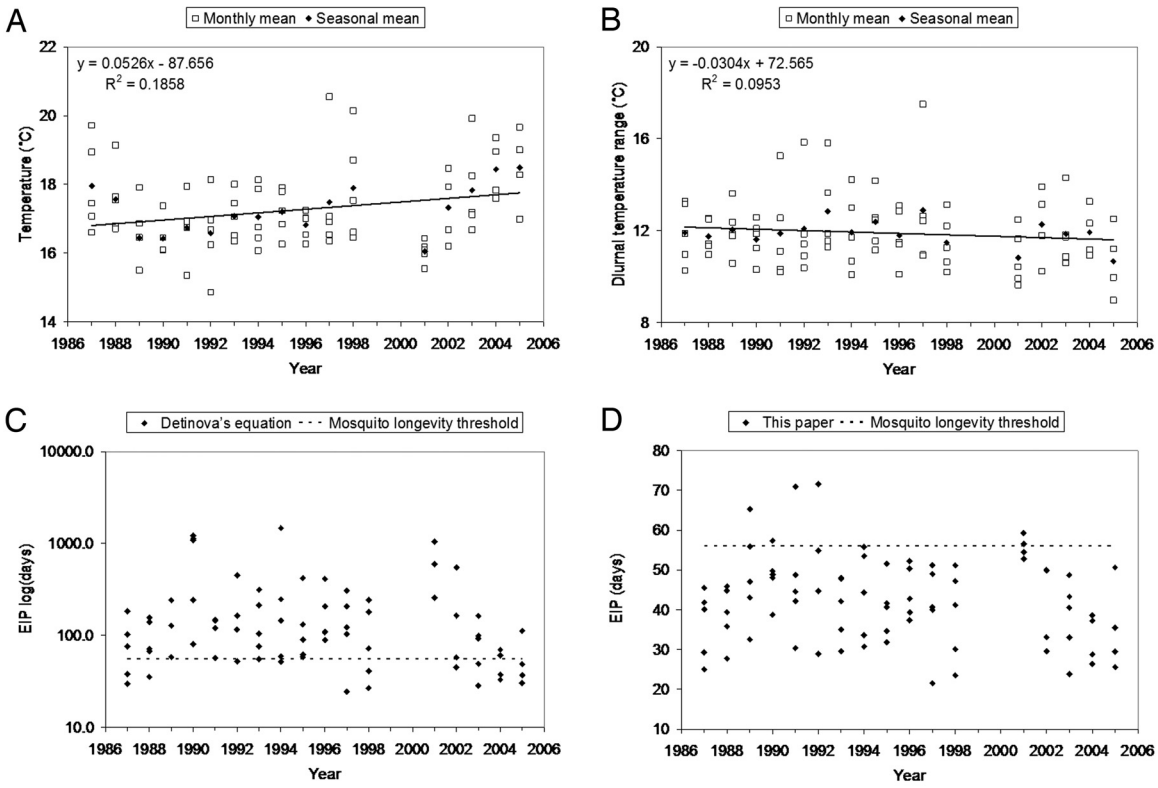

Fig. 2. Actual temperature conditions in a Kenyan highland area and the predicted length of malaria parasite development with and without the implementation of temperature fluctuation. (*A*) The mean air temperature and (*B*) the mean diurnal temperature range during malaria transmission seasons (March–July) between 1986 and 2006 in Kericho, and the corresponding length of the extrinsic incubation period of *Plasmodium falciparum* based on (*C*) Detinova's equation (12) and (*D*) the thermodynamic model described in the current paper, allowing temperature fluctuations.

to be less than expected when daily temperature fluctuation is taken into account. However, there is a further complication as global warming is unlikely to result in a symmetrical shift in maximum and minimum temperatures. Several studies predict proportionately greater increases in daily minima than in daily maxima, resulting in decreases in DTR (26–28), although the reverse is possible at local scales (28, 29). Defining the nature of these changes at particular sites will be important for predicting local changes in disease burdens because changes in DTR can exacerbate or mitigate the influence of increases in mean temperatures, depending on initial starting conditions (Fig. 1*A*).

To explore these arguments further we examine the predicted influence of temperature fluctuation on seasonal malaria at Kericho, a site in the Kenyan Highlands that has been at the centre of the debate on whether climate change has already impacted on malaria dynamics (6, 30–35). We derived estimates of mean daily temperatures and DTR from the Kericho meteorological station data (obtained from the National Climatic Data Center) for the main malaria transmission season for the years 1987–2005 (see *Methods*). These data indicate mean monthly temperatures over the transmission season ranging from 14.9–20.5°C, with DTR from 9.0–17.5°C (Fig. 2*A* and *B*). With these mean temperatures, the Detinova curve (12) predicts EIP to last between 24 and 1,475 days (Fig. 2*C*). Given mosquito mortality rates of 10–20% per day and an upper limit for survival of 56 days (13), these incubation periods make transmission unlikely (and often impossible) in all but 16 of a total of 82 potential transmission months for which data are available (Fig. 2*C*). Assuming that transmission is not possible in years where EIP falls below maximum mosquito longevity for less than 1 month (unless the EIP itself is less than a month), this translates at the annual level to transmission during only 6 of the 17 years (Fig. 2*C*). Yet seasonal malaria epidemics have occurred annually throughout the 17 years (36). This mismatch between theory and observation can be rectified by incorporating the observed DTR, which reduces the length of the EIP considerably to 22–72 days (Fig. 2*D*). These shorter periods of the EIP now make transmission possible in every year, with just 6 unsuitable months of the total; a result consistent with the frequency of epidemics observed at Kericho during this period (36).

Simple linear regressions fitted to the Kericho temperature data reveal a marginally positive trend in the mean temperatures (R^2 = 0.1858) and a marginally negative trend in DTR (R^2 = 0.0953) over the 19-year time period. Conventional extrapolation from the Detinova curve would lead to the conclusion that the warming would have reduced the EIP from 129 to 61 days. While this represents a substantial reduction, in line with the interpretation above, the absolute EIP is still too long to make the change biologically meaningful. Including the influence of fluctuation together with the modest decline in DTR reduces the magnitude of the relative change, with the EIP reduced from 42 to 36 days. However, although smaller, this relative change is now within biologically meaningful limits for mosquito survival, and importantly is associated with very substantial relative increase of 150% in R_0.

Discussion

Our analysis reveals that diurnal temperature fluctuation will alter the length of parasite incubation compared with estimates based on the equivalent means, with both DTR and day length shaping the relationship. Under warmer conditions, for example, diurnal fluctuation increases the EIP due to the nonlinear effects of short-term exposure to sub- and superoptimum temperatures. Consequently, in areas with mean temperatures in the range of 22–28°C (representative of large parts of sub-Saharan Africa),

estimates of R_0, or other metrics of malaria risk, based solely on measures of mean temperature could be too high so that by extension, malaria may be potentially more controllable than currently assumed. The effect is likely to be greatest for mean temperatures >26°C, which tend to be representative of areas with high transmission intensities. A more pronounced effect, however, occurs at lower temperatures, where malaria transmission is more likely to be epidemic rather than endemic. In these transition environments, EIP becomes markedly shorter as day length and DTR increase. Indeed, temperature fluctuation could enable parasites to complete development within the lifespan of their vector at lower mean temperatures than previously predicted. Hence, in areas with mean temperatures below 20°C, current estimates of risk could be too low.

This latter point is supported by the data from Kericho. The relatively low mean temperatures revealed at Kericho over the last c.20 years are similar to those reported by Zhou et al. (31) from meteorological station data at 4 East African Highland sites (including Kericho) for the period 1978–1998, and by Shanks et al. (35) from a separate station at Kericho for the period 1966–1995. The malaria developmental period estimated from the Detinova curve (12) for the cool mean temperatures at Kericho is too long to allow malaria transmission in most years. However the regular malaria epidemics observed there since the 1980s can be explained by the observed diurnal temperature fluctuations; at these cooler altitudes, there is nonetheless sufficient heat during part of the day to allow EIP to be routinely completed within the lifespan of adult *Anopheles* mosquitoes.

Indoor temperatures could also play a role. These can differ from outdoor temperatures, both in terms of mean and DTR, depending on altitude (19, 37) and the nature of the building structure (38) and surroundings (37). Incorporating this source of temperature variation is not straight forward. Some studies indicate that *An. gambiae* and *An. funestus*, the principal malaria vectors in Kericho (39, 40), spend time feeding and resting indoors (41–46). Other studies suggest a tendency for outdoor biting and resting, or no clear preference between environments (43, 45, 47, 48). In addition, the ability of anopheline mosquitoes to maintain steady-body temperatures by behavioral thermoregulation is limited (49). The importance of temperature fluctuation we have shown here makes a strong case for trying to determine how mosquito behavior impacts on the temperature conditions actually experienced by developing malaria parasites, and further emphasizes that mean monthly temperature is but one determinant of the thermal regime determining malaria transmission.

In previous studies of climate change in the East African Highlands (e.g., 32, 34) mean temperatures were estimated using the global 0.5 × 0.5° [≈55 × 55 km at the equator (32)] gridded data set of terrestrial surface climate (extracted from the Climate Research Unit, Norwich, U.K.). These interpolated means appear 2–4°C higher than actual mean temperatures derived from site specific meteorological stations [see (35) for explicit comparison and also (30)]. Although the extent of these differences will depend in part of the exact location (especially altitude) of the meteorological stations, the deviations from actual temperature data [shown to be significant by Shanks et al. (35)] raise questions over use of global gridded data for any quantitative evaluations influenced by absolute temperature.

Beyond helping to explain the presence of malaria under cool conditions, the role of temperature fluctuations appear central for interpreting the consequences of climate change. The trends in the Kericho data, for example, suggest a marginal increase in the mean temperatures and a marginal decrease in DTR during the transmission season over the last 20 years. The changes are small, and we acknowledge that alternative analyses could reveal different patterns and that additional factors, such as intermonth or interannual variability (51), can also be important. Even so, an increase in mean temperature is consistent with the analysis of Pascual et al. (34) and predicted for the region using general circulation models (52). Moreover, our aim is not to argue for or against the presence of a climate change signal (31, 32, 34, 30), but rather, to ask how daily temperature fluctuation might affect the biological significance of any change that might have already occurred, or which will do so in the future. From mean temperature alone, the increase from 16.8–17.8°C seen at Kericho (Fig. 2*A*) is predicted to halve the extrinsic incubation period, an apparently striking change. However, the resultant EIP is still too long for parasite development to be completed within the mosquito lifespan, so the probability of transmission remains effectively nil. Hence, this large predicted effect of climate change on EIP will have little biological significance. When the effects of diurnal temperature fluctuation are included, the predicted shortening in incubation period for that same 1°C change in mean temperature is relatively smaller (Fig. 2*B*). Nonetheless, because the baseline EIP is already much shorter due to the effects of temperature fluctuation, even this more modest change now has biological significance since parasites are developing fast enough to transmit routinely. This result supports the argument for a recent climate-induced increase in malaria transmission intensity in the Kenyan Highlands (34).

The influence of short-term temperature fluctuation suggests an important but largely unexplored mechanism via which environmental temperature can affect disease transmission, and adds a layer of complexity to the potential influence of climate change on dynamics of vector-borne diseases such as malaria. In general, diurnal temperature fluctuation reduces the impact of a change in mean temperature, although the nonlinearities, together with possible changes in DTR, make patterns complex. For example, if we consider a 3°C rise in temperature [the median increase in terrestrial temperature predicted by the IPCC for the months March–May in East-Africa by 2100 (52)] then for a site with a current mean temperature of 18°C, the standard Detinova equation (12) predicts a shortening of EIP of 34 days (from 56 to 22 days). However, allowing for a typical DTR of 12°C, the reduction in EIP would only be 13 days (from 34 to 21). Whether DTR changes simultaneously over this range is relatively unimportant as 21°C is on the linear part of the development curve (Fig. S1) and so daily temperature variation has negligible effect on ultimate EIP (Fig. 1). Alternatively, if we consider an equivalent 3°C increase in temperature for a hypothetical malaria-free area with a current mean of just 14°C, then although EIP is predicted to be reduced dramatically, malaria transmission would remain extremely unlikely based on mean temperatures alone because EIP still exceeds the lifespan of most mosquitoes. However, with a DTR of 12°C, EIP is reduced to 42 days making malaria transmission theoretically possible. If DTR was to simultaneously increase at this transition site by 2°C, then EIP would be reduced to 37 days, further increasing risk of transmission. On the other hand, if warming reduced DTR by 2°C, then EIP would increase to 47 days, lessening the effect of the increase in mean temperature.

These theoretical arguments rest on the assumption that parasite development within the mosquito is sensitive to short-term variation in temperature. Remarkably, we can find no studies quantifying this effect; a substantial knowledge gap that needs to be filled and tested. However, the so-called Kaufmann-effect (53), whereby biological processes appear to be faster under fluctuating low temperatures, and slower under fluctuating high temperatures, has long been recognized and the influence of diurnal temperature variation established in a range of other host/vector-pathogen/parasite systems (54–60), and for many insect-related rate processes in general (61). As such, there is every reason to expect malaria parasite development to be affected by temperature fluctuation and reciprocally, little empirical support for the prevailing use of mean monthly (or even annual) temperatures for estimating disease risk. Indeed, the effects we describe result only from basic rate

summation of the generic nonlinear development model and take no account of additional physiological mechanisms, which could further exacerbate the influence of temperature variation (61). Furthermore, while our focus has been on the extrinsic incubation period, the effects of daily temperature dynamics have not been thoroughly explored for any of the key entomological parameters (e.g., development rate, adult size, length of feeding cycle, biting rate, adult longevity) that combine to determine vectorial capacity and R_0, and are likely highly dynamic, especially in environments where malaria is seasonal [see (34)]. Analysis of these nonequilibrium conditions will require development of models beyond the standard Ross-MacDonald framework. In either case, given the need to understand malaria dynamics for setting operational control objectives and for predicting consequences of climate change, this study highlights an urgent need to develop a better mechanistic understanding of vector-parasite interactions with improved integration of the biological and environmental parameters.

Methods

Temperature Model. A good representation of both the phase and form of the diurnal rhythm of the air temperature (T) is given by a sinusoidal progression during daytime and a decreasing exponential curve during the night (21, 22):

$$T = T_{\min} + (T_{\max} - T_{\min})\sin\left[\pi\,\frac{t - 12 + D/2}{D + 2p}\right]$$

$$t_{rise} \le t < t_{set}$$

$$T = \frac{T_{\min} - T_{set}\exp(-N/\tau) + (T_{set} - T_{\min})\exp(-(t - t_{set})/\tau)}{1 - \exp(-N/\tau)}$$

$$t_{set} \le t < t_{rise}$$

where T_{min} and T_{max} (°C) are the minimum and maximum daily air temperature, respectively, t (hours) the time, D (hours) the day length, p (1.5 h) the time duration between solar noon and maximum air temperature, t_{rise} (hours) the time of sunrise, t_{set} (hours) the time of sunset, T_{set} (°C) the temperature at sunset, N (hours) the duration of the night and τ the nocturnal time constant.

This model accurately predicts the daily temperature progression as observed during clear days in a lowland (Kisian; 1,126 m above mean sea level) and highland site (Fort Ternan: 1,552 m amsl) in western Kenya [Fig. S2; data from (62), D = 12]. The observed average temperatures were 23.0°C (model prediction 23.3°C) and 20.3°C (model prediction 20.6°C) in the lowland and highland site, respectively.

We modeled the air temperature at 30-min intervals for a wide range of mean temperature - diurnal temperature range combinations. Mean temperatures varied from 12 to 28°C and the DTR from 0 to 16°C. To assess the effect of seasonality and/or latitude, 3 different day lengths of 10, 12, and 14 h were tested.

Extrinsic Incubation Period. The development of a malaria parasite within its mosquito host is highly temperature-sensitive. Available functional forms that have been used to describe the relationship between malaria development rate (r) and temperature (T) are either not valid near the temperature limits of development [Detinova's formula (12)] or, we feel, do not accurately mimic the pattern generally observed in cold-blooded species (63). Therefore we fitted the temperature-development function proposed by Brière et al. (20) to a set of empirical data (64–69) and the appropriate linear range derived from the Detinova function (12) (Fig. S1) to give:

$$r(T) = 0.000112T(T - 15.384)\sqrt{(35 - T)} \qquad (R^2 = 0.924)$$

Development rate was calculated at 30-min intervals using the temperature model described above. Growth rates were accumulated until they reached a value of 1, which defines the completion of the extrinsic incubation period.

This type of approach has been widely used to explore a number of temperature-dependent rate effects for insects (e.g., 20, 70–72) and more fundamentally for exploring the effect of temperature on a wide range of ecological and evolutionary questions (e.g., 59, 73, 74). Brière's model is just one of the possible models that captures the characteristic nonlinear and asymmetric influence of temperature, with a sharper decline in development at temperatures above the optimum than below. Other alternative functions such as those proposed by Logan (70) and Lactin (71) are more complex and provide no better fit to the available data. While having equivalent fit alone does not ensure identical model behavior (61), exploring the effects of temperature fluctuations on EIP with an alternative thermodynamic model for *Plasmodium falciparum* (63), produces qualitatively similar results (Fig. S4 *A* and *B*) due to the fundamental nature of the rate summation effect with nonlinear development functions (61).

Basic Reproduction Rate. The mean number of secondary cases a single infectious person will cause in a population is important for setting proper disease control targets. R_0 is determined by a range of entomological and epidemiological parameters:

$$R_0 = \frac{1}{r}\left[\frac{ma^2bcp^{EIP}}{-\ln p}\right]$$

where r is the recovery rate of hosts from infection, m the vector:host ratio, a the biting rate of the vector, b the transmission coefficient from vertebrate to vector, c the transmission coefficient from vector to vertebrate, p the daily survival rate of the vector and EIP, the extrinsic incubation period or development time of the parasite within the vector. The relative consequences of changes in extrinsic incubation period on R_0 can be assessed using the relationship between EIP and p derived from the R_0 equation: p^{EIP}/-ln$p$. We assume that all other parameters in the R_0 equation (10) are similar and can therefore be omitted; that is, estimates based on the mean and estimates based on a fluctuating temperature regime only affect the duration of *EIP* in this study.

Temperature Data from Kericho. The Kericho district ranges from 1,600–3,000 m (40). Available daily surface data for the years 1986–2006, including minimum, maximum, and mean air temperatures, were obtained from the National Climatic Data Center (http://www.ncdc.noaa.gov/oa/mpp/freedata.html). The meteorological station is situated at an altitude of 2,184 m. We restrict our analysis to data from March to July, the months of the main transmission season. The approach of focusing on the transmission season differs from most other studies that tend to consider conditions across the whole year, or even multiple years (31, 34, 35, 50). However malaria in the East African Highlands is not holoendemic (35) and entomological surveys in Kericho indicate the principal mosquito vectors are largely restricted to a 4–5-month window (39, 40). Thus, from the perspective of mosquito-parasite biology, it is most relevant to consider conditions during this discrete season. Months with less than 7 days of temperature data were omitted from the analysis (May 2002, April 2004, and June 2005).

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