THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENTAL RATE AND SURVIVAL OF *AEDES ALBOPICTUS* IN THAILAND

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Abstract. Global climate change has impacted public health that including vector borne disease. In particular, changing temperatures have altered insect vector life cycles and viral incubation periods. Several methods have been used to estimate changes in the worldwide distribution of dengue in various global climate change scenarios. In this study, we investigated the effect of three different temperatures (normal, 26ºC; moderate, 30ºC; and high, 33ºC) on the wild and laboratory population dynamics of *Aedes albopictus,* focusing on the larval developmental rate from the first instar to adulthood and survival at the immature and mature stages. *Aedes albopictus* underwent more rapid development at a high temperature. Conversely, survival was highest at a normal temperature and lowest at a high temperature. Our findings provide insight into the effect of temperature on the life cycle of *Aedes albopictus* in Thailand, and illustrate the biologic changes that this mosquito may undergo in response to global warming.

Keywords: *Aedes*, temperature, climate change, development rate, survival

INTRODUCTION

Billions of insects exist in the global ecosystem, and their numbers and behaviors are controlled by environmental factors. Insects of medical significance, such as vectors of dengue virus, reside in many locations worldwide. The *Aedes albopictus* mosquito is a vector of dengue virus widely distributed in urban, peri-urban, and rural areas. Females feed on the blood of a relatively broad range of vertebrate hosts, including humans. *Ae. albopictus* has adapted well to the activities of humans (Gould and Higgs, 2009). *Ae. albopictus* can survive outside and around the homes. Their development process of immature and mature stages are controlled by envelopment. Increasing night temperatures due to climate change have impacted the global ecosystem (Peng *et al*, 2004).

Ae. albopictus, a member of a homogeneous group within the subgenus *Stego-*

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myia, is known as the "Asian Tiger" Mosquito" because of its striped appearance. The species has spread around the world (Fontenille and Rodhain, 1989; Delatte *et al*, 2009; Guo *et al*, 2013). Temperature can affect the population size and distribution of *Ae. albopictus*. Expansion of the *Ae. albopictus* population occurred with warmer winter temperatures in the northeastern United States (Rochlin *et al*, 2013). The developmental rate of *Ae. albopictus* has a negative relationship with temperatures (Alto and Juliano, 2001a). In addition, high temperatures have increased the rate of spread of *Ae. albopictus* by increasing their biting and reproductive rates, enhancing colonization and population growth (Alto and Juliano, 2001b).

In Thailand, incidences of dengue and other vector-borne diseases have increased in recent years. Global warming has affected the distribution of dengue and other arthropod-borne diseases. Vector control is the most effective way to combat dengue because the disease has no effective treatment. Monitoring the development of insect vectors enables the prediction and control of disease outbreaks. In this study, we investigated larval developmental rate and adult survival at different temperatures in immature- and adult-stage wild and laboratory strains of *Ae. albopictus*. We aimed to provide information on changes in the vector's geographic range and the adaptation of local species of *Ae. albopictus* in response to global warming.

MATERIALS AND METHODS

Mosquito strains and rearing

One laboratory strain and two wild strains of *Ae. albopictus* were used in this study. The laboratory strain (LAB) originated from Kanchanaburi Province, Thailand. The two wild strains originated from Prachuab Khiri Khan (PCK) and Phuket (PKT) Provinces, Thailand. Colonies of all strains were established by larval collection from containers in villages experiencing dengue outbreaks, and reared under insectarium conditions (environmental temperature, 26 ± 2°C; relative humidity, 70 ± 10%; and photoperiod, 12:12 h light:dark) at the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Ground cat food tablets (Me-O®, PCGperfectcompanion, Bangkok) were fed to larvae (0.2 g/100 larvae), and sucrose solution was fed to adults. Human blood was fed to females for egg production using an artificial feeder. Gravid females were allowed to lay eggs on moist filter paper in oviposition bowls. Dried oviposition papers from each strain were stored separately in closed plastic boxes, and submerged dried oviposition papers in immature-rearing trays when a new generation was needed or when experiments began.

Environmental chambers

Three incubators (Accuplus™ model i250, with International Organization for Standardization 17025 certification of calibrated temperature accuracy; Entech Associate, Bangkok, Thailand) were used to maintain chambers of three different temperatures as follows: normal, 26°C; moderate, 30°C; and high, 33°C. The relative humidity and photoperiod of each chamber were identical to insectarium conditions.

The effects of normal, moderate, and high temperatures on the three strains of *Ae. albopictus* were assessed in two ways, as shown in Fig 1. Survival at all stages and developmental rate were investigated using one set of a single generation of the laboratory (F_{23}) and wild (F_3) strains, whereas adult survival was investigated

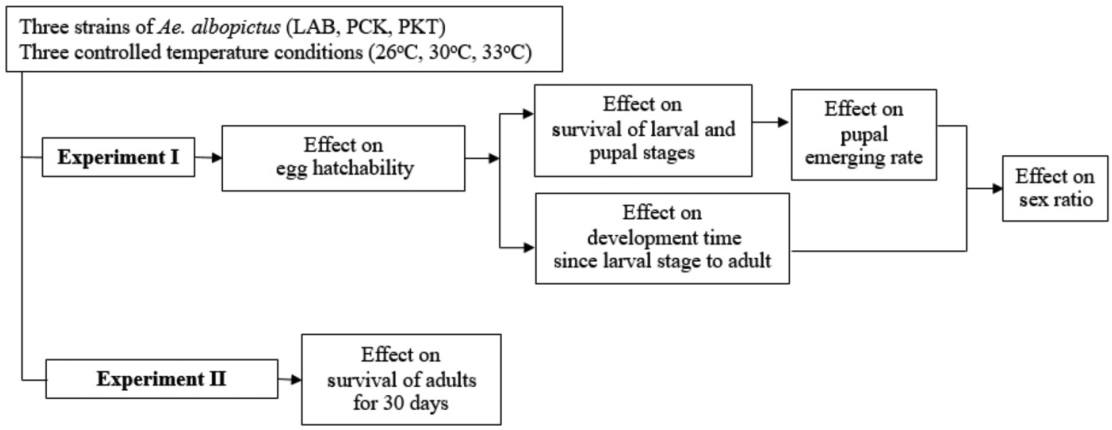

Fig 1–Experimental design and data analysis.

using another set of the same generation of specimens. The method used to rear immature and adult specimens in the environmental chambers was identical to that used in the insectarium. Three replications of the experiments for each strain were performed to prevent contamination among the different strains. Survival at immature stages consisted of: 1) the egg-hatching rate or survival at first instar larval stage; 2) survival from first to fourth instar larval stages; 3) survival at fourth instar larval stage; 4) survival at the pupal stage.

The statistical analyses used in this study comprised analysis of variance, with the one-sample Kolmogorov-Smirnov test to analyze whether the data were normally distributed, and the nonparametric Kruskal-Wallis test for comparisons among sex, temperature, and population groups.

Effect on egg-hatching rate

Four hundred eggs were counted on a dried oviposition paper. The selected area of paper was cut and submerged in an egg-hatching tray for 24 hours in each environmental chamber. The first instar larvae were counted and transferred to trays daily.

Assessment of developmental rate and survival in the immature stages

In each temperature-controlled chamber, 100 L_1-stage larvae were reared per tray. The L_4 larvae were counted and transferred to the L_4-rearing tray daily. The individual pupae were separated into vials covered with gauze. The vials were coded, and the pupa collection date, adult sex, and emergence date were recorded. Newly emerged adults were reared in mosquito cages in the temperature-controlled chambers for one day.

Assessment of survival at the adult stage

In each temperature-controlled chamber, 50 female and 50 male mosquitoes that emerged on the same day were released into mosquito cages and reared for 30 days. The adults were examined daily to record and remove from the cage any dead males or females. The recorded data were used to quantify survivorship.

RESULTS

Egg-hatching rate and survival in the immature stages

The mean number of eggs hatched did not significantly differ at normal (26°C) and moderate (30°C) temperatures.

Table 1
Hatching rate of *Aedes albopictus* larvae reared at different temperatures (26°C, 30°C, and 33°C) under controlled conditions. (LAB, Laboratory strain; PCK, Prachuabkhirikhan; PKT, Phuket).

A Temperature (°C)	Egg-hatching rate (%)			*F*	*p*-value
	LAB	PKN	PKT		
26	2,823/3,540 (79.75)[a]	1,163/1,430 (81.33)[a]	987/1,190 (82.94)[a]	0.402	0.685
30	3,718/4,520 (82.26)[a]	1,331/1,550 (85.87)[a]	1,256/1,455 (86.32)[a]	0.503	0.628
33	2,727/5,585 (48.83)[a]	1,780/2,550 (69.80)[b]	1,180/1,665 (70.87)[b]	6.237	0.034
B Strain	Egg-hatching rate (%)			*F*	*p*-value
	26°C	30°C	33°C		
LAB	2,823/3,540 (79.75)[a]	3,718/4,520 (82.26)[a]	2,727/5,585 (48.83)[b]	12.921	0.007
PCK	1,163/1,430 (81.33)[a]	1,331/1,550 (85.87)[a]	1,780/2,550 (69.80)[b]	6.213	0.035
PKT	987/1,190 (82.94)[a]	1,256/1,455 (86.32)[a]	1,180/1,665 (70.87)[a]	3.185	0.114

One-way analysis of variance (ANOVA), letters in superscript indicate statistical significance at the $p<0.05$.

In contrast, at a high temperature (33°C), significantly fewer eggs hatched, especially of the LAB strain ($F = 6.237$, $p = 0.034$; Table 1A). High temperature did not affect the egg-hatching rate of the PKT strain, whereas it significantly affected that of the LAB and PCK strains ($F = 12.921$, $p = 0.007$ and $F = 6.213$, $p = 0.035$, respectively; Table 1B).

All strains exhibited decreased survival with increasing temperature (Fig 2). However, the PKT strain was significant differences in survival: this strain demonstrated the lowest survival at a normal temperature in the L_1-L_4 period ($F = 8.333$, $p = 0.019$) and at moderate and high temperatures in the L_4-pupa period ($F = 11.727$, $p = 0.008$ and $F = 37.202$, $p \leq 0.001$, respectively).

Emergence of adults and sex ratio

At normal and moderate temperatures, significantly fewer adult mosquitoes of the PCK and PKT strains emerged ($F = 40.158$, $p \leq 0.001$ and $F = 26.882$, $p = 0.001$, respectively), whereas there was no significant difference at a high temperature.

Regarding the sex ratio, males of the LAB and PKT strains were less numerous than females at a high temperature (36.35% and 32.84%, respectively), whereas males of the PCK strain were less numerous than females at a moderate temperature (33.85%).

Developmental rate in the immature stages

In this study, we used a total of 2,020 males and 2,457 females. To calculate population growth, we used nonparametric testing because the durations of their life cycles exhibited non-normal distribution according to the one-sample Kolmogorov–Smirnov test. Moreover, a

Table 2
Survival of *Aedes albopictus* specimens reared at different temperatures.

A. Males and females of the laboratory strain

Temperature (°C)	Sex	Survival time (days)[a]	95% CI	χ^2 (p-value)[b]
		Estimate (SE)		
26	Male	22.00 (0.54)[a]	(20.94-23.06)	279.01
30	Male	12.00 (0.48)[b]	(11.06-12.94)	(< 0.001)
33	Male	8.00 (0.33)[c]	(7.35-8.65)	
26	Female	27.00 (0.99)[a]	(25.06-28.94)	286.82
30	Female	13.00 (0.85)[b]	(11.34-14.66)	(< 0.001)
33	Female	8.00 (1.12)[c]	(5.81-10.20)	

B. Males and females of the wild strain from Prachuabkhirikhan Province

Temperature (°C)	Sex	Survival time (days)[a]	95% CI	χ^2 (p-value)[b]
		Estimate (SE)		
26	Male	20.00 (0.73)[a]	(18.58-21.42)	279.18
30	Male	15.00 (0.51)[b]	(14.00-16.00)	(< 0.001)
33	Male	6.00 (0.35)[c]	(5.31-6.70)	
26	Female	22.00 (0.58)[a]	(20.87-23.13)	280.72
30	Female	19.00 (0.52)[b]	(17.98-20.02)	(< 0.001)
33	Female	8.00 (0.57)[c]	(6.88-9.12)	

C. Males and females of the wild strain from Phuket Province

Temperature (°C)	Sex	Survival time (days)[a]	95% CI	χ^2 (p-value)[b]
		Estimate (SE)		
26	Male	16.00 (0.37)[a]	(15.28-16.72)	281.7
30	Male	11.00 (0.29)[b]	(10.44-11.56)	(< 0.001)
33	Male	5.00 (0.83)[c]	(3.38-6.62)	
26	Female	18.00 (2.09)[a]	(13.90-22.11)	248.9
30	Female	13.00 (0.68)[b]	(11.67-14.32)	(< 0.001)
33	Female	9.00 (0.37)[c]	(8.28-9.73)	

[a]Median survival time; [b]Overall log-rank test. CI, Confidence interval; SE, Standard error; letters in superscript indicate statistical significance at the $p<0.05$.

significant difference in life cycle duration between the three temperatures was detected by the Kruskal-Wallis test.

The duration of development in males was shorter than that in females. In both sexes, the duration of development was shorter at a high temperature. The duration of development in the L_1-L_4, L_4-pupa,

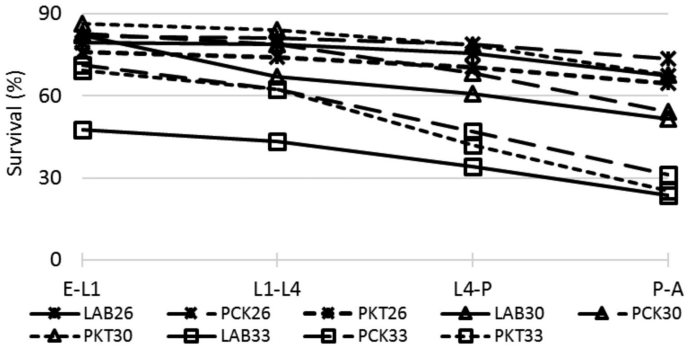

Fig 2–Survival of *Aedes albopictus* larvae reared at different temperatures (26°C, 30°C, and 33°C) under controlled conditions. E, egg; L4, fourth-instar larvae; P, pupae; and A, adults. LAB, Laboratory strain; PCK, Prachuab Khiri Khan; PKT, Phuket; temperatures (26°C, 30°C, and 33°C).

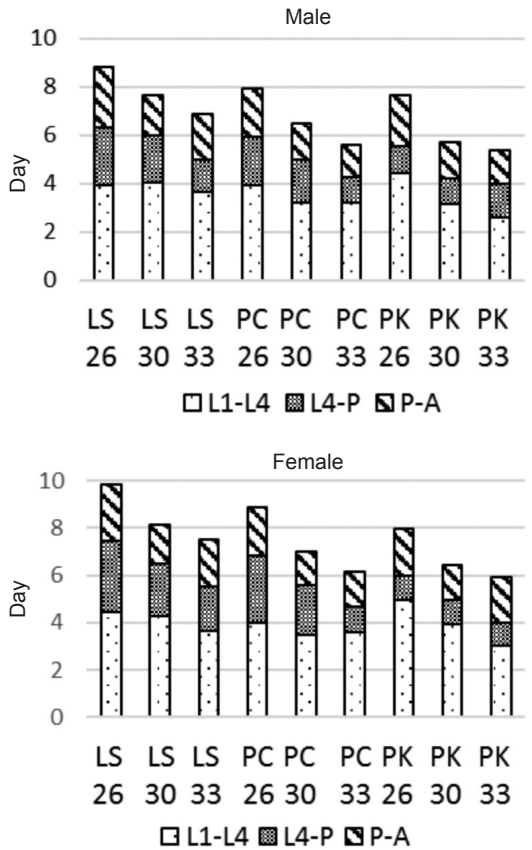

Fig 3–Larval instar durations of *Aedes albopictus* larvae reared at different temperatures (26°C, 30°C, and 33°C) under controlled conditions. LS, laboratory strain; PC, wild strain from Prachuab Khiri Khan Province; PK, wild strain from Phuket Province.

and pupa-adult stages was shortest at a high temperature, especially in the PKT strain (5.39 days in males versus 5.91 days in females; $\chi^2 = 285.348$, $p \leq 0.001$ and $\chi^2 = 80.036$, $p \leq 0.001$, respectively).

A high temperature decreased the developmental rate of the L_1-L_4 and L_4-pupa stages of males of the LAB strain ($\chi^2 = 123.48$, $p \leq 0.001$ and $\chi^2 = 348.999$, $p \leq 0.001$, respectively) and a moderate temperature impacted the duration of the pupa-adult stage in this strain ($\chi^2 = 306.258$, $p \leq 0.001$). A high temperature also affected the developmental rate of the L_1-adult stages in males of the PCK and PKT strains ($\chi^2 = 557.049$, $p \leq 0.001$ and $\chi^2 = 285.348$, $p \leq 0.001$, respectively). However, in the PKT strain, a high temperature prolonged the duration of the L_4-pupa stage. Developmental rate during the L_1-adult stages was influenced by temperature in female mosquitoes, but in females of the PKT strain, the L_4-pupa stages lasted 2 days at all treatment temperatures, as shown in Fig 3.

Survival at the adult stage

Based on 30 days observation, lower survival was evident at a high temperature in all three strains. A higher percentage of survivors were female. Males of the PKT strain showed the shortest median survival time (in days). At a high temperature, both males and females of the LAB strain

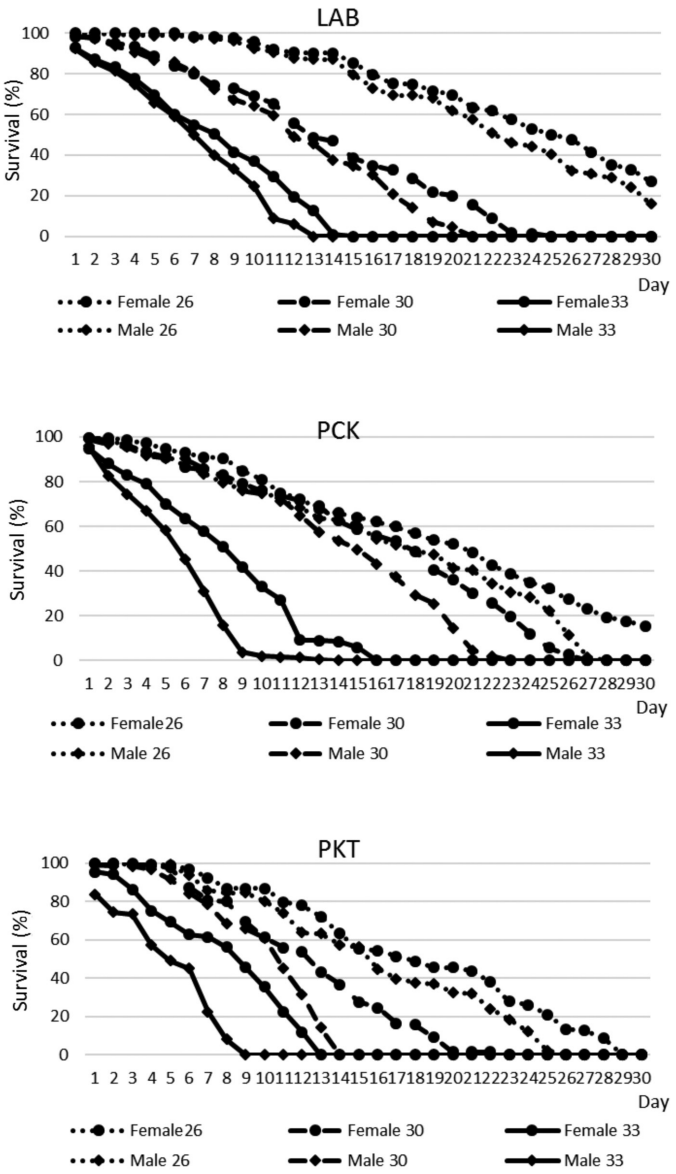

Fig 4–Survival of adult *Aedes albopictus* specimens. LAB, Laboratory strain; PCK, Prachuab Khiri Khan; PKT, Phuket; temperatures (26°C, 30°C, and 33°C).

presented the same survival time (median = 8 days), whereas females of the PCK and PKT strains exhibited a longer survival time (Table 2). At a normal temperature, both males and females of LAB strain can be survived longer than other strains (22 days and 27 days, respectively). Moreover, 16% of the males and 27% of the females can be survived longer than 30 days. The shortest survival of males was evident at a high temperature (PCK and PKT strains).

Both males and females of the LAB strain showed a clear pattern in which increased temperature led to decreased survival (Fig 4). However, at a moderate temperature, the PCK strain exhibited a similar survival to that at a normal temperature and the PKT strain displayed the lowest survival, as shown in Fig 4.

DISCUSSION

Flooding and drought are known to stimulate insect embryogenesis and egg-hatching. The threshold hatching temperature differs between insect species. The hatching response of *Aedes* mosquito eggs can also be influenced by humidity and temperature, especially temperatures between 25°C and 28°C (Judson *et al*, 1965; Huang *et al*, 2006; Thomas *et al*, 2012). Unsurprisingly, our data found that the wild strains have a higher hatching rate than the LAB strain. Wild strain has exposed under climatic stress for many years while laboratory strain has rearing under stable condition. Both strains were different genetic background because of environmental condition that might be the reason why they showed unlike of responsiveness.

Temperature exerted a marked effect on developmental rate and longevity dur-

ing the immature stages in *Ae. albopictus*. At a high temperature, larval development was fastest and survival was lowest. Higher temperatures are frequently encountered during the dry season in most tropical zones. Our data show that, at a high temperature, there is more variability in the duration of larval development (5.39-7.49 days) compared with at a normal temperature (7.64-9.84 days).

At higher temperatures, fewer larvae survive the period necessary for adult development, despite the fact that the duration of development is shorter (Bayoh and Lindsay, 2003). Insect growth depends on temperature because biochemical reactions control development and metabolism (Byrd and Castner, 2001). The developmental rate of *Anopheles gambiae s.s.* is greatest at temperatures of between 28°C and 32°C (Bayoh and Lindsay, 2003), that of *Culex quinquefasciatus* is greatest between 20°C and 30°C, that of *Aedes aegypti* is greatest between 20°C and 27°C (Rueda *et al*, 1990), and that of *Ae. albopictus* is greatest between 25°C and 30°C (Delatte *et al*, 2009). In this study, we used the same food throughout because diet and nutrient quality have known associations with developmental rates (Couret *et al*, 2014). The duration of the aquatic to mature stages of development was shortest in the PKT strain. Thus, both temperature and strain influenced the development of *Ae. albopictus* in this study. Our data imply that wild strains of *Ae. albopictus* exhibit greater adapt themselves than the LAB strain under stress envelopment and temperature.

When the external temperature of an insect rises, its respiration rate drops as the rates of metabolism and respiration increase to their threshold limits, leading to death. Even if the insect returns to a normal temperature, the systematic cell death caused by the high temperature continues. High temperatures also affect the nervous and endocrine systems involved in insect metamorphosis, causing death. In this study, we found that some pupae failed to emerge as adults, and some adults died within 24 hours. At high temperatures, insects have less time to accumulate sufficient mass, which impedes pupation: larvae must consume nutrients for metabolism before pupation.

Our data demonstrate that a constant temperature is a critical factor determining the developmental rate and survival of *Ae. albopictus*. Survival was highest at a normal temperature and lowest at a high temperature. Rozilawati *et al* (2016) reported that survival of *Ae. albopictus* decreases with increasing age and temperature. In this study, the proportion of males was higher than that of females at a high temperature, which may reflect their reduced survival at the immature stages, especially between the pupa and adult stages, at a high temperature.

Geographic distribution and population growth are important to both the occurrence and control of a disease outbreak (Richards *et al*, 2010). The numbers and survival of females have a positive relationship with offspring turnover. Rising temperatures may cause a rapid increase in mosquito abundance. Temperature is not the only factor affecting survival at the immature and mature stages in *Ae. albopictus*. Crowding of larvae, water quality, food quality, predation, and pathogens all interact to limit mosquito development and survival.

In addition to human, biologic, and ecologic determinants, climatic factors also influence the emergence and re-emergence of infectious diseases (Anderson *et al*, 2004; Fuller *et al*, 2012; Lafferty, 2009). The incidences of mosquito-borne diseases, including malaria, dengue, and viral encephalitis,

are among the most sensitive to climate (Russell *et al*, 2009; Gilbert, 2010; Bouma *et al*, 2011). Climate change may directly affect disease transmission by shifting the vector's geographic range. In this study, the temperature of the mosquito collection sites was lower than 30°C, but the wild populations exhibited higher hatching rates and more rapid development at a high temperature. Temperature fluctuation is a natural mechanism of developmental regulation in this mosquito. The laboratory population was reared at a constant temperature, and may have lost some of its sensitivity to temperature stimulation. Our findings in the three strains suggest not only that shifting of the vector's geographic range may occur with global warming, but also that resident species may adapt to climate change.

In conclusion, global warming affects the distribution of arthropod-borne diseases. Monitoring the development of insect vectors may enable the prediction and control of disease outbreaks. *Ae. albopictus* develops more rapidly at higher temperatures, but its development may become too fast for the accumulation of a food reserve sufficient to support its metamorphosis. In this study, we analyzed both wild and laboratory strains of *Ae. albopictus*. The wild strains displayed a significantly faster developmental rate at moderate and high temperatures. Shifting of the vector's geographic distribution may occur in response to global warming, but adaptation of the endemic population should be considered. In future, we aim to analyze *Ae. albopictus* in other parts of Thailand to assess the pattern of adaptation. Population genetics should also be performed.

ACKNOWLEDGEMENTS

We would like to thank all of the staff at the Department of Medical Entomology for their support. This study was financially supported by the National Research University Project of Thailand, Office of the Higher Education Commission, Ministry of Education, Thailand.

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