EFFECT OF TEMPERATURE ON LABORATORY REARED *ANOPHELES DIRUS* PEYTON AND HARRISON AND *ANOPHELES SAWADWONGPORNI* RATTANARITHIKUL AND GREEN

Siriporn Phasomkusolsil[1], Kriangkrai Lerdthusnee[1], Benjawan Khuntirat[1,2], Weeraphan Kongtak[1], Kanchana Pantuwatana[1] and Jittawadee R Murphy[1,3]

[1]Department of Entomology, [2]Department of Virology, US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand;
[3]Department of Mosquito Biology, Division of Entomology, Walter Reed Army Institute of Research, Silver Spring, MD, USA

Abstract. Investigations have shown that female mosquitoes with a larger body size (determined by wing length) exhibit higher feeding rates and greater fecundity relative to smaller mosquitoes. In this study, *Anopheles dirus* and *An. sawadwongporni* were reared in the laboratory at two different temperatures (23ºC and 30ºC). Effects of the rearing temperature on body size, fecundity, and larval development period were examined by measuring wing length, adult body weight at emergence, the number of eggs produced and the length of time from the first to the fourth instar. Rearing temperature had a direct effect on body size, fecundity and larval development period for both species. Mosquitoes of both species reared at 23ºC were larger in body size, experienced prolonged development and produced a larger clutch of eggs relative to mosquitoes reared at 30ºC. However, there was no temperature effect on egg hatching rate and sex ratio.

Keywords: *Anopheles dirus*, *Anopheles sawadwongporni*, temperature, body size, fecundity

INTRODUCTION

Factors that generally influence mosquito size include nutritional conditions, photoperiod, and temperature (Farkas and Brust, 1985; Oda and Nuorteva, 1987; le Sueur and Sharp, 1991; Ameneshewa and Service, 1996). Several studies (Haramis, 1983; O'Meara *et al*, 1993; Nasci and Mitchell, 1994; Grech *et al*, 2007) have shown that larger body size is generally associated with larger blood meals, higher fecundity and a longer life span, since female mosquitoes with larger body sizes tend to live longer than smaller-sized females (Kitthawee *et al*, 1992). Temperature and photoperiod have been shown to affect wing length, body weight, and the morphology of developing mosquitoes (Canyon *et al*, 1999; Lardeux and Cheffort, 2002; Mourya *et al*, 2004; Menge *et al*, 2005). *Anopheles quadrimaculatus* reared under shortened photoperiods and lower tem-

Correspondence: Siriporn Phasomkusolsil, Department of Entomology, US Army Medical Component, Armed Forces Research Institute of Medical Sciences, 315/6 Ratchawithi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2696 2817; Fax: 66 (0) 2354 7885.
E-mail: siripornp@afrims.org

peratures have greater wing lengths and heavier weights than those reared under prolonged photoperiod and higher temperatures (Lanciani, 1992). Wing length is often used as a reliable index of body size (Nasci, 1990; Suwanchaichinda and Paskewitz, 1998). Other studies have shown similar effects on wing length and body size when mosquitoes were reared at lower temperatures (Siegel *et al*, 1994; Atkinson, 1995; Nasci *et al*, 1996; Gleiser *et al*, 2000; Bochdanovits and De Jong, 2003). An inverse correlation between temperature and body size has been reported for several mosquito species (Petrarca *et al*, 1998). In the current study, we examined the effects of rearing temperature on body size (measured by wing length and weight), fecundity, and larval developmental period for *An. dirus*, and *An. sawadwongporni.*

MATERIALS AND METHODS

Anopheles rearing procedures

An. dirus and *An. sawadwongporni* were reared in a permanent insectary located at the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand. The mosquitoes were blood-fed on anesthetized hamsters for 15 minutes. Twenty blood-engorged female mosquitoes of each species were randomly selected. The first batch/clutch of eggs laid by these females (approximately 500 eggs) was divided into two groups of relatively equal quantities and placed in larval rearing water. One group was reared at 23ºC and the other at 30ºC ambient temperature. Each group was divided into two cohorts. The temperature in each room was checked and monitored daily by a hygrothermograph. On Day 2 after the eggs hatched, 0.01 g ground fish food (HIPRO®) was added to each container daily until all of the larvae pupated. Debris and dirty water were removed daily. Pupae were collected and then aspirated into a plastic cup using a dropper. After 2 days, most adult mosquitoes had emerged and the water was then gently drained from the plastic cup. Adults were provided with cotton balls soaked in a mixture of 5% multivitamin syrup in water.

Egg hatching rate, larval period, and sex ratio. Egg hatching rates were determined from 100 eggs reared at each tested temperature (23ºC and 30ºC) for *An. dirus* and *An. sawadwongporni*. The numbers of newly hatched larvae were recorded daily. The larval developmental period was determined from 50 first instars, randomly selected from the pool of larvae used in the hatching rate determinations. Time spent from first instar to pupation was recorded. Sex ratios were determined for 25 randomly-selected adults that had emerged from pupae.

Body weight. Fifty newly-emerged adults reared at each tested temperature (25 males and 25 females) were randomly selected and weighed individually.

Wing length. Fifty adult mosquitoes reared at each tested temperature (25 males and 25 females) were randomly selected from a pool of 100 mosquitoes for wing length measurement. The measurement was made on their right wing from the auxillary incision to the apical margin as described previously (Nasci, 1986b).

Fecundity. Twenty-five 7-day old females were randomly selected from a pool of 100 females and fed with the blood of golden hamsters (*Mesocricetus auratus*) from the National Laboratory Animal Center of Mahidol University, Salaya Campus, Nakhon Pathom, Thailand. Mosquitoes were mated manually using an artificial insemination method (Yang *et al*, 1963).

Each blood-fed and mated female was held in an individual vial containing a piece of moist filter paper as an oviposition substrate. the numbers of eggs laid were recorded daily for 48 hours post-blood feeding.

Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) by SPSS for Windows (version 16.0).

RESULTS

Hatching rate, larval period, and sex ratio (Table 1)

An. dirus. No significant difference ($p > 0.05$) was found between the mean hatching rate of eggs reared at 23ºC (66.1% hatching rate) and 30ºC (74.0% hatching rate). Mosquitoes reared at both temperatures showed a sex ratio of 1:1. Larvae reared at 23ºC displayed a significantly longer developmental time (11.8 days) compared to the larvae reared at 30ºC (8.1 days) ($p< 0.05$).

An. sawadwongporni. No significant difference ($p>0.05$) was found between the mean hatching rate of eggs reared at 23ºC (41.1%) and 30ºC (45.9%). Mosquitoes reared at both temperatures had a sex ratio of 1:1. Larvae reared at 23ºC had a significantly longer developmental time (13.7 days) compared to larvae reared at 30ºC (7.3 days) ($p<0.05$).

Body weight (Table 2 and Fig 1)

An. dirus. Body weights of female and male mosquitoes reared at 23ºC (2.32 and 1.51 mg, respectively) were significantly heavier than those reared at 30ºC (1.69 and 1.22 mg, respectively) ($p<0.05$).

An. sawadwongporni. Body weights of female and male mosquitoes reared at 23ºC (1.27 and 1.16 mg, respectively) were significantly heavier than those reared at 30ºC (1.00 and 0.88 mg, respectively) ($p<0.05$).

Wing length (Table 3 and Fig 2)

An. dirus. Female and male mosquitoes reared at 23ºC had significantly longer wings (3.65 and 3.35 mm, respectively) than those reared at 30ºC (3.44 and 3.09 mm, respectively) ($p<0.05$).

An. sawadwongporni. Female and male mosquitoes reared at 23ºC had significantly longer wings (3.09 and 3.04 mm, respectively) than those reared at 30ºC (2.78 and 2.71 mm, respectively) ($p<0.05$).

Fecundity (Table 4 and Fig 3)

An. dirus. The mean number of eggs laid by females reared at 23ºC (201.4 eggs) was significantly higher than the number reared at 30ºC (103.3 eggs) ($p<0.05$).

An. sawadwongporni. The mean number of eggs laid by females reared at 23ºC (177.5 eggs) was significantly higher than the number reared at 30ºC (140.8 eggs) ($p<0.05$).

DISCUSSION

A critical factor that affects mosquito growth and development throughout its life cycle is temperature which has been shown to regulate the length of the gonotrophic cycle (Depinay *et al,* 2004). As previously demonstrated for *Aedes albopictus* females, mean wing length is longer during the spring (mild conditions), declines during midsummer (hot conditions) and increases slightly in the fall (cooler conditions) (Willis and Nasci, 1994). Factors which directly affect egg-batch sizes include nutritional conditions, blood volume and blood meal source. The larval development period has also been shown to impact adult size and fecundity (Nasci, 1986a; Briegel, 1990; Clements,

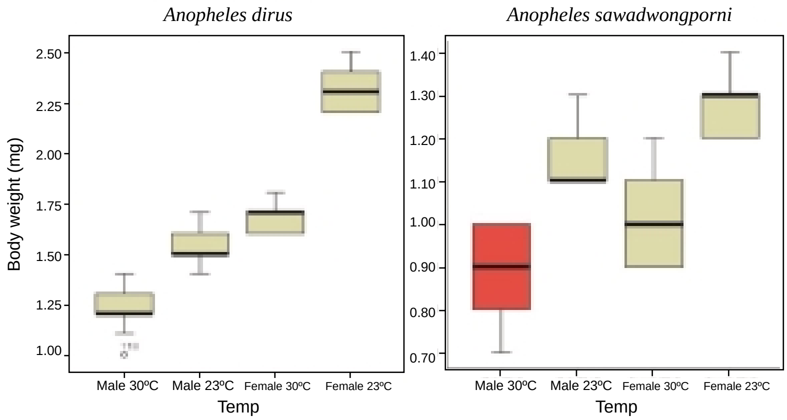

Fig 1–Body weight of *An. dirus* and *An. sawadwongporni* reared at 23ºC and 30ºC.

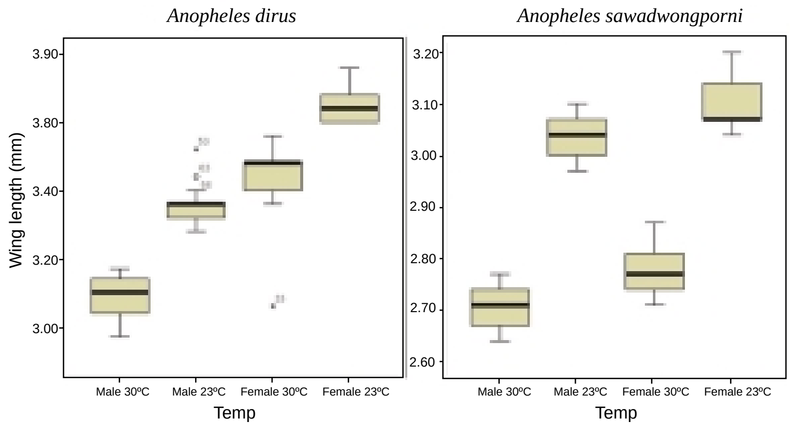

Fig 2–Wing length of *An. dirus* and *An. sawadwongporni* reared at 23ºC and 30ºC.

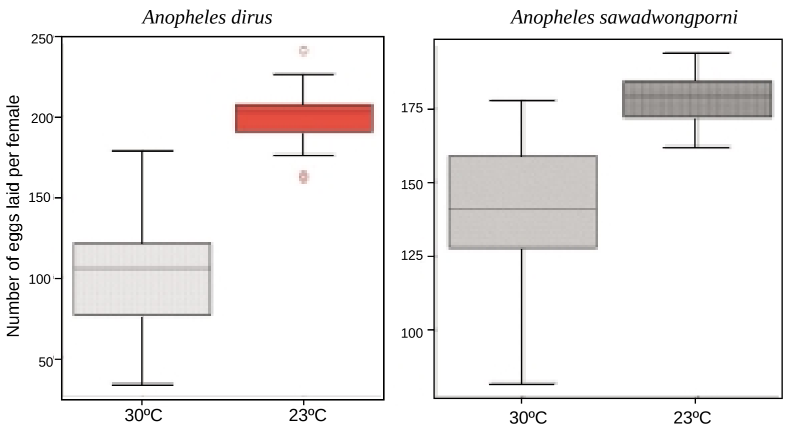

Fig 3–Relationship between rearing temperatures and numbers of eggs laid by *An. dirus* and *An. sawadwongporni* at 23ºC and 30ºC.

1992). Previous studies demonstrating the role of temperature on mosquito development have largely focused on its effects on the epidemiology of mosquito-borne viruses (Brubaker and Turell, 1998), population dynamics or population growth (Alto and Juliano, 2001; Kirby and Lindsay, 2009), survival rate and adult size (Loetti *et al*, 2007).

Our study clearly demonstrates that a lower rearing temperature results in larger mosquitoes in terms of body weight and wing length, a prolonged period of larval development, and higher fecundity compared with higher temperatures for both *An. dirus* and *An. sawadwongporni*. Larger-sized females are more likely to become infectious relative to smaller females when fed on hosts with viremias (Nasci and Mitchell, 1994; Schneider *et al*, 2007). Results from our study have important implications for future studies investigating the relationship of temperature and adult size on the susceptibility of *An. dirus* and *An. sawadwongporni* to infection by the malaria parasite.

Table 1
Hatching rates, larval periods and sex ratios for *An. dirus* and *An. sawadwongporni* at 23ºC and 30ºC.

Rearing temperature							
An. dirus	Mean±SD		*p*-value	*An. sawadwongporni*	Mean±SD		*p*-value
	23ºC	30ºC			23ºC	30ºC	
[a]Hatching rate (%)	66.1 ± 17.8	74 ± 11.6	>0.05	[a]Hatching rate (%)	41.1 ± 15.7	45.9 ± 17.7	>0.05
Larval period (days)	11.8 ± 0.6	8.1 ± 0.7	0.00[b]	Larval period (days)	13.7 ± 0.7	7.3 ± 0.8	0.00[b]
Male : Female	1:1	1:1		Male : Female	1:1	1:1	

[a]Hatching rate = no. of live larvae/total no. of eggs laid x 100
[b]Comparison of means within groups indicate differences (p<0.05) when tested by one-way ANOVA.

Table 2
Average body weights (mg) of *An. dirus* and *An. sawadwongporni* reared at 23ºC and 30ºC.

Body weight (mg)							
An. dirus	Mean±SD		*p*-value	*An. sawadwongporni*	Mean±SD		*p*-value
	23ºC	30ºC			23ºC	30ºC	
Female (*n*=25)	2.32 ± 0.09	1.69 ± 0.08	0.00[a]	Female (*n*=25)	1.27 ± 0.07	1.00 ± 0.09	0.00[a]
Male (*n*=25)	1.51 ± 0.09	1.22 ± 0.11	0.00[a]	Male (*n*=25)	1.16 ± 0.07	0.88 ± 0.11	0.00[a]

[a]Comparison of mean within groups indicate differences (p<0.05) when tested by one-way ANOVA.

Table 3
Mean wing lengths (mm) for *An. dirus* and *An. sawadwongporni* reared at 23°C and 30°C.

Wing length (mm)							
An. dirus	Mean±SD		*p*-value	*An. sawadwongporni*	Mean±SD		*p*-value
	23°C	30°C			23°C	30°C	
Female (n=25)	3.65 ± 0.04	3.44 ± 0.09	0.00[a]	Female (n=25)	3.09 ± 0.05	2.78 ± 0.05	0.00[a]
Male (n=25)	3.35 ± 0.06	3.09 ± 0.06	0.00[a]	Male (n=25)	3.04 ± 0.04	2.71 ± 0.04	0.00[a]

[a]Comparison of means within groups indicate differences (p<0.05) when tested by one-way ANOVA.

Table 4
The effect of temperature on numbers of eggs laid by *An. dirus* and *An. sawadwongporni* at 23°C and 30°C.

Number of eggs laid per female							
An. dirus	Mean±SD		*p*-value	*An. sawadwongporni*	Mean±SD		*p*-value
	23°C	30°C			23°C	30°C	
	201.4 ± 19.0	103.3 ± 35.9	0.00[a]		177.5 ± 9.3	140.8 ± 26.2	0.00[a]

[a]Comparison of means within groups indicate differences (p<0.05) when tested by one-way ANOVA.

ACKNOWLEDGEMENTS

All the experiments involving animals were approved by the AFRIMS's Institutional Animal Care and Use Committee. Funding for this project was partially provided by the US Army Medical and Material Command. The views expressed in this paper are those of the authors and do not purport to represent the position and/or reflect the official policy of the Department of the Army, the Department of Defense or the US Government. We would like to thank Ms Jaruwan Tawong and Ms Nantaporn Monkanna for providing technical support and Ms Somporn Krasaesub for her helpful advice on data analysis. The authors wish to express their gratitude to Maj Brian P Evans, Department of Entomology, US Army Medical Component of the Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS) and Col Chaiyaphruk Pilakasiri, Royal Thai Army, Armed Forces Research Institute of Medical Sciences (RTA-AFRIMS), Bangkok, Thailand, for their constructive comments and valuable support.

REFERENCES

Alto BW, Juliano SA. Temperature effects on the dynamics of *Aedes albopictus* (Diptera: Culicidae) populations in the laboratory. *J Med Entomol* 2001; 38: 548-56.

Ameneshewa B, Service MW. The relationship between female body size and survival rate of the malaria vector *Anopheles arabiensis* in Ethiopia. *Med Vet Entomol* 1996; 10: 170-2.

Atkinson D. Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule. *J Thermal Biol* 1995; 20: 61-74.

Bochdanovits Z, De Jong G. Temperature dependent larval resource allocation shaping adult body size in *Drosophila melanogaster*. *J Evol Biol* 2003; 16: 1159-67.

Briegel H. Fecundity, metabolism, and body size in Anopheles (Diptera: Culicidae), vectors of malaria. *J Med Entomol* 1990; 27: 839-50.

Brubaker JF, Turell MJ. Effect of environmental temperature on the susceptibility of *Culex pipiens* (Diptera: Culicidae) to Rift Valley fever virus. *J Med Entomol* 1998; 35: 918-21.

Canyon DV, Hii JL, Muller R. Adaptation of *Aedes aegypti* (Diptera: Culicidae) oviposition behavior in response to humidity and diet. *J Insect Physiol* 1999; 45: 959-64.

Clements AN. The biology of mosquitoes. Vol I. Development, nutrition and reproduction. *Am J Trop Med Hyg* 1992; 52: 579 pp.

Depinay JM, Mbogo CM, Killeen G, *et al*. A simulation model of African Anopheles ecology and population dynamics for the analysis of malaria transmission. *Malar J* 2004; 3: 29.

Farkas MJ, Brust RA. The effect of a larval diet supplement on development in the mosquito *Wyeomyia smithii (Coq.)* under field conditions. *Can J Zool* 1985; 63: 2110-3.

Gleiser RM, Urrutia J, Gorla DE. Body size variation of the floodwater mosquito *Aedes albifasciatus* in Central Argentina. *Med Vet Entomol* 2000; 14: 38-43.

Grech K, Maung LA, Read AF. The effect of parental rearing conditions on offspring life history in *Anopheles stephensi*. *Malar J* 2007; 6: 130.

Haramis LD. Increased adult size correlated with parity in *Aedes triseriatus*. *Mosq News* 1983; 43: 77-9.

Kirby MJ, Lindsay SW. Effect of temperature and inter-specific competition on the development and survival of *Anopheles gambiae* sensu stricto and *An. arabiensis* larvae. *Acta Trop* 2009; 109: 118-23.

Kitthawee S, Edman JD, Upatham ES. Relationship between female *Anopheles dirus* (Diptera: Culicidae) body size and parity in a biting population. *J Med Entomol* 1992;

29: 921-6.

Lanciani CA. Photoperiod and the relationship between wing length and body weight in *Anopheles quadrimaculatus*. *J Am Mosq Control Assoc* 1992; 8: 297-300.

Lardeux F, Cheffort J. Age-grading and growth of *Wuchereria bancrofti* (Filariidea: Onchocercidae) larvae by growth measurements and its use for estimating blood-meal intervals of its Polynesian vector *Aedes polynesiensis* (Diptera: Culicidae). *Int J Parasitol* 2002; 32: 705-16.

le Sueur D, Sharp BL. Temperature-dependent variation in *Anopheles merus* larval head capsule width and adult wing length: implications for anopheline taxonomy. *Med Vet Entomol* 1991; 5: 55-62.

Loetti MV, Burroni NE, Schweigmann N, de Garin A. Effect of different thermal conditions on the pre-imaginal biology of *Culex apicinus* (Philippi, 1865) (Diptera: Culicidae). *J Vector Ecol* 2007; 32: 106-11.

Menge DM, Guda T, Zhong D, *et al*. Fitness consequences of *Anopheles gambiae* population hybridization. *Malar J* 2005; 4: 44.

Mourya DT, Yadav P, Mishra AC. Effect of temperature stress on immature stages and susceptibility of *Aedes aegypti* mosquitoes to chikungunya virus. *Am J Trop Med Hyg* 2004; 70: 346-50.

Nasci RS. Relationship between adult mosquito (Diptera: Culicidae) body size and parity in field populations. *Environ Entomol* 1986a; 15: 874-6.

Nasci RS. The size of emerging and host-seeking *Aedes aegypti* and the relation of size to blood-feeding success in the field. *J Am Mosq Control Assoc* 1986b; 2: 61-2.

Nasci RS. Relationship of wing length to adult dry weight in several mosquito species (Diptera: Culicidae). *J Med Entomol* 1990; 27: 716-9.

Nasci RS, Berry RL, Restifo RA, Moore CG. Population size, parity structure, and wing length of *Coquillettidia perturbans* in an Ohio focus of eastern equine encephalitis. *J Am Mosq Control Assoc* 1996; 12: 64-8.

Nasci RS, Mitchell CJ. Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. *J Med Entomol* 1994; 31: 123-6.

O'Meara GF, Larson VL, Mook DH. Blood feeding and autogeny in the peridomestic mosquito *Aedes bahamensis* (Diptera: Culicidae). *J Med Entomol* 1993; 30: 378-83.

Oda T, Nuorteva P. Autumnal photoperiod and the development of follicles in *Culex pipiens pipiens* L. (Diptera, Culicidae) in Finland. *Ann Entomol Fennici* 1987; 53: 33-5.

Petrarca V, Sabatinelli G, Toure YT, Di Deco MA. Morphometric multivariate analysis of field samples of adult *Anopheles arabiensis* and *An. gambiae* s.s. (Diptera: Culicidae). *J Med Entomol* 1998; 35: 16-25.

Schneider JR, Mori A, Romero-Severson J, Chadee DD, Severson DW. Investigations of dengue-2 susceptibility and body size among *Aedes aegypti* populations. *Med Vet Entomol* 2007; 21: 370-6.

Siegel JP, Novak RJ, Ruesink WG. Relationship between wing length and dry weight of mosquitoes. *J Am Mosq Control Assoc* 1994; 10: 186-96.

Suwanchaichinda C, Paskewitz SM. Effects of larval nutrition, adult body size, and adult temperature on the ability of *Anopheles gambiae* (Diptera: Culicidae) to melanize sephadex beads. *J Med Entomol* 1998; 35: 157-61.

Willis FS, Nasci RS. *Aedes albopictus* (Diptera: Culicidae) population density and structure in southwest Louisiana. *J Med Entomol* 1994; 31: 594-9.

Yang CKO, Maria FLS, Wharton RW. Maintenance of a laboratory colony *Anopheles maculatus* Theobald by artificial mating. *Mosq News* 1963; 23: 34-5.