How do I bite thee? Let me count the ways: Heterogeneity and temperature dependence in Ae. aegypti biting habits drive individual mosquito arbovirus transmission potential

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Abstract (203 words)

In models of mosquito-borne transmission, biting rate is the most influential parameter, and understanding the role of temperature on this process is important for assessing the transmission potential of arboviruses like dengue (DENV). Further, the process of biting is assumed to be relatively homogeneous across individuals, with time-between-bites described by an exponentially distributed process. However, these assumptions have not been addressed through laboratory experimentation. We experimentally determined daily biting habits of *Ae. aegypti* at three temperatures (24°C, 28°C, and 32°C) and determined that there was a high degree of individual heterogeneity in biting habits (number of bites, timing of bites, etc.). There was a significantly higher proportion of females that bit at 28°C compared to both 24°C and 32°C. We further explored the consequences of biting heterogeneity using an individual-based model designed to examine whether a particular biting profile determines whether a mosquito is more or less likely to 1) become exposed given a single index case of DENV and 2) transmit to a susceptible individual. Our results indicate that biting is heterogeneous among individuals and this heterogeneity affects transmission potential of DENV. Understanding individual-level heterogeneity in biting is important and may suggest a role for high-frequency biters as significant contributors to the transmission of DENV.

Keywords: Aedes aegypti, arbovirus, biting, dengue, temperature, transmission

Introduction

Aedes aegypti are the primary vectors for several arboviruses of public health importance and are primarily found in tropical regions. *Ae. aegypti* tend to be in urban areas and often live close to or within human dwellings with typically limited flight ranges. There are usually abundant opportunities for daily blood meals from human hosts [1-4]. Bloodmeal analyses from mosquito trapping experiments have identified that the majority of bloodmeals in female *Ae. aegypti* mosquitoes are taken from humans that live in the same household as where the mosquito was found [5]. Moreover, *Ae. aegypti* are known to take multiple bloodmeals – sometimes from multiple individuals – within a single gonotrophic cycle [5, 6]. These bites provide opportunity for transmission of *Aedes*-borne viruses such as dengue (DENV), which is primarily moved by humans rather than mosquitoes among households [7]. The transmission system is therefore reliant upon contact between susceptible mosquito vectors and infectious and susceptible humans.

Biting rate is a critical component of the transmission cycle of *Ae. aegypti*-borne viruses [8, 9]. Mosquitoes must bite once to acquire a virus and again to transmit that virus [10]. The timing of these two events is also important, as these two bites must be separated by a sufficient period such that the virus can disseminate through the mosquito and establish infection in the salivary glands [10]. After this period of time, called the extrinsic incubation period (EIP), the virus is transmissible via the next bite from that mosquito to a susceptible human [11]. Vector competence – the ability of a mosquito to transmit the virus – and EIP are both temperature dependent where higher temperatures in general mean that vector competence increases and the EIP is shortened [11-13]. Similarly, mosquito life traits are temperature dependent, and in combination with viral-vector kinetics define the transmission potential of the virus-vector pairing [10, 14, 15].

Heterogeneity in the biting habits of *Aedes aegypti* mosquitoes has been noted [5, 6] but current modeling frameworks ignore the potential for individual-level *Ae. aegypti* heterogeneity in biting, and any heterogeneity that is included is almost always attributed to the host [16, 17]. Oversimplified model assumptions and/or parameterization can lead to misinterpreting the drivers of transmission dynamics, as well as inaccurate projections about the impact of control measures or environmental changes on arboviral outbreaks.

In this effort, we address three critical gaps in knowledge. First, we experimentally measure the range of heterogeneity among individual biting habits. Second, we determine the effect of temperature on this process, and lastly, we use an individual-based model (IBM) to determine how differences in biting habits translate to small-scale transmission potential.

Methods:

Determination of Individual Biting Habits:

Aedes aegypti eggs were vacuum hatched and placed in a rearing pan with deionized water and fish food as in [10]. The aquatic stages were held at a constant temperature of 28°C in environmental chambers with a photoperiod of 16:8 L:D (light:dark) [10]. On the day of emergence, adults were cold anesthetized, and females were placed in 4-ounce, white disposable paper cartons with screen fastened around the top to provide containment. Cartons were placed in 24°C, 28°C, or 32°C environmental chambers with 16 individual females per temperature group. Two females died within four days from unnatural causes (got stuck in the screen) in the 24°C group and were removed from the study. Recently, it was determined that female *Ae. aegypti* will take a blood meal in the absence of water to satisfy thirst [18], so mosquitoes were maintained on 10% sucrose solution at all times (i.e., not starved prior to blood-meal offering).

Beginning on day 2 post-emergence, mosquitoes were offered a 15-minute blood meal consisting of Bovine blood in Alsever's anticoagulant via Hemotek feeding device, daily between 8:00 and 9:00 a.m. for 25 days (ending on day 27 post emergence). *Ae. aegypti* were previously determined to bite repeatedly with highest frequency at 24-hour intervals [19]. The Hemotek arms were threaded through a port in the environmental chamber so that mosquitoes were never removed from the chamber, and thus temperature remained consistent. Cartons were blown on to introduce CO_2 cues and then the discs were placed directly on top of the screen. Blood feeding was recorded at each blood meal offering when the presence of fresh (bright red) blood in the abdomen was observed. Only one person observed each feed to eliminate that as a source of variation. Deaths were documented as they occurred. In addition, egg papers were placed into the cups and exchanged at 7, 14, and 21 days of the study and observed for the presence of eggs.

We recognize that blood feeding as a proxy for "biting" is likely a conservative estimate as it does not take into account probing that does not involve the uptake of blood. Other proxies, such as human landing rates, etc. have similar caveats of not explicitly measuring probing versus feeding or multiple hosts [5], and recent data demonstrate that blood feeding resulted in transmission approximately twice as often as probing [20]. As an additional validation of our observation method, we tracked egg production at 7, 14, and 21 days. We found that only mosquitoes observed to have bitten at least once during the preceding period were observed to have laid eggs, indicating that we had correctly identified those mosquitoes who had taken a bloodmeal in the preceding period.

Model Structure and Parameterization: To investigate the role of mosquito biting heterogeneity on small-scale, household transmission, an individual-based model was developed that simulated household transmission patterns using the heterogeneity in biting behavior observed in our experiments. A schematic of the model is given in Supplemental Figure S1. Briefly, a household of two human individuals was assumed, one of whom is initially infectious (the index case), with a single susceptible mosquito which is allowed to bite according to her experimentally-determined bite profile. The mosquito may bite each person with equal probability, regardless of infection status. If the mosquito bites the index case, she is exposed until completion of the EIP, at which time she is infectious. The additional human is initially susceptible until bitten by an infectious mosquito. The simulation stopped once a secondary case was achieved or at the end of 25 days, whichever came first. We also assumed that contact with an infected human resulted in perfect transmission to the mosquito and, likewise, that a bite from an infectious mosquito always resulted in transmission to the susceptible human.

Model parameters are given in Table 1. But briefly, the DENV latent period in humans was assumed to be 6.5 days and the infectious period was 8 days [13, 21]. We varied the mosquito biting behavior in separate simulations to match the different mosquitoes in our experiment. The model was realized for 1000 simulations per single mosquito, with its biting parameterized according to its distinct profile. The mosquito bites with a probability of 0 or 1 determined by the experimental data. That is, if a particular mosquito from the data bites on days 2 and 15 from the experimental data, she will only bite on days 2 and 15 of the simulation. The temperature dependent EIP for DENV virus was defined as 11.5, 7.9, and 6.4 days for 24°C, 28°C, and 32°C, respectively [22].

Table 1: Parameter values for the models were either generated in the Christofferson laboratory or taken from the literature.

Temp	Parameter	Value (rounded up to nearest day)
24	EIP [11]	12 days
28		8 days
32		7 days
All	Infectious Period of Human [21]	8 days
	Latent Period in Human [13]	7 days

All individuals (humans and mosquitoes) are initialized at day 1 of the simulation, which was run for 25 days and the total number of transmission events to the mosquito and secondary transmission events to the susceptible human among all simulations was recorded. The probability of secondary transmission was calculated as the number of simulations where the susceptible human was infected divided by the total number of simulations.

The output from these models was used to determine which aspects of the bite profiles were most correlated with transmission using the “rcorr” function in R. Specifically, correlation matrices were produced to determine the association between the probability of the total number of bites a mosquito performed, time to first bite, the total number of times the mosquito became infected out of 1000 simulations, and the total number of times out of 1000 simulations that the susceptible human became infected.

Exploring experimental data under common mathematical model assumptions

Commonly used compartmental models assume that the time-between-bites for a given population is distributed exponentially with a single rate parameter, λ. We tested this assumption by first fitting the empirical data to a null model of exponentially distributed time-between-bites, using a non-linear model in the *nlsLM* function (minpack.lm package, [23]), to obtain estimates of temperature-dependent λ_{TEMP}. This λ_{TEMP} parameter was then used to define a theoretical probability density function of time-between-bites, ~EXP(λ_{TEMP}). Because the experimental data were measured discretely (daily), we discretized the continuous ~EXP(λ_{TEMP}) to an analogous geometric distribution. The resulting probability distribution was then tested against the time-between-bites of the experimental data using the Chi-square test for goodness-of-fit (*chisq.test* function with simulated p-values) to determine whether we could reject the null hypothesis that the experimental data could be derived from the discretized ~EXP(λ_{TEMP}) distribution.

Local Sensitivity Analysis:

A sensitivity analysis was conducted for the human infectious period (+/-2 days) and the EIP (+/-2 days), for each temperature, compared to the parameter values presented in Table 1. The same metrics were calculated and observed for changes from the base scenario.

Results:

Experimentally measured heterogeneity in biting among individual mosquitoes

The experimental data showed that biting is highly heterogenous among mosquitoes and across temperature conditions (**Figure 1**). At 32°C, mosquitoes were less likely to bite overall and died within the timeline of the study (25 days). The preferred temperature for mosquito biting was 28°C, as all (16/16) mosquitoes bit at least twice. At 24°C, we found that 93% (13/14) of the mosquitoes bit more than once with only a single mosquito not biting at all. At 32°C there was a significant reduction in the overall number of mosquitoes that bit with only 37.5% (6/16) taking a bloodmeal. Mosquitoes that bit did so with an empirical average of 4.5 times over the 25 days of the study period at both 24°C and 28°C each and 2.3 times at 32°C. This would convert to an average of one bite every 5.5 days at 24°C and 28°C and one every 10.9 days at 32°C if bites were evenly distributed. To contribute to the transmission cycle, a mosquito must bite once to get exposed to an arbovirus and then again to transit and this subsequent bite must be past the EIP. When we compared the proportion of mosquitoes at each temperature that had a subsequent bite outside the EIP window (assuming exposure occurred on the first bite), there was a significant difference between 28°C (100%) and both 24°C (71.42%) and 32°C (31.25%) (Supplemental Figure S2).

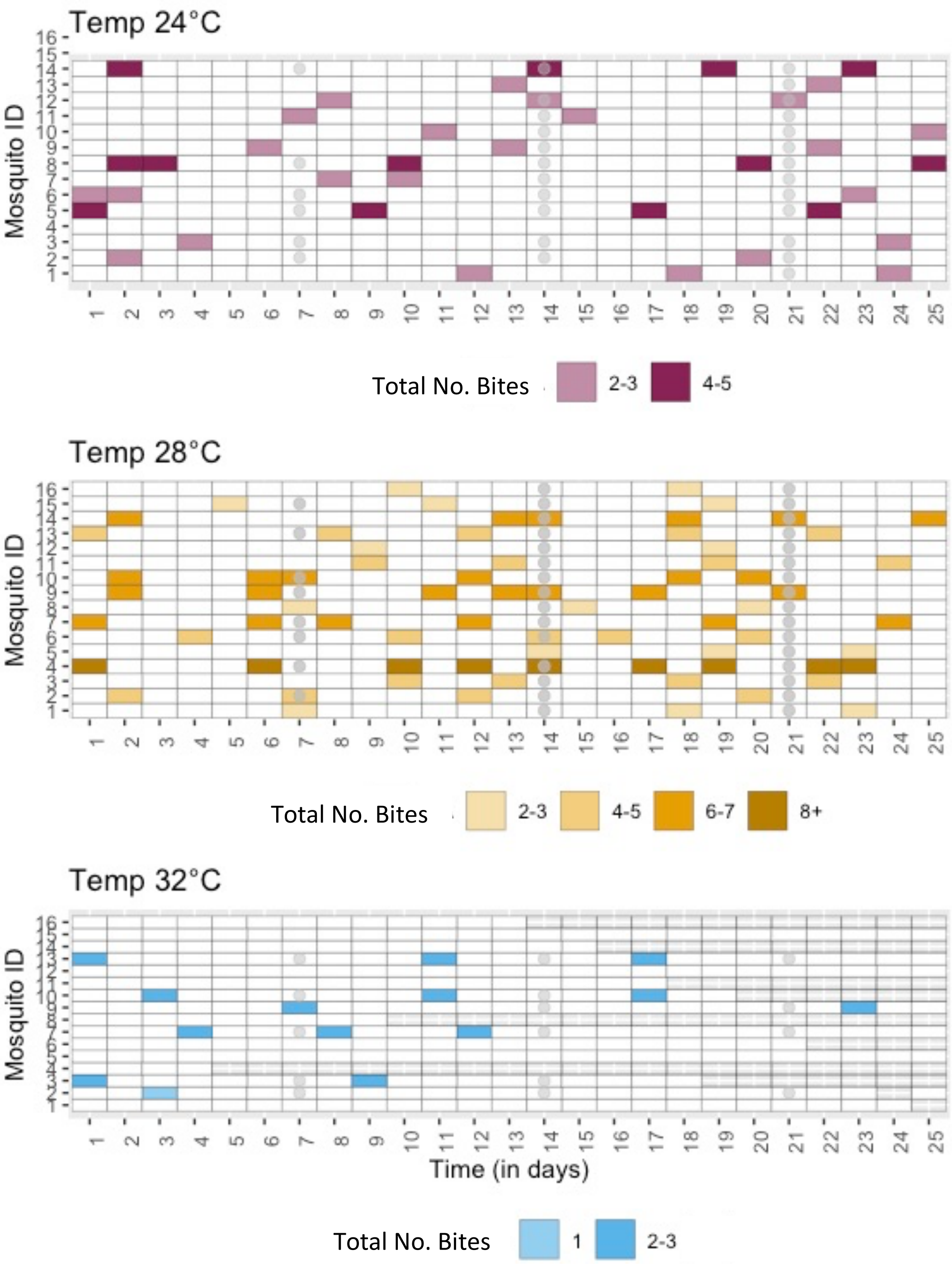

***Figure 1:** Biting patterns of individual mosquitoes (rows) for 24°C (top panel), 28°C (middle panel), and 32°C (bottom panel). Colored tiles indicate an observed bite, white indicates no observed biting, and greyed out tiles indicate mosquito had died (thus no observation). Grey dots are days on which egg papers from that mosquito had presence of eggs. Different shades of colors delineate the total number of bites per mosquitoes over her lifetime.*

Biting patterns that correlate with mosquito exposure and secondary transmission

To demonstrate the differential patterns of bite profile characteristics that support a particular mosquito's role in transmission, we measured the contribution of a particular mosquito bite profile to the likelihood that a secondary (human) infection occurs given the introduction of a single infectious index case. Not all mosquitoes that bit did so with patterns that supported transmission (**Figure 2**), meaning they either did not bite soon enough to become exposed by the viremic index case or there were no bites after the EIP resulting in subsequent transmission. At 28°C, five mosquitoes never became exposed, at 24°C, 5 mosquitoes that bit did not become

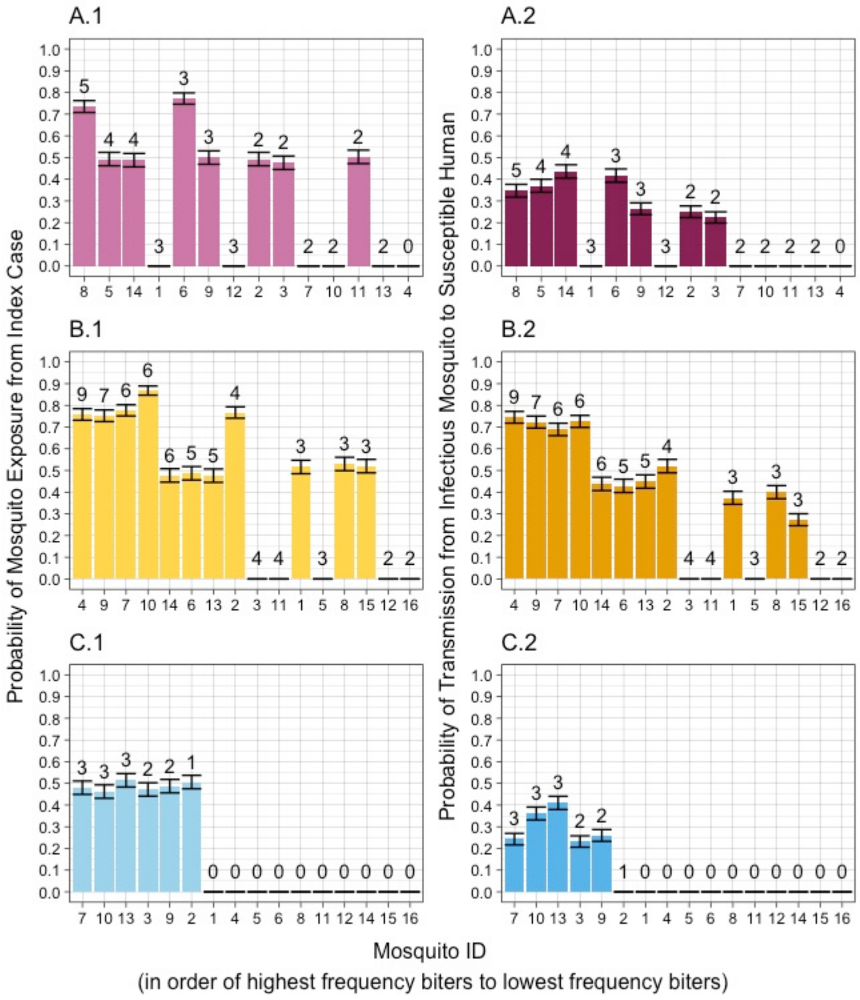

Figure 2: The proportion of mosquito-specific simulations (y-axes) that resulted in successful mosquito exposure from the index case (A-C.1) and subsequent transmission to the susceptible human (A-C.2) at each temperature: 24°C (A), 28°C (B), and 32°C (C). X-axes are ordered according to the number of bites (numbers above bars) and error bars are 95% binomial confidence intervals.

exposed, while all mosquitoes that bit at 32°C became exposed (**Figure 2A-C.1**). Similarly, six mosquitoes never transmitted at 24°C, including one mosquito that was successfully exposed in approximately 50% of simulations (#11). One mosquito at 32°C who was successfully exposed in >50% of simulations also did not transmit (#2), but she only bit once, and thus could not complete the transmission cycle. At 28°C, only mosquitoes that were not successfully exposed did not transmit to susceptible humans (**Figure 2A-C.2**).

The highest proportion of biting mosquitoes that supported transmission under model conditions was 32°C where 6/6 mosquitoes became exposed in a maximum of 51.4% of simulations (mosquito #13). Subsequent transmission to susceptible individuals occurred in a maximum 41.0% of simulations (also mosquito #13). Of the 8/14 mosquitoes that bit at 24°C, mosquito exposure occurred in a maximum of 77.2% of simulations (mosquito #6) and human infections from mosquitoes occurred at a maximum of 43.6% of simulations (mosquito #14). At 28°C, maximum mosquito exposures occurred in 86.8% simulations of mosquito #10 and human secondary infections maximally occurred in 74.5% simulations of mosquito #4.

To determine whether these differences in patterns were related to the static parameters of EIP and human infectious period, we tested the sensitivity of the model to these quantities. We found that the system was relatively insensitive to small changes in either parameter (**Supplemental Figures S3-4**). The system was not sensitive to changes in EIP, as the number of secondary transmission events did not change at all. However, the number of secondary human infections was more sensitive to the human infectious period, especially at 28°C. But how widely this period varies is not known, though it is likely less variable than EIP.

Visualizing the contribution of individual bites

Decoupling of the bite process allows for attribution of the timing of those bites, which can be important for transmission determination [10]. To show the hypothetical role of each bite within a biting profile of an individual mosquito, we calculated the proportion of transmission events to a particular mosquito out of all simulations at each bite and did the same for mosquito to human transmission events (**Figure 3**). In most cases, the first bite did result in transmission when exposure was limited to a single index case, especially at 32°C where 100% of mosquito exposure occurred upon the first bite. At 28°C and 24°C, there was more variability in the bite at which a mosquito was exposed, though the first bite still accounted for more than other bites.

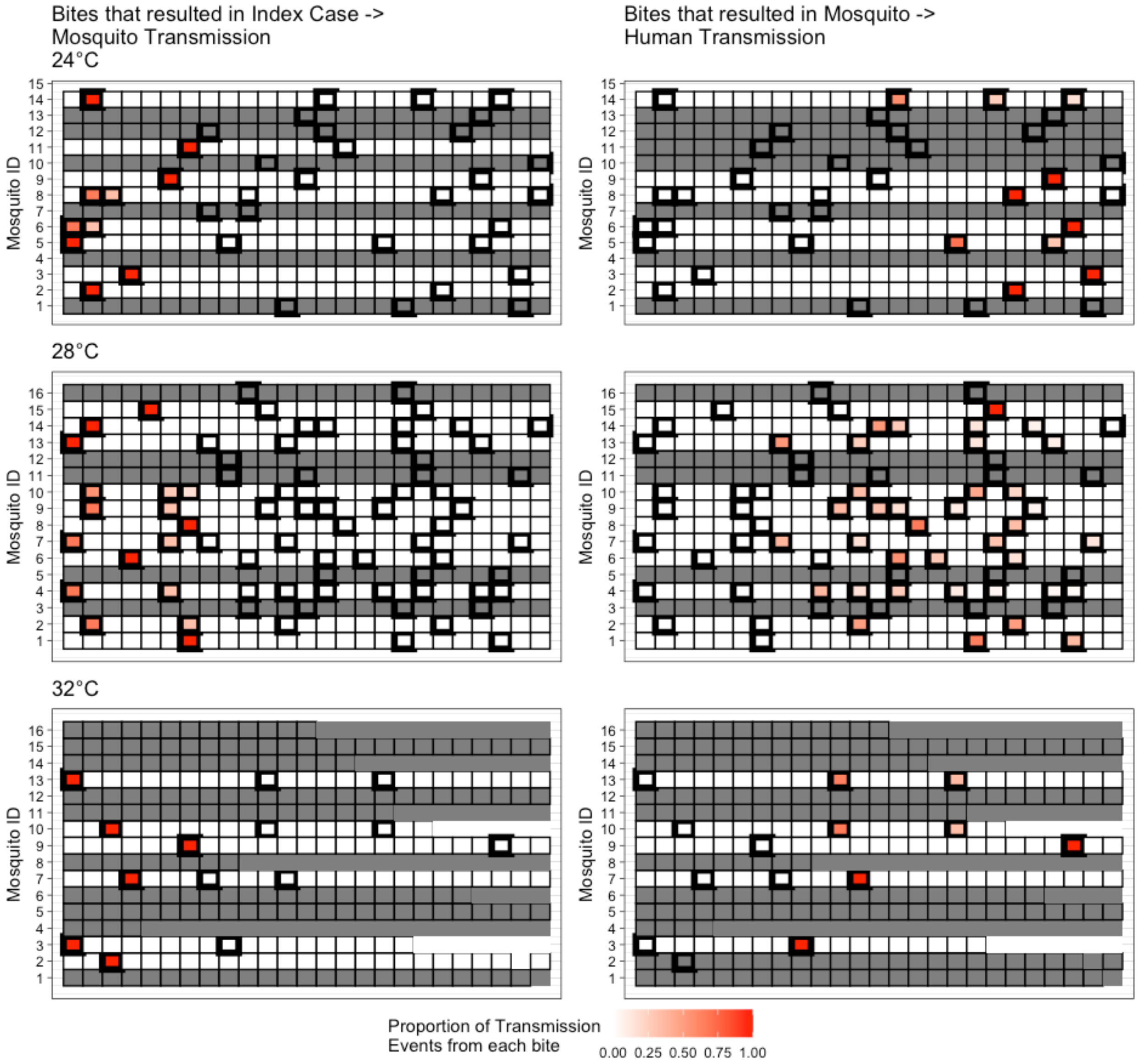

Figure 3: Bold outlines denote bites for a particular mosquito (y-axis) while the gradient denotes the proportion of (Left column) index case to mosquito transmission events or (Right Column) infectious mosquito to susceptible human transmission events occurring at each bite for each temperature: 24°C, top row; 28°C, middle row; 32°C bottom row.

Correlation of biting profile with simulated transmission potential

While probability of mosquito exposure and probability of subsequent transmission to a secondary human are obviously correlated, it should be noted that this correlation was not perfect across all temperatures and only at 32°C did the same mosquito have the highest probability in both events. **Table 3** shows the correlation between mosquito exposure and subsequent transmission at each temperature. Though variable, the correlation was uniformly positive and relatively high, peaking at 28°C.

Table 3: Pearson's correlation coefficient shows the positive, high correlation between the probability of a mosquito becoming infected and the probability that the mosquito subsequently transmitted the virus

Temp (C°)	Corr between p(mosquito exposure) and p(secondary human infection)
24	0.85 (p-value < 0.001)
28	0.97 (p-value < 0.001)
32	0.83 (p-value < 0.001)

The traits that were most correlated with successful exposure of the mosquito and then successful transmission to the susceptible human were not uniform across all temperatures (**Table 4**). For example, given that biting behavior was so reduced at 32°C the total number of bites was highly, positively correlated with the probability of secondary human infection. The correlation was much lower for mosquito exposure, which may be due to the small range in the number of total bites for mosquitoes at 32°C (1-3). However, none of these correlations were significant ($p>0.05$) likely due to the small number of mosquitoes that actually bit ($n=6$) at this temperature. At 28°C, time-to-first bite had the highest magnitude correlation with mosquito exposure probability ($p<0.05$). This was negatively correlated with both probability of mosquito exposure and transmission, meaning that a shorter time to first bite was correlated with higher probabilities of both mosquito exposure and secondary human infection. Similarly, the time between first and last bites was strongly, positively correlated with both transmission events, as was total number of bites ($p < 0.05$). Thus, at 28°C the characteristics defining the bite process are more evenly influential and all but the time between the first and second bites were significantly correlated with the likelihood of secondary human transmission. At 24°C, time-to-first bite was the highest magnitude and only significantly correlated variable, again indicating that shorter times to first bite was important for mosquito exposure and subsequent secondary human infection.

Table 4: Variables significantly correlated with successful exposure/ transmission demonstrate that factors associated with successful transmission are not uniform across all temperatures.

Event	Temp	Variable	ρ
Mosquito exposure	24°C	Time to 1st bite	-0.89
		Total # Bites	0.64
		Time b/t 1st and last bites	0.67
	28°C	Time to 1st bite	-0.89
		Time b/t 1st and last bites	0.82
		Total # Bites	0.79
	32°C	No significant correlations	
Mosquito to Susceptible Human Transmission	24°C	Time to 1st bite	-0.87
	28°C	Time to 1st bite	-0.87
		Time b/t 1st and last bites	0.75
		Total # Bites	0.66
	32°C	No significant correlations	

Null model assumptions about biting do not represent the observed process

We tested whether the distribution of time-between-bites is consistent with the commonly assumed distribution: $\sim EXP(\lambda_{\text{Temp}})$. Theoretical time-between-bites was not significantly different from the experimental data at 28°C ($p \approx 0.11$) indicating that time-between-bites could be appropriately modeled by an exponential distribution at this temperature. However, at 24°C and 32°C, the probability distribution of time-between-bites generated under this assumption differed significantly from the experimental data (p ≈ 0.01 and p ≈ 0.03, respectively) (**Figure 4**).

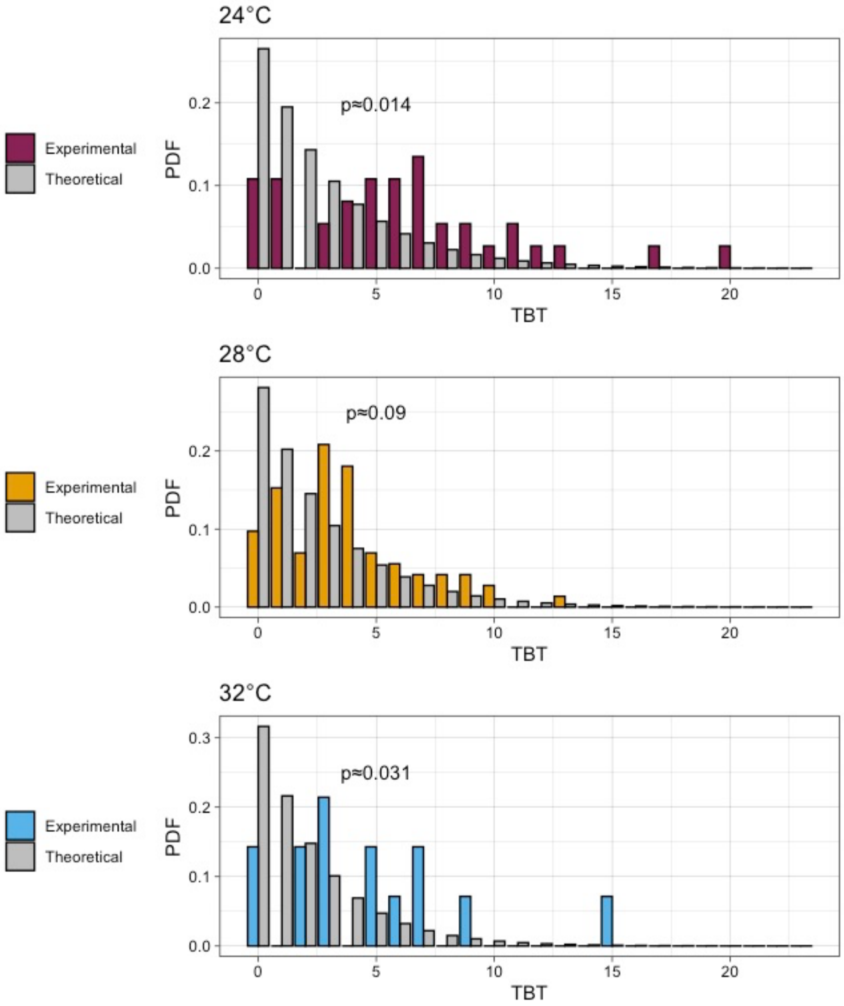

Figure 4: The probability distribution of the time between bites (TBT, in days) observed experimentally and generated theoretically under common model assumptions (grey bars). Comparisons of distributions within each temperature was done with the Chi Square goodness of fit.

Discussion

Biting is often thought about as a singular process, and while transmission is known to require two bites, rarely are independent bites decoupled. These data represent a novel quantification of individual biting habits of *Ae. aegypti*. At 28°C, several biting characteristics were significantly correlated to the probability that a mosquito would become exposed and subsequently transmit, indicating that at this temperature, both the processes of mosquito exposure and subsequent secondary human infection is a nuanced interaction of the number and timing of bites. At 24°C, successful transmission to the mosquito was completely dependent on the time to first bite, indicating either the mosquito bites the index case at the right time or not. Transmission to a susceptible human was more like 28°C in that it was correlated with both the magnitude and timing of bites. However, at 32°C transmission appears to be a matter of luck: either the mosquito bites enough to become infected or doesn’t. Similarly, either she bites a second time to transmit or not.

Commonly used compartmental models of arbovirus transmission assume that 1) all mosquitoes have the same chance of biting each day and 2) the time-between-bites for a given population is distributed exponentially with a single rate parameter. Our experimental data suggests that this first assumption holds at the two lower temperatures of 24°C and 28°C, but is likely inappropriate for more extreme temperatures such as 32°C where only 6/16 mosquitoes bit at all. Further, we tested the second assumption of whether time-between-bites was consistent with an exponentially-distributed process. We found that there was a statistical difference between the distribution of the data and that expected from an exponential distribution at 24°C and 32°C. While there was no significant difference at 28°C, other distributions may better represent the process over many temperatures for generalizability.

The impact of using individual-level data rather than population averages (such as for compartmental models) remains to be elucidated, but it is important to note that the simple act of biting twice does not translate to a mosquito becoming part of a transmission cycle because the timing of bites is critical. A bite profile must allow for “catching” an infectious human during active infection and for a mosquito living long enough to transmit [10]. This has implications for studies that rely on bloodmeal analysis and gravid trap counts to evaluate transmission parameters from these coarse human biting estimates, as well as the way simple quantitative frameworks such as vectorial capacity might be interpreted.

The lack of model system sensitivity to the infectious period of the index case may be attributable to the synchrony of initialization, and exploration of the timing of human introduction is thus warranted. However, the lack of sensitivity of the system to EIP was surprising as this metric describes the temporal process of infection within the mosquito and was expected to be more impactful on the success of transmission.

While these data represent novel individual-level data on the frequency of biting, we recognize caveats of the study. First, laboratory settings represent ideal conditions for mosquitoes, and partial and full bloodmeals are probably a conservative estimate of mosquito-human contact. However, *Ae. aegypti* biting in the field was estimated as 0.63-0.76 bloodmeals per day (a population estimate) within a single gonotrophic period [24]. Further, recent published data demonstrate that while there are likely differences in biting habits across geographic and laboratory populations, a degree of heterogeneity was observed in a field-derived colony, indicating that heterogeneity among individuals is not limited to laboratory colonies [20]. Future studies are needed to determine the magnitude of differences among populations and the effects on individual transmission contributions, as well as correlations with geographically proximal outbreak data.

Our study also demonstrates that the most influential traits associated with successful transmission events were variable across temperatures. At 32°C the overall number of mosquitoes that bit was very low, suggesting that too-high temperatures will have a deleterious effect on transmission. Though larger studies may reveal more heterogeneity among those mosquitoes that bite at higher temperatures than is demonstrated here, the lack of overall biting in the experimental data is important to understand as climate change increases temperatures in the global tropics. At 24°C and 28°C, the proportion of mosquitoes that bit was higher, and the timing of bites rather than number of bites was most correlated with successful transmission.

We reveal here the importance of understanding the nuances of the bite process as influential characteristics of this process are not uniform across all temperatures. While we demonstrate this in a laboratory system, temperature fluctuations – especially at the margins of vector distributions and in the face of climate change – may shift the relative importance of these characteristics. Understanding these in the broader contexts of mosquito density, variable incubation periods, and other factors is critical for understanding the transmission system and informing future models.

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