

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☒ | ☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☒ | ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☒ | ☐ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☐ | ☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	N/A These data were collected experimentally.
Data analysis	All analyses were conducted in R (v4.3.1) using the 'tidyverse' package (v2.0.0). TPCs were fit using R and JAGS (v4.3.2) via the 'R2jags' package (v0.7.1.1). The TPC and suitability model analysis and figures also used the R packages 'progress' (v1.2.3), 'cowplot' (v1.1.1), 'patchwork' (v1.1.2), and 'gridExtra' (v2.3). Raster calculations and mapping analysis also used the R packages 'raster' (v3.6.26), 'terra' (v1.7.78), 'sf' (v1.0.17), 'mapproj' (v1.2.11), 'mapdata' (v2.3.1), 'ggthemes' (v5.1.0), and 'rnatuarearth' (v1.0.1). All code is available in the project GitHub repository: https://github.com/mshocket/anopheles-rate-summation-release ; DOI: 10.5281/zenodo.15026846 .

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

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Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

N/A

Reporting on race, ethnicity, or other socially relevant groupings

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

We conducted an experiment using the mosquito *Anopheles stephensi* to investigate the effects of variation in mean temperature and diurnal temperature range on the performance of three life history traits (lifespan, lifetime egg production, and daily biting rate) that are relevant for transmission of vector-borne diseases like malaria. Our experiment included three diurnal temperature ranges (DTR: 0 C, DTR: 9 C (+ or - 4.5 C), DTR: 12 C (+ or - 6 C) that fluctuated around one of five mean temperatures (16 C, 20 C, 24 C, 28 C, 32 C). 3 day old female mosquitoes were provided a bloodmeal and then sorted randomly into each of these environmental treatments. Females were offered a bloodmeal and oviposition site daily, and we noted when females fed, when they laid as well as how many eggs were laid, and when they died. We conducted two full biological replicates of this experiment. We then used a Bayesian framework to fit either symmetrical or asymmetrical non-linear unimodal functions to generate a thermal performance curve that predicted trait values across temperatures. Functions (e.g., quadratic or Briere) were restricted from becoming negative by assuming a trait value to be zero if $T < T_{min}$ or $T > T_{max}$. We fit trait thermal responses to individual-level data used different probability distributions for each trait based on the data type and observed distribution. For biting rate we used a normal distribution truncated at zero, for lifespan we used a gamma distribution and for lifetime egg production we used a negative binomial distribution. For each trait we selected the best-fitting functional form using the deviance information criterion (DIC). For each parameter in the mean response function (i.e., c , T_{min} , T_{max}) and the additional parameter required to specify each probability distribution (i.e., the variance for the truncated normal distribution, the rate parameter for the gamma distribution, and the r parameter for the negative binomial distribution), we assumed low-information uniform priors ($T_{min} \sim \text{uniform}(0, 20)$, $T_{max} \sim \text{uniform}(28, 45)$, $c \sim \text{uniform}(0, 10)$, variance $\sim \text{uniform}(0, 1000)$, rate $\sim \text{uniform}(1, 100)$, $r \sim \text{uniform}(1, 100)$) that restricted the range of parameters to biologically or statistically meaningful values. For each TPC, we used the posterior distributions for the parameters to generate posterior distributions over a temperature gradient from 0-45°C at 0.1°C intervals, which we then used to calculate the mean, median, and 95% credible intervals. To test for the statistical significance of fluctuation treatment, we used the Deviance Information Criterion (DIC) output from JAGS. For each trait, we compared: 1) the sum of DIC values for the three models fit separately to data from each treatment (constant, DTR 9°C, and DTR 12°C) and 2) the DIC of a model fit to the combined data from all treatments. Fluctuation treatment is significant if the sum of the separate models is ≥ 2 DIC units lower than the DIC value for the combined model. To calculate the trait thermal responses predicted by rate summation (Equation 1) we used the 7,500 posterior samples from the Bayesian fitted TPCs for each trait measured at constant temperatures. First, we used a Parton-Logan model 65 to calculate a temperature profile for each mean temperature spanning 0-50°C with 0.1°C increments, assuming a DTR of 9 or 12°C

across a 24-hour period (see SI Methods). Second, we calculated predicted trait values at each hour using the TPC for trait performance at constant temperatures. Third, a daily mean value for each trait was calculated by averaging the predicted hourly values for that trait over the 24-hour period for each mean temperature. When fluctuating temperatures extended beyond the range of our constant temperature TPCs ($0^{\circ}\text{C} \leq T \leq 45^{\circ}\text{C}$), we used the trait value predicted at the corresponding edge temperature, which was always equal or approximately equal to zero. Lastly, since rate summation was conducted for each posterior sample, we calculated the mean, median, and 95% credible interval of the resulting rate summation estimates for each mean temperature. We use a modified relative R0 expression that incorporates the thermal responses of mosquito and parasite traits to evaluate the combined effects of temperature and temperature fluctuation on the predicted thermal suitability (S(T)).

Research sample	Anopheles stephensi, the S. Asian urban malaria mosquito, from a long-standing colony (~40 years) were reared in Murdock's facility and used for this experiment. Mosquito strain was the urban type form originally sourced from Walter Reed Army Institute of Research, Silver Spring, MD, USA. Field collections of Anopheles stephensi to generate recently field derived colonies are impossible to obtain due to strict regulations that prohibit the export of any biomaterial from India. Only female mosquitoes were used, as they are the only sex that takes blood meals and transmits pathogens. Pupae were collected after 9 days of larval development. Adult mosquitoes were 3-days post-emergence at the start of the experiment.
Sampling strategy	This work is building on a large body of previous work in Murdock's research program that have demonstrated that these sample sizes and replication are sufficient to power any frequentist statistical analyses.
Data collection	Data on whether or not females took a bloodmeal, whether they laid eggs, how many eggs they laid, and if they were alive or dead were recorded by Murdock's former PhD student Kerri Miazgowiec at the University of Georgia. Data were initially recorded using pen and paper and then entered into an spreadsheet.
Timing and spatial scale	This study was conducted throughout 2017 and 2018. For each treatment, two biological replicates were performed that ranged from 60-70 days. Data were recorded on a daily basis throughout the course of each experiment from individually housed females for the duration of their lifespan in each environmental treatment.
Data exclusions	Data were not excluded.
Reproducibility	We conducted two full biological replicates of this experiment, which took approximately 1 year to run in full. Both replicates were successful. One treatment, 28 C had three biological replicates.
Randomization	3-day old females were randomly assigned into their respective environmental treatments after successfully bloodfeeding by using a random number generator to generate the order in which cups were filled after verifying for sufficient interspersions. further, the order in which cups of females were removed on a daily basis to be provided a bloodmeal was also randomized using a random number generator.
Blinding	We did not blind this study and based on how it was designed, our randomization process, as well as the metrics we were recording we did not think this was necessary.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Anopheles stephensi (urban type form)
Wild animals	N/A

Reporting on sex

We only used females in this study as they are the only sex that take bloodmeals, determine population dynamics, and transmit mosquito-borne disease.

Field-collected samples

N/A

Ethics oversight

All work was done in accordance to the Institutional Biosafety Committee at the University of Georgia

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A