*Correspondence:
stephen.beverley@wustl.edu
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**Forum**

# Multiple Blood Feeding: A Force Multiplier for Transmission

Riley E. Tedrow,[1,2,*]
Peter A. Zimmerman,[1,2]
and Karen C. Abbott[2]

*Anopheles* mosquitoes employ complex behavioral and physiological strategies to adapt to their environment. Here we show how altering the number of bites a mosquito takes per gonotrophic cycle (gonotrophic discordance) could raise the transmission potential of a mosquito population far above model predictions.

## Violating a Common Assumption

Malaria transmission models rely on entomological parameters extrapolated from field-collected data [1]. Considering the complexity of mosquito behavior [2] and the paucity of data from the field, it is crucial to evaluate how model predictions rely on unknown quantities or uncertain assumptions. The malaria reproduction rate, $R_0$, represents the chance that a malaria parasite will find a competent vector, mature, and infect another human. So long as $R_0 > 1$, transmission occurs, with new cases emerging faster than existing cases resolve. Drop below $R_0 = 1$, and net transmission ceases. One of the classic entomological parameters [3] used to compute $R_0$ is the human feeding rate ($a$), calculated as the product of the proportion of mosquito bites taken on a human ($Q$) and the number of total bites taken per gonotrophic cycle ($f$). However, $f$ is overwhelmingly taken to equal 1, implying gonotrophic concordance (i.e., that the number of blood meals taken by a mosquito is assumed to be equivalent to the number of gonotrophic cycles) [2]. Numerous field studies indicate a violation of this assumption [2,4–6]. Gonotrophic discordance, the phenomenon of taking multiple blood meals per gonotrophic cycle, is also known as multiple blood feeding (MBF). This is distinct from interrupted feeding, where defensive behavior from the host causes temporary disengagement followed by a resumption of feeding [2]. Multiple blood feeding appears to be beneficial for *Anopheles* females, increasing fecundity, longevity, and resistance to insecticides [2,7], all of which could contribute to increased disease trans-mission. These factors notwithstanding, MBF directly increases the number of potentially infective bites, the impact of which we highlight here.

To assess the impact of MBF on model predictions, we show the effect of raising the number of bites per gonotrophic cycle on $R_0$, the entomological inoculation rate (EIR), the vectorial capacity ($V$), and the age-specific proportion and total number of living, infectious vectors in a population ($I_i$ and $N_I$, respectively) (see Table S1 in the supplemental information online). We consider the effects of MBF in low-, moderate-, or high-transmission environments by altering values for other parameters that determine overall transmission intensity.
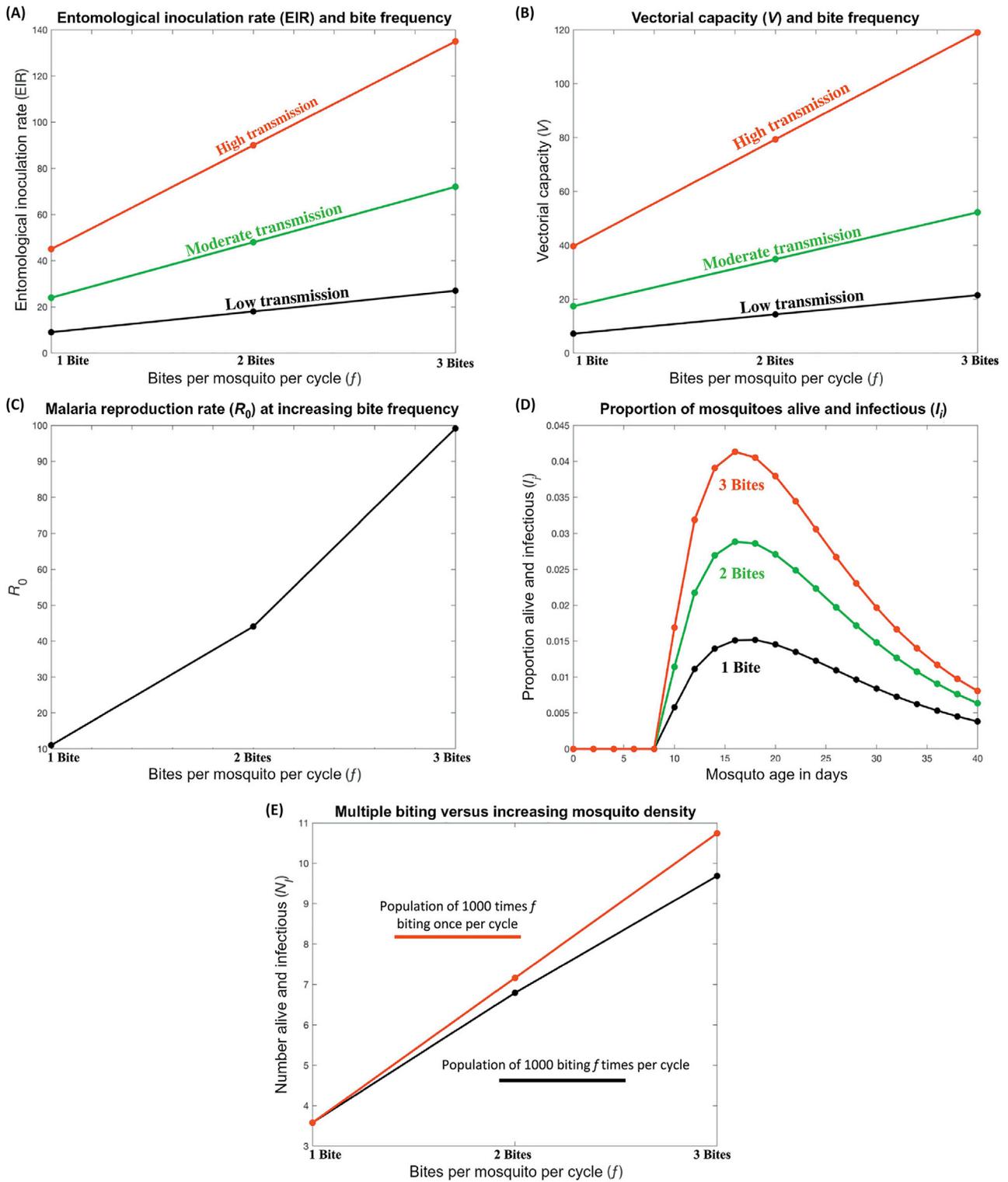
## The Impact of Multiple Blood Feeding on Modeled Malaria Transmission

The equations evaluated here incorporate components of the Ross–MacDonald model [8]. The original purpose of these equations was to address a major shortcoming of Ross's theory: a lack of field-based entomological measurements [8]. Increasing the EIR, vectorial capacity, or $N_I$ would be expected to increase $R_0$.

The EIR represents the number of infective bites an individual human receives per unit time. Adding additional bites per cycle (by increasing the feeding rate $a$) raises the EIR at a given transmission intensity (Figure 1A). Underestimating the number of bites per gonotrophic cycle unrealistically lowers the EIR, an effect intensified by increased transmission intensity.

The vectorial capacity ($V$), or daily reproduction rate of the disease, is the expected number of infective mosquito bites that would eventually arise from all the mosquitoes that bite a single

(A) Entomological inoculation rate (EIR) and bite frequency

(B) Vectorial capacity (V) and bite frequency

(C) Malaria reproduction rate ($R_0$) at increasing bite frequency

(D) Proportion of mosquitoes alive and infectious ($I_i$)

(E) Multiple biting versus increasing mosquito density

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*(Figure legend at the bottom of the next page.)*

fully infectious person on a single day. More simply, it is a measure of transmission intensity. The equation for $V$ (Table S1) uses the entomological components of the Ross–MacDonald model [8,9]. Transmission intensity likewise increases proportionately with the number of blood meals taken per gonotrophic cycle (Figure 1B).

The malaria reproductive rate, $R_0$, is the expected number of new human infections from a single infected human in a naïve population. It increases quadratically with the human feeding rate $a$, meaning that MBF accelerates transmission through the population (Figure 1C).

Next, we consider the prevalence of infectious vectors in a population over a mosquito generation, $I_i$ (Table S1), comparing the number of living, infectious vectors for a given MBF scenario (Figure 1D). At age 18 days, there is a peak in $I_i$, ranging from 1.52% (one bite) to 4.13% (three bites).

Finally, we compare the number of infectious vectors ($N_I$) under two scenarios representing the same total number of bites: one in which 1000 mosquitoes exhibit MBF by biting $f$ times each per gonotrophic cycle, and a second wherein 1000$f$ mosquitoes each bite once per cycle (Figure 1E). MBF is less efficient, in that we find fewer infected mosquitoes for the same total number of bites in the scenario with multiple bites per gonotrophic cycle. However, for modest MBF (two or three bites per cycle), the two scenarios are only marginally different, suggesting

that a mosquito which bites two or three times has nearly the same malaria transmission potential as two or three mosquitoes biting once.

## Implications for Malaria Control

Our results suggest that MBF could substantially impact malaria transmission, raising the EIR and vectorial capacity by a factor equal to the number of bites per gonotrophic cycle, and raising $R_0$ by a factor equal to the number of bites per cycle squared. This increase can be explained by a rise in the number of living, infectious vectors ($N_I$) in the mosquito population. Only a fraction of mosquitoes will live long enough (~9 days) to allow *Plasmodium* parasites to reach their infective stages. The proportion of surviving infectious vectors, $I_i$, peaks at 18 days, dropping off quickly thereafter. This suggests that a given cohort has a brief burst of infectious mosquitoes, after which background mortality quickly removes them from the population. While we do not expect to observe such a burst in natural mosquito populations with overlapping generations, this observation could be operationally useful in the context of severe weather that could cause synchronized spikes in mosquito recruitment. These data allow us to estimate the window of time before this population peaks in transmission potential, allowing us to set clear goals for disease mitigation.

MBF could drastically impact the per vector impact of an individual mos-

quito. We show that the impact of each mosquito biting two or three times is almost equivalent to having two or three times as many mosquitoes in the population. This behavior might help to untangle the seemingly paradoxical observation of high malaria prevalence in low-transmission settings [10,11]. Accounting for MBF in these settings could lessen the disparity between transmission and prevalence measures. This could mitigate the possibility of underestimating transmission intensity, which could lead to a mischaracterization of the impact of vector control. If we underestimate the importance of vector control, we are liable to prematurely reduce or remove targeted interventions by assuming we have achieved an $R_0$ below one. Premature cessation of vector control can lead to catastrophic spikes in malaria morbidity and mortality [12], requiring us to ensure cessation of net transmission before these reductions are considered. Furthermore, even if we believe we have interrupted transmission, the limitations of our ability to detect malaria could result in this status being quite fragile [13]. In our models, we set values for the proportion of bites taken on humans. Mosquitoes in nature lie on a continuum of host preferences, and this could temper or exacerbate the effect of MBF.

Effective control of *Anopheles* mosquitoes is the most important facet of our malaria-control arsenal [10]. Considering the complex behavioral and physiological strategies mosquitoes use to adapt to their environments, it is vital

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**Figure 1. The Impact of Multiple Blood Feeding on Malaria Transmission.**
(A) The entomological inoculation rate (EIR) at increasing transmission intensity with one to three bites per gonotrophic cycle. Transmission intensity was raised by increasing $m$ and $Z$ (Table S1). (B) Vectorial capacity at increasing transmission intensity with one to three bites per gonotrophic cycle. Transmission intensity was raised by increasing $m$ and lowering $g$. (C) Malaria reproduction rate ($R_0$) at one to three bites per gonotrophic cycle. (D) Proportion of living infectious vectors at each age, 1–40 days, considering one to three bites per gonotrophic cycle. (E) Total number of living and infectious mosquitoes in an arbitrary population, assuming a stable age distribution and age-specific infectiousness as given in (D). The population either contains 1000 mosquitoes who each bite $f$ times per gonotrophic cycle, or 1000$f$ mosquitoes who each bite once per cycle.

that we emphasize entomological surveillance in developing countries to enhance our understanding of gonotrophic discordance and other quantities that determine local transmission dynamics. This strategy could lead to actions more proactive than reactive, a far more sustainable approach [14]. Furthermore, to spur investment in building entomological capacity, these data should be synthesized and presented in a format useful to institutions responsible for introducing policy and developing strategies for malaria control.

## Supplemental Information

Supplemental information associated with this article can be found online at https://doi.org/10.1016/j.pt.2019.08.004.

[1]The Center for Global Health and Diseases, Case Western Reserve University, Biomedical Research Building 4th Floor, 10900 Euclid Avenue LC: 4983, Cleveland, OH 44106, USA

[2]Department of Biology, Case Western Reserve University, 10900 Euclid Avenue LC: 4983, Cleveland, OH 44106, USA

*Correspondence:
ret31@case.edu

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