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Large Diurnal Temperature Fluctuations Negatively Influence *Aedes aegypti* (Diptera: Culicidae) Life-History Traits

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ABSTRACT Seasonal variation in dengue virus transmission in northwestern Thailand is inversely related to the magnitude of diurnal temperature fluctuations, although mean temperature does not vary significantly across seasons. We tested the hypothesis that diurnal temperature fluctuations negatively influence epidemiologically important life-history traits of the primary dengue vector, *Aedes aegypti* (L.), compared with a constant 26°C temperature. A large diurnal temperature range (DTR) (≈18°C daily swing) extended immature development time (>1 d), lowered larval survival (≈6%), and reduced adult female reproductive output by 25% 14 d after blood feeding, relative to the constant 26°C temperature. A small DTR (≈8°C daily swing) led to a negligible or slightly positive effect on the life history traits tested. Our results indicate that there is a negative impact of large DTR on mosquito biology and are consistent with the hypothesis that, in at least some locations, large temperature fluctuations contribute to seasonal reduction in dengue virus transmission.

KEY WORDS *Aedes aegypti*, Thailand, dengue virus, life-history trait, temperature fluctuation

Seasonal changes in dengue virus (DENV) transmission are often linked with climatic factors (Hales et al. 2002; Johansson et al. 2009a, b; Colón–González et al. 2011). Although the influence of climatic factors on the biology of mosquito vectors has been intensely studied, conditions under which they have been examined in the laboratory often do not accurately represent the environment to which the mosquitoes are exposed in the field. In particular, natural mosquito populations are exposed to daily fluctuations in water and air temperature in their immature and adult stages, respectively.

Natural temperature profiles have been described using a variety of models (e.g., Parton and Logan 1981, Knight et al. 1991). Temperature typically fluctuates asymmetrically throughout the day. A sinusoidal increase in temperature begins after the sun rises, before it reaches a peak and drops in the evening and early morning after a negative exponential curve. Only in recent years have researchers begun to examine mosquito biology under realistic temperature profiles.

A direct, and well-established relationship between constant temperatures and numerous adult traits has been reported for some mosquito species (Reisen et al. 1984, Tun–Lin et al. 2000, Muturi and Alto 2011, Richardson et al. 2011, Williams and Rau 2011). There is a small, but growing body of literature exploring relative changes in mosquito life-history traits and vector competence under constant versus fluctuating temperatures (Bates and Roca–Garcia 1946, Turell and Lundstrom 1990, Maharaj 2003, Paaijmans et al. 2010b, Fischer et al. 2011, Lambrechts et al. 2011, Mohammed and Chadee 2011, Richardson et al. 2011).

In a recent study, Lambrechts et al. (2011) described the impact of large magnitude temperature fluctuations on *Aedes aegypti* (L.) DENV interactions using realistic, diurnally fluctuating temperatures. The two sinusoidal profiles used represented recorded temperatures from Mae Sot, Thailand, during high and low DENV transmission seasons, which are associated with small (10°C daily swings) and large (20°C daily swings) DTRs, respectively, although the mean temperature remains ≈26°C across seasons. DENV vector competence and survival of *Ae. aegypti* were tested under these two symmetrical temperature profiles and compared with a constant 26°C. The hypothesis that large fluctuations negatively influenced vector competence was supported and the amplitude of the fluctuation negatively affected adult survival of infected mosquitoes. The researchers did not determine the effect of real-world, asymmetrical temperature fluctuations on mosquito life history traits, such as development and survival of immature *Ae. aegypti*, or adult body size and egg production.

Mohammed and Chadee (2011) performed laboratory experiments to test the effect of constant and fluctuating temperature regimes on egg viability, lar-

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val development, and survival. There were five cyclical temperature regimes, each with a different thermal maximum. They identified altered sex ratios under variable DTRs with high maxima and an inverse relationship between body size and constant temperatures, but not under fluctuating temperatures.

Tun–Lin et al. (2000) assessed development time for Australian *Ae. aegypti* under field conditions (and hence considered fluctuating temperatures) in various sized containers, with comparisons to pre-established laboratory estimates for size and development time. Water temperature measurements were recorded once a day, so limits of thermal exposure for mosquitoes were unknown (Tun–Lin et al. 2000).

Investigators in another Australian study (Richardson et al. 2011) considered immature development time and survival under various temperature regimes with respect to predicting the geographic distribution of *Ae. aegypti* across the country. They determined development and survival rates across a wide range of constant temperatures (12–40°C) and found that development time estimates under constant temperatures accurately predict development rates under fluctuating field conditions. They did not, however, report which constant temperature was the most appropriate descriptor of fluctuating conditions or the extent of the diurnal temperature ranges that were experienced by the immature mosquitoes in the field.

We were able to identify only one study comparing the effects of constant and fluctuating temperatures on the reproductive traits of mosquitoes. Joshi (1996) exposed *Ae. krombeini* (Huang) to 4- to 8-degree DTRs around five mean temperatures ranging from 14 to 30°C and directly compared results to their respective mean constant temperature control. At constant temperatures, females were unable to lay eggs at or above 33.5°C, but were able to recover egg-laying potential when 33°C was the peak temperature of an eight-degree diurnal temperature swing. At low temperatures, male and female egg to adult development time was shorter under fluctuating than constant temperatures.

Maharaj (2003) investigated the effect of seasonal temperature variation on *Anopheles arabiensis* (Patton), a primary malaria vector in Sub-Saharan Africa. His detailed study examined adult and immature survival, reproduction and egg viability under temperatures representing four climatic seasons in South Africa. Results were associated with some seasonal changes in malaria transmission in the region. Unfortunately, the magnitude of temperature and humidity fluctuations to which mosquitoes were exposed were not reported.

Limitations of previous studies provide rationale for the present work. A number of the studies do not disclose the temperature profiles to which immature mosquitoes were exposed (e.g., Tun–Lin et al. 2000, Richardson et al. 2011, and Maharaj 2003). This makes comparing results difficult because neither the mean, nor minimum, and maximum temperatures can be compared. A potentially confounding factor in the Lambrechts et al. (2011) study is that only the adult mosquitoes were moved to incubators with fluctuating temperatures a few days before the vector competence experiments, as opposed to being reared under fluctuating temperatures. Additionally, that study focused on vector competence and did not, except for adult survival, consider life-history traits. This is analogous to the studies of Mohammed and Chadee (2011) and Richardson et al. (2011), who concentrated on immature development and did not study the adult mosquito. Of those studies that did consider adult life-history traits (i.e., fecundity and aspects of the gonotrophic cycle) the target species was not *Ae. aegypti* (Joshi 1996, Maharaj 2003), which restricts our ability to predict the impact of temperature fluctuations on *Ae. aegypti*-DENV interactions.

Herein we report results from our investigation of fluctuating versus constant temperatures effects on selected life-history traits of larval and adult *Ae. aegypti*. We tested the hypothesis that large temperature fluctuations, around a common mean of 26°C, will slow development time and reduce survival of immature *Ae. aegypti*, decrease female fecundity, and increase the length of the gonotrophic cycle. This study is an extension of our previous research on *Ae. aegypti*-DENV interactions (Lambrechts et al. 2011). We used averaged temperature profiles recorded during the high and low DENV transmission season in Mae Sot, Thailand, to determine whether detected temperature effects on life-history traits are consistent with seasonal variation of DENV transmission in northwestern Thailand.

Materials and Methods

Experimental Design. We investigated the effect of fluctuating diurnal temperatures on selected life-history traits of *Ae. aegypti* from Thailand, using three environmental temperature treatments, each created using programmable incubators (Binder KBF 115, Tuttlingen, Germany). The first was a constant 26°C, and the remaining two closely mimicked observed temperatures that were measured in Mae Sot, Thailand, with a common mean of 26°C. Profiles emulated the average of 500 hourly observations (≈3 wk) during the high and low dengue transmission seasons in Mae Sot, which were 26.8 and 26.2°C, respectively. The actual mean air temperatures inside the incubators across experiments averaged 26.4 ± 0.3°C under the small DTR, and 25.9 ± 0.2°C under the large. Temperature profiles were programmed to follow a sinusoidal progression in the day, and exponential decrease at night (Parton and Logan 1981), with hourly temperature input. Temperature ramped between set temperatures each hour, with the peak temperature of each day reached at 2:00 p.m., and the minimum at 5:00 a.m. We programmed a photoperiod of 12:12 (L:D) h cycle, with alternations occurring at 8:00 a.m. and 8:00 p.m. Relative humidity was maintained between 70 and 80% across all experiments. We measured relative humidity, air and water temperature using HOBO data loggers (Onset, Cape Cod, MA). Air temperature and humidity were measured

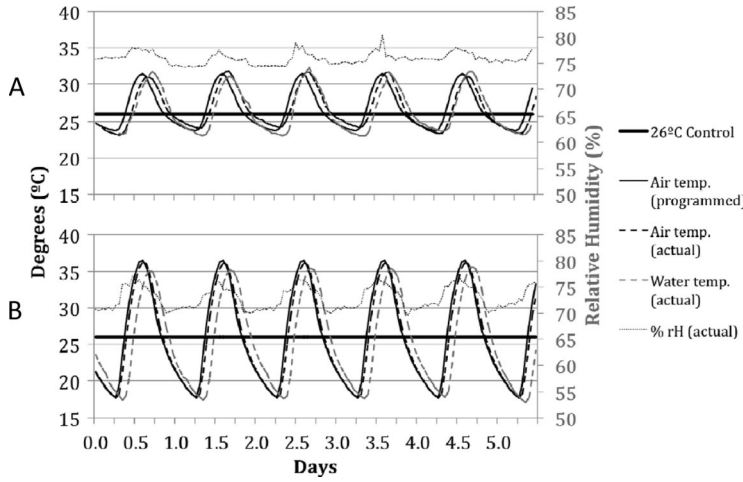

Fig. 1. Environmental conditions to which mosquitoes were exposed during experiments. The top panel shows the programmed and recorded temperatures (primary axis) and relative humidity (secondary axis) in incubators with small fluctuations. The lower panel is the same, but for large fluctuations.

using the External Temperature/Relative Humidity data logger (U23 proV2) and water temperature was measured using the Pendant Temperature/Light data logger (8K-UA-002-08). Because of the small volume of water in larval rearing cups, water temperature followed air temperature closely. There was an average 1- to 2-h lag between air and water maximum and minimum temperatures (Fig. 1). Despite the lag, water temperature averaged within 0.6°C of the programmed air temperature. As for the slight delay in air temperature under the small diurnal temperature regime, we suspect that the internal clocks for data loggers and incubators were not synchronized. We cannot reaccess the original data to confirm this because the loggers have since been reset. Nonetheless, recorded temperatures still indicate that mosquitoes were exposed to the intended temperature profile.

Under each of these three temperature regimes, termed *constant temperature* (DTR = 0°C), *small DTR* (DTR = 7.6°C), and *large DTR* (DTR = 18.6°C), we conducted two experiments. We measured immature survival and development time, fecundity, clutch size, and length of the gonotrophic cycle during 14 d of daily access to human blood. We used a subset of the females that emerged from the development time experiment, of known pupation time, in the fecundity experiment.

Mosquitoes. We used *Ae. aegypti* that were collected from Kamphaeng Phet, Thailand on two different occasions. The first colony was collected in August 2009 and the second in January 2011. Both colonies were maintained under standard insectary conditions (28 ± 1.5°C; 70 ± 3% relative humidity [RH]) with a photoperiod of 12:12 (L:D) h cycle. The first was maintained in the laboratory from collection until testing (generation 23) for immature development time and fecundity. We reassessed these traits in a second experiment using the newly established second colony, which was presumably more genetically representative of the original field population (generation 2). Because each experiment used a single generation of mosquitoes, we place greater importance on the results from the second set of experiments. Eggs were hatched in water using a vacuum manifold and reared under a controlled density (≈200 larvae per tray) in containers (≈33 × 29 × 6 cm) with 1.5 liters of deionized water. Colonies were maintained with a population size of >500 individuals per generation. Larvae were fed as previously described in Styer et al. (2007).

Immature Development Time and Survival. The length of development time from egg hatching to pupation and emergence was measured twice. For each experiment, we had between 16–20 replicate cups in each of the three temperature regimes. Every replicate cup had a starting density of 20 larvae, 100 ml deionized water, and a standardized amount of food (proportional to the amount of food per larvae provided for the colony maintenance as described in Styer et al. [2007]) across all treatments. Because of water evaporation under high temperatures, the volume of each cup was checked daily and fluid was added as needed with prewarmed water to a total volume of 100 ml. Survival of larvae was monitored daily (except for the first day after hatching, because of the fragility of the first instar larvae) until the end of the experiment. We assessed larval survival by counting all remaining larvae in a cup each day and subtracting this number from the previous count for that cup.

When the first pupa was observed, the frequency of monitoring events increased from once per day to every 6 h, to enhance the precision of our measurements. At each monitoring event, pupae from each temperature were pooled into a single cup, placed inside a 1-pint carton, and returned to their respective temperatures for continued development. Pupal cups were subsequently checked every 6 h for pupal sur-

Table 1. MANOVA results for larval development time and survival

Factor	Wilk's Λ	df (num, den)	*P*	SCC (larval dev. time)	SCC (larval survival)
DTR	4.64	3, 200	0.0013	2.001	0.346
Generation	164.84	2, 100	<0.0001	2.089	−0.063
DTR × generation	4.77	4, 200	0.0011	2.087, 0.236	−0.072, 1.004

Means for each replicate rearing cup were used for data analysis. Standardized canonical coefficients for each factor are presented at the right; the larger the no., the greater the effect of the factor on the trait.

vival and adult emergence. When adults emerged, a subset was selected for fecundity experiments.

Fecundity and Length of Gonotrophic Cycle. We tested the effect of fluctuating temperatures on overall fecundity, clutch size, length of gonotrophic cycles, and number of completed gonotrophic cycles. For each temperature and experiment, we set up 10 single pair matings in 1-pint cartons, using females and males reared at controlled densities. The individuals collected after emergence from the immature development time experiments formed subpopulations with known pupation and emergence times. We used a random sample of individuals from specified subpopulations within each experiment to minimize the potential effects of body size because of development time differences. Females were offered blood from a human arm or leg (L.B.C.) for 12 min every day, from the day of emergence (day 1) for 14 d. The order in which groups were fed and the part of the body on which they were fed, was randomized each day. Oviposition papers that lined a 30 ml cup inside the carton were replaced daily, for up to a maximum of 20 d. Each day, eggs that were laid in the preceding 24 h-period were removed, counted, and recorded.

The length of the gonotrophic cycle was determined as the period between the day of the first bloodmeal to the day that the first eggs were observed (Wong et al. 2011). Subsequent gonotrophic cycles were also measured, and separated from one another by a 1-d pause in egg laying (calculated retrospectively), when the first bloodmeal of the subsequent cycle occurred or after the last day of egg laying of the previous cycle. We counted the total number of completed gonotrophic cycles only for females that survived to the end of the experiment (a maximum of 20 d) because including the estimates for females that died prematurely does not accurately measure their reproductive potential. We did, however, include all data on when blood meals were taken and the length of each gonotrophic cycle for all females.

The University of California at Davis Institutional Review Board determined that this experiment (allowing mosquitoes to take bloodmeal from people) did not meet the criteria for human subjects research and thus, did not require human subjects approval.

Statistical Analysis. Analyses were conducted using SPSS (version 15; SPSS Inc., Chicago, IL) and SAS (version 9.3, SAS, Inc., Cary, NC). For immature stages, we used a multivariate analysis of variance (MANOVA) to test egg-pupa development time and larval survival estimates. The MANOVA was performed on summary data from each replicate cup of 20 individuals and tested for the effects of DTR, generation, and DTR × generation. The standardized canonical coefficients (SCCs) are reported for each of the dependent variables of each independent variable. The MANOVA, however, compares mean development times, but not rates of development across treatments; for example, development could be extended over a longer period of time under one treatment and still have the same mean development time as another that occurred more quickly (a steeper development curve). Thus, we carried out a Kaplan–Meier (K–M) analysis (with a log-rank test) in addition to the MANOVA, to test for DTR effects on egg-pupa and egg-adult development time stratified by sex. Because we are particularly interested in the effect of DTR on the youngest colony (second colony), we analyzed only adults from generation two in this manner. Log-rank tests were used to assess differences between development rates of DTR treatment groups.

We tested fecundity data (log-transformed number of eggs and the number of gonotrophic cycles completed) using a MANOVA, testing for DTR, generation and DTR × generation, and report the SCCs for significant effects. The log egg numbers were Winsorized (transformed to minimize outliers in the data) before the MANOVA, because of the presence of a few large outliers among the responses. We conducted a repeated-measure ANOVA on specific gonotrophic cycle data (eggs per cycle and length of cycles), as long as that cycle was clearly finished. Observed differences were further tested to identify variation between treatments using Bonferroni post hoc tests.

Results

Immature Life Stages. Development time can be influenced by larval density; therefore, we considered the effect of DTR on mean larval mortality and egg to pupa development time for each replicate in the same analysis, using a MANOVA. We identified highly significant effects of DTR, generation and an their interaction on immature survival and development time (Table 1). Standardized canonical coefficients for the independent variables are also presented. Our results indicate that DTR and generation strongly influence larval development time, and to a lesser extent, immature survival. There was also a significant interaction between DTR and generation. Figure 2 summarizes mean larval survival (±1 SE).

We identified consistent effects of DTR across egg to pupa and egg to adult using log-rank tests (egg-pupa: $\chi^2 = 173.9$, df = 2, $P < 0.001$; egg-adult: $\chi^2 =$

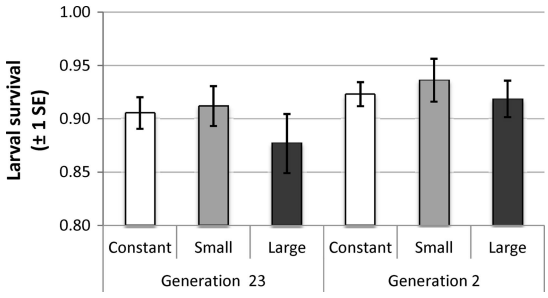

Fig. 2. Proportion of *Aedes aegypti* larvae surviving to pupation. Estimates are based on between 16 and 20 replicate cups for each DTR in each experiment. The x-axis groups DTR within generation. Error bars represent ±1 SEM.

209.5, df = 2, $P < 0.001$). For adults, a larger DTR consistently extended development time (males = 11.28 d; females = 12.38 d), relative to a small DTR (males = 9.56 d; females = 10.62 d) and constant 26°C (males = 9.82 d; females = 10.58 d). In a pairwise analysis of male development, all treatments were different from each other (constant vs. small $\chi^2 = 6.26$, df = 1, $P = 0.012$; small vs. large $\chi^2 = 152.97$, df = 1, $P < 0.001$; constant vs. large $\chi^2 = 113.99$, df = 1, $P < 0.001$). For females, only the large DTR produced significantly different estimates of development compared with constant and small DTR (constant vs. small $\chi^2 = 0.50$, $P = 0.479$; small vs. large $\chi^2 = 85.70$, $P < 0.001$; constant vs. large $\chi^2 = 105.56$, $P < 0.001$). Figure 3A, B summarize these differences.

Adult Life Stages. Using a MANOVA, we observed an effect of DTR and generation, but no interaction between these two factors (Table 2) on total fecundity

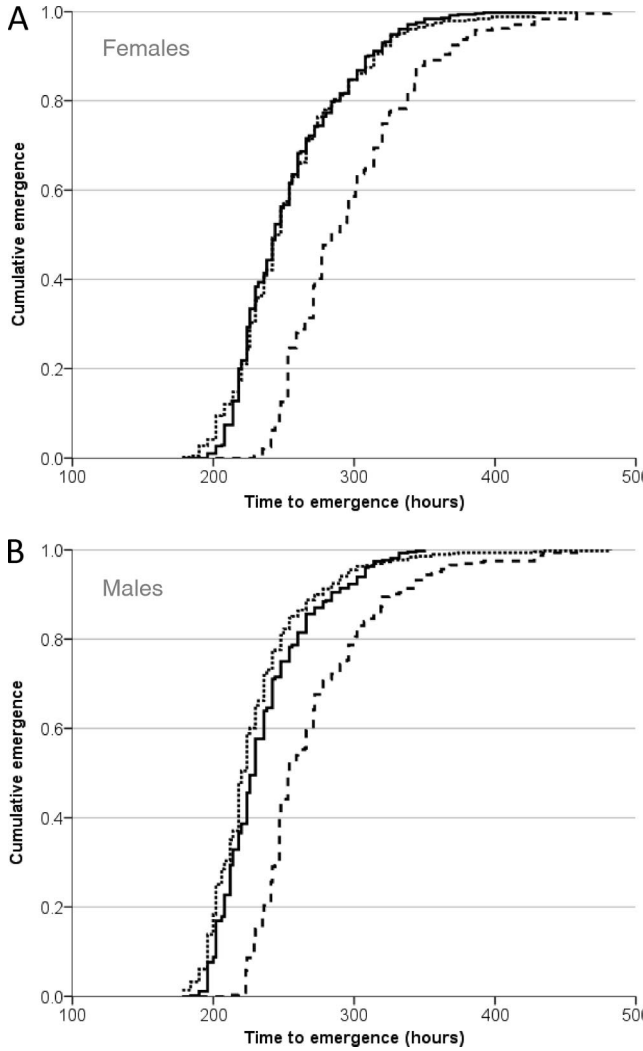

Fig. 3. Development curves of *Aedes aegypti* exposed to three diurnal temperature ranges with a mean of 26°C. (A) Females. (B) Males. Solid black lines denote constant 26°C temperatures, small dotted lines represent small DTR (26 ± 7.6°C) and large dotted lines represent large DTR (26 ± 18.6°C).

Table 2. MANOVA results for fecundity and the no. of gonotrophic cycles completed by females

Factor	Wilk's Λ	df (num, den)	*P*	SCC (log.fecundity)	SCC (no. gonotrophic cycles)
DTR	3.45	4, 74	0.0122	1.886	−0.430
Generation	22.07	2, 37	<0.0001	2.206	−0.926
DTR × generation	0.34	4, 74	0.8596	−1.353, 1.923	1.804, −0.479

The standardized canonical coefficients, indicative of the strength and direction of the effect, are presented for each trait, at right.

and the number of completed gonotrophic cycles. The positive SCCs reported for DTR and generation on log-transformed egg counts indicate that generations and DTR differ primarily with respect to the number of eggs and with a secondary effect, in the opposite direction, on the number of cycles completed.

Mean fecundity for each DTR is summarized in Fig. 4A. Mosquitoes reared under a small DTR completed an average of 3.62 (±0.13 SE; $n = 26$) gonotrophic cycles across both experiments, compared with 3.30 (±0.13; $n = 27$) and 3.10 (±0.19; $n = 21$) cycles for a constant and large DTR, respectively (Fig. 4B).

We used a repeated measures analysis to explore the response of several aspects of the gonotrophic cycle to treatments, but did not identify differences because of DTR treatments or generation on the number of eggs laid per gonotrophic cycle or the length of each gonotrophic cycle. There was no interaction between these two factors for any of the dependent variables.

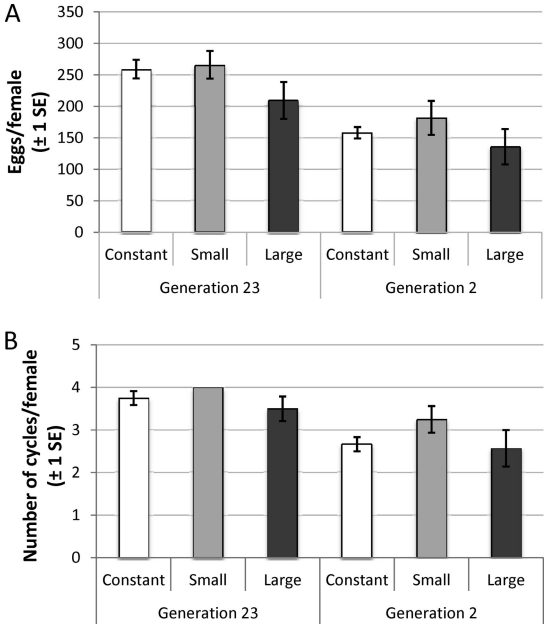

Fig. 4. Estimates for reproductive capabilities of *Aedes aegypti* under varying DTRs, after daily access to human blood for 14 consecutive days postemergence. Two generations of mosquitoes were tested: generation 23 from colony 2 and generation two from colony 2. (A) Total female reproductive output. (B) Average number of eggs per gonotrophic cycle. The x-axis groups DTR within generation. Error bars represent ±1 SEM.

The average batch of eggs across the three experiments was 66.0 eggs (±6.04) for constant temperature, 64.84 (±4.51) for small DTR, and 57.81 (±7.07) for large DTR.

Discussion

In this study, we examined the effect of large and small temperature fluctuations with a common mean, relative to a constant temperature, on the biology of the primary DENV vector, *Ae. aegypti.* Our study was designed to assess the effects of temperature variations to which mosquitoes are exposed in nature across their immature and adult life stages. We detected a trend of negative effects that large temperature fluctuations have on the mosquito life-history traits, relative to a constant temperature. The direction of the results was consistent across the old and new mosquito colonies. Effects were observed in immature survival and development time, and adult reproductive output. Conversely, a small DTR had a negligible or slightly positive effect on the life-history traits we tested, relative to constant 26°C.

Our study design followed discrete generations of mosquitoes through their immature development to adulthood under a single temperature profile. One of the limitations of this design is that we were not able to isolate effects of temperature occurring at specific mosquito life stages through a full-factorial analysis that would have compared different combinations of temperature profiles and life stages. We did not test life stage-specific effects of temperature fluctuations because rarely, if at all, would a mosquito naturally experience a transition from one temperature regime during the aquatic, immature stages, to a dramatically different environment after adult emergence. Another consideration for our experimental design is that because of space limitations we were unable to directly follow mosquitoes from larval rearing to adulthood; that is, the progression of mosquitoes in each replicate cup.

Air temperatures used in our study were recorded in natural, *Ae. aegypti* indoor environments during high and low DENV transmission seasons in Thailand. The thermal dynamics of air and water are, however, different. Investigators in two field studies examined the aquatic environment of mosquitoes in nature and the association between water and air temperatures in the field (Hemme et al. 2009, Paaijmans et al. 2010a). Although volume and surface area of the water will influence thermal dynamics, the water temperatures recorded in our experiments are within the

ranges of those presented by Hemme et al. (2009) and Paaijmans et al. (2010a). The temperature inside the 26°C constant temperature incubator was not recorded. Daily checking of the climatic data logger built-in to the incubators, however, demonstrated that the temperature and humidity in the previous 24-h period was consistent with the programmed climatic conditions. We did not investigate relative humidity as a factor that influences mosquito biology, although we expect that it can affect adult mosquitoes, because changes in humidity have been observed to alter mosquito fecundity and longevity (Reiskind and Lounibos 2009, de Almeida Costa et al. 2010). We are unaware of any experiment testing the effects of changes in relative humidity on vector competence.

The origin of the mosquitoes we studied was <150 km away from where we recorded experimental temperatures. Annual temperatures (mean, minimum, and maximum temperatures at all times of the year) in Kamphaeng Phet are similar to that of Mae Sot. We assume mosquitoes from both locations would respond similarly to the environmental treatments we tested. Using a more geographically isolated (and potentially genetically distinct) population of mosquitoes from other parts of Thailand (or elsewhere) could alter outcomes of this kind of experiment. Other investigators have reported differences in traits of *Ae. aegypti* from different geographic locations, when tested at the same temperatures (Bar–Zeev 1958, Rueda et al. 1990, Focks et al. 1993, Tun–Lin et al. 2000, Richardson et al. 2011). This could be because of differing levels of genetic variation among locations (Endersby et al. 2009) or plasticity within the population (Schneider et al. 2011). Although we acknowledge that a colony from a different geographical location may result in variable results, we expect that the relative directions of the effects of small and large DTR will remain the same.

We further recognize that conditions in nature are likely suboptimal for mosquitoes compared with those provided in our laboratory experiments. Thus, our estimates of life-history trait parameters should be considered as conservative (i.e., likely to be an overestimate of real trait values), and thus provide more of a relative measure of the temperature effects under laboratory conditions rather than absolute effects that one could expect to see in nature.

Immature Development Time and Survival. We identified a significant negative effect of DTR on larval development time and survival. Larval development time was extended and survival was reduced in response to increasing amplitude of temperature fluctuations. There was discrepancy in the comparison of development time, however, under constant versus small DTRs across generations, with constant temperatures resulting in faster development for mosquito colonies in generation 23, but slower in generation 2. We attribute this variation to the colonization history of the mosquitoes, but speculate that larval development rates of mosquitoes under constant and small DTRs are in fact quite similar.

A few investigators studied how DTR influences development time, very few have considered multiple DTR amplitudes or directly compared fluctuating to constant temperatures. Paaijmans et al. (2010b) tested immature development time in *Anopheles stephensi* (Liston), a malaria vector, after exposure to fluctuations with a DTR of 12°C around two constant means (20 and 27°C). They found that fluctuations of 12°C around lower temperatures increased survival and, in general, tended to decrease development time relative to the constant temperature. Fluctuations of the same magnitude around the higher mean had the opposite effect. Given our higher mean temperature, our results are consistent with the idea that large fluctuations around a relatively high mean slow immature development. In another study, Mohammed and Chadee (2011) compared the effect of several fluctuating rearing temperatures on *Ae. aegypti* larval development under laboratory conditions. Mean water temperatures used were higher than in our study and, as expected, higher means resulted in faster development. We obtained greater estimates of immature survival, however, indicating a possible trade-off between speed of development and probability of survival.

Richardson et al. (2011) reported estimates of development time from constant temperatures were used to accurately predict rates under fluctuating temperatures. Unfortunately, they did not report the mean or magnitude of fluctuations for which this comparison was made. Our results suggest that their fluctuations were comparable to our small DTR treatment (in the order of <10°C), which resulted in similar estimates of immature development time.

Egg Production. Our relative estimates of various aspects of the gonotrophic cycle indicated that temperature (and generation) did not alter the number of eggs per cycle or the length of the female reproductive cycle. Despite this, we did detect an effect of DTR on the total number of cycles completed by females (with the large DTR completing the smallest number), and accordingly, there was a change in the total number of eggs counted. Generation also influenced our measurements of egg related traits. Because we did not identify a significant interaction between generation and DTR, our results support a consistent effect of increasing DTR on reproductive fitness. Results demonstrating that large fluctuations reduce total egg productivity are consistent with that seen in the field during the low DENV transmission season in Thailand (Koenraadt et al. 2008). Although we are confident the effects would remain significant had we maintained adults separately according to their replicate rearing cups, because of space limitations we were not able to account for the potential effect of replicate rearing cups in our analysis. Significance values, therefore, may be slightly inflated given our design and analyses.

de Almeida Costa et al. (2010) similarly examined the effect of small temperature fluctuations (DTR = 4°C) around two relative humidity treatments on *Ae. aegypti* fecundity and survival. They determined that high temperatures reduced egg productivity and adult

survival, with the intensity of the reduction influenced by humidity. Although direct comparisons between the two studies cannot be made because their temperature treatments varied only the means and not the magnitude of fluctuations, results provide support for the prediction that fluctuations in both temperature and humidity can affect mosquito biology and, therefore, merit additional study.

Implications and Future Directions. The importance of this work is that it explores environmentally relevant variation in temperature conditions to which mosquitoes are naturally exposed. Improving our understanding of *Ae. aegypti* population dynamics under such conditions will help elucidate the complexities associated with *Ae. aegypti*'s role in DENV transmission. Our data indicate that because of reduced egg production and larval and pupal survival rates, the resulting number of adult females in the next generation will be reduced under a large diurnal temperature regime relative to a constant temperature. This is not the case when temperature fluctuations are relatively small. In addition to altering population dynamics, rearing temperature can influence adult size, susceptibility to viral infection, development time, and egg viability (Alto et al. 2008, de Almeida Costa et al. 2010, Westbrook et al. 2010), highlighting the need for increasingly rigorous investigation into the effects of diurnal temperature fluctuations on specific vector-virus interactions. We plan to address this issue in future *Ae. aegypti*-DENV studies.

Our results are consistent with conclusions by Lambrechts et al. (2011) and their prediction that DENV transmission patterns in Mae Sot, Thailand, are influenced by seasonal variation in the range of temperature fluctuations. Changes in DTR affected *Ae. aegypti* life-history traits in ways that can directly or indirectly influence virus transmission; that is, immature and adult survival, speed and success of immature development, production of adults, and speed and magnitude of reproductive output. Thus, in addition to reducing vector competence and survival of infected adult females (Lambrechts et al. 2011), large DTRs can contribute to decreased DENV transmission through an overall negative effect on mosquito vector population density. In Kamphaeng Phet densities of *Ae. aegypti* pupae and adult females have been shown to vary spatially and temporally (Koenraadt et al. 2008).

A key unanswered question concerns the effect of DTR relative to the 26°C mean temperature we studied. Future experiments should investigate vector competence and life-history traits using a wider range of natural temperature regimes that are selected based on well-supported associations with DENV transmission dynamics and epidemiologically relevant variation in mosquito biology.

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