TEMPERATURE AND PHOTOPERIOD EFFECT ON DURATION OF GONOTROPHIC DEVELOPMENT AND FECUNDITY OF A LABORATORY COLONY OF *AEDES ALBOPICTUS*

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ABSTRACT

The impact of temperature and photoperiod on duration of gonotrophic development and fecundity in a Gainesville strain of *Aedes albopictus* Skuse were observed in laboratory settings. Photoperiodic regimens at 24L:0D, 14L:10D, 12L:12D, 8L:16D, and 0L:24D were tested on females reared at 25º C. A series of temperatures 15° C, 20° C, 25° C, 30° C, and 33º C were tested on females reared at 16L:8D. The gonotrophic development duration showed a significant difference only between 8L:16D and 0L:24D which had the longest and shortest cycles, respectively. Fecundity was highest at 14L:10D and lowest at 0L:24D without significant differences between different photoperiodic regimens. Both 1st and 2nd gonotrophic cycle durations differed significantly only between 15° C/ 20° C and 33º C which had the longest and shortest cycles, respectively. The highest temperature had the highest fecundity in the 1st gonotrophic cycle whereas it had the lowest fecundity in the 2nd cycle. The findings of this study would benefit in estimating field *Ae. albopictus* population for control and for rearing purposes.

Key Words: *Aedes albopictus*, fecundity, temperature, photoperiod, gonotrophic cycle

Aedes albopictus Skuse was first introduced into North America in the middle of 1980's. There are many studies regarding the impact of temperature and photoperiod on the survival, longevity, blood feeding, diapause, ovarian development, and fecundity in the mosquito, *Ae. albopictus* (Delatte et al. 2009, Li et al. 2015, Xue 2016). The temperature of the environment is one of the most important abiotic factors affecting the life cycle and spread of *Aedes* mosquitoes (Reinhold et al. 2018). Based on the recent continuing geographic spread of *Ae. albopictus* in Southern Europe (Lesto et al. 2022) and the importance and health risk considerations of the species as a vector of several pathogens, the major factors of temperatures and photoperiod on the gonotrophic development and fecundity in different strains are still needed to be addressed. Although the influences of temperature, photoperiod, and larval nutrition on fecundity (Zhong and He 1990) and egg diapause in *Ae. albopictus* (Pumpuni et al. 1992) have been documented, some results are unclear because the small observing number and different strains of mosquitoes were used in each experiment. A Gainesville strain of *Ae. albopictus* showed a diel pattern in pupation, emergence, biting, and oviposition in the laboratory (Xue and Barnard 1997). However, no data is available about the impact of temperature and photoperiod on the duration of gonotrophic development and fecundity in this strain of *Ae. albopictus*. Here we present the results of a study on effects of the temperature and photoperiod on duration of gonotrophic and the fecundity during each gonotrophic cycle in a laboratory colony of *Ae. albopictus*.

Ae. albopictus utilized in this experiment were colonized from larvae and pupae collected from the city of Gainesville, Florida in 1994 and the experiment was conducted at USDA, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, in 2000-2002. In the laboratory, stock colony adults were held in a screened cage (45 cm L X 38 cm W X 35 cm H) under a photoperiod of 14L:10D at 27ºC. Sugar water (3% sucrose impregnated cotton balls) was available to adults at all times. Blood was offered for stock cage, periodically, by placing about 5-week-old chicken, restrained by a stick tape, in the adult mosquito cage.

A total of five temperature and photoperiod-controlled chambers (Hotpack Corporation) were used in each study. The photoperiod regimens at 24L:0D, 14L:10D, 12L:12D, 16L:8D, and 0L:24D were selected for the experiment of photoperiodic impacts. Five hundred 4th instar larvae per white pan (30 cm L x 19 cm W x 5 cm H) with 1,000 mL well water (six pans/chamber) were exposed to five chambers with different photoperiod regimens. All chamber temperatures were set up at 25° C. The photoperiod regimens and temperatures were controlled by designed and adjustable program on each chamber. Relative humidity was kept between 60-70 % by

putting a water pan (30 cm L X 19 cm W X 5 cm H) in the chamber bottom, and the water was available to evaporate at all times. Daily relative humidity was read and recorded by a hydrate meter at each chamber. Pupae were collected and introduced into respective adult cages. Once emerged, 300 females and 300 males were collected and transferred to three new cages at 600 total mosquitoes per cage. The new cages were kept under the same photoperiod regimens. Sucrose solution (3%) was available all times in each cage. After 3-4 days of transferring, females were blood fed for 1 hr with a restained baby chicken in each cage. Blood engorged 250 females per cage (total 3 cages) were transferred to a new cage with sugar supply under the same photoperiod conditions for further observation on duration of gonotrophic development and fecundity of the 1st gonotrophic cycle. The gonotrophic development duration was measured from the fresh engorgement to oviposition. A piece of black filter paper (33 cm L X 8 cm W) was placed in 500 mL black plastic cups containing 200 mL of well water for egg depositing in cages. The oviposition was checked and counted by collecting and changing the filter paper hourly after 40-hours of blood meals. All egg papers were air dried and the eggs were counted under 20 X using a binocular microscope. The collection and replacement of egg papers were terminated at 5-days post-first oviposition as no further eggs were deposited on filter papers for 12 hours. The fecundity of female *Ae. albopictus* was determined as the eggs/female.

In another experiment, a series of temperatures of 15° C, 20° C, 25° C, 30° C, and 33º C, was set up for study on effects of temperature on the duration of gonotrophic development and fecundity of female mosquitoes. The photoperiod was 16L:8D for all temperatures. The larval rearing and blood feeding procedure were the same as mentioned above. A total of 450 blood engorged females were transferred to three new cages at 150 females/cage (45cm L x 38cm W x 35cm H) for each temperature treatment group. The gonotrophic development duration and fecundity in 1st and 2nd gonotrophic cycles were determined as described above.

All the data analyses were performed using IBM®SPSS® statistics (version 20). Data were first tested for normality and parametric and non-parametric tests were used accordingly. Table 1 shows the duration and fecundity of the 1st gonotrophic cycle of *Ae. albopictus* under all photoperiodic regimens tested. Kruskal Wallis test performed on data of photoperiod and gonotrophic development duration demonstrated an overall significant difference (χ^2= 12.456, df=4, P = 0.014). According to *post hoc* tests the difference was significant only between 8L:16D (longest cycle) and 0L:24D (shortest cycle) (P=0.017). The one-way ANOVA test showed that there was no significant difference in fecundity at any of the photoperiodic regimes ($F_{4,10}$ = 2.226, P = 0.139).

Table 2 shows the duration and fecundity of the 1st and 2nd gonotrophic cycles in *Ae. albopictus* under all temperatures tested. Kruskal Wallis test demonstrated overall significant differences in the duration of both 1st and 2nd gonotrophic cycles (χ^2 = 13.087, df=4, P = 0.01 and χ^2 = 13.665, df=3, P = 0.003, respectively). According to *post host* tests, the difference in the 1st cycle was only between the longest duration at 15° C and shortest duration at 33° C (P = 0.01), while in the 2nd cycle, the duration was significantly longer at 20° C than at 33° C (P=0.003) (15° C was not tested in the 2nd cycle). Fecundity in the 1st gonotrophic cycle was significantly impacted by temperature (ANOVA, $F_{4,10}$ = 50.802, P<0.001). *Post hoc* analysis showed that the fecundity at all temperature pairs except 15° C/20° C, 25° C/30° C, and 30° C/33º C were significantly different (P<0.05 for all). In the 2nd

Table 1. Effects of photoperiod on gonotrophic development duration and fecundity of *Aedes albopictus* (Gainesville Strain, FL) in the 1st gonotrophic cycle in the laboratory chambers (temperature at 25° C).

Photoperiod Regime (hour)	Number of mosquitos used	Gonotrophic development duration (hr., mean ±SD)**	Fecundity (eggs/female) (mean ±SD)*
24L:0D	120	75.0±0.0	55.9±24.6
14L:10D	150	71.6±2.1	60.3±10.4
12L:12D	120	73.4±0.0	53.0±13.2
08L:16D	90	97.7±0.58**	44.3±6.5
0L:24D	150	70.0±0.58**	29.0±10.0

L = Light, D = Dark.
* no significant difference in the fecundity. ** There is a significant difference in the gonotrophic development duration between all dark and 8L:16D photoperiods.

Table 2. Effects of temperature on gonotrophic development duration and fecundity of *Aedes albopictus* (Gainesville strain, FL.) in the 1st and 2nd gonotrophic cycles in the laboratory chambers. Photoperiod at 16L:8D for all temperatures.

(˚C)	Number of Mosquitoes used	Gonotrophic development duration (hr., mean ±SD)@	Fecundity (eggs/ female (mean ±SD)@@	Number of Mosquitoes used	Gonotrophic development duration (hr., mean ±SD)*	Fecundity (eggs/ female (mean ±SD)**
15	150	338.7±0.58@	40.3±3.9	-	-	-
20	150	121.3±0.58	47.3±5.5	70	120.0±0.0*	45.7±0.3
25	150	51.0±0.0	86.3±4.7	63	49.7±0.3	69.7±0.3
30	150	50.3±0.58	94.0±1.2	70	50.0±0.0	86.3±3.2**
33	150	45.0±0.0@	108.3±4.4	70	46.0±0.0*	35.3±3.0**

"-" no data due to no enough number of mosquitoes availabale.
1st gonotrophic cycle: @ There are significant differences in the duration of gonotrophic development). @@ There are significant differences in the fecundity (see the text for significantly different pairs)
2nd gonotrophic cycle: *There is a significant difference in the gonotrophic development duration between 20˚ C and 33˚C. ** There is a significant difference in the fecundity between the temperatures at 30˚ C and 33˚ C.

gonotrophic cycle, the temperature impact was significant (Kruskal Wallis, χ^2 = 12.94, df = 3, P=0.005) only between 30˚ C (highest) and 33º C (lowest) (*post hoc*, P=0.005).

Temperature influences many biological parameters (Delatte et al. 2009) of *Ae. albopictus*. Also, the temperature variations influence dengue and chikungunya transmission (Mercier et al. 2022) and susceptibility to insecticides (Salinas et al 2021). Observation of environmental temperature variation may benefit to population estimation based on variation in gonotrophic development duration and fecundity. A female *Ae. albopictus* laid 40-80 eggs per batch or during a single gonotrophic cycle in some Asian countries (Hawley 1988, Fu 1990, Zhong and He 1990). A strain of Guangzhou *Ae. albopictus* laid 229.9-403.33 eggs/female during their whole life in laboratory and the temperature affected the eggs/female (Fu 1990, Zhong and He 1990). Our results showed that the lower temperature decreased the number of eggs/ female in the strain of Gainesville *Ae. albopictus* during both 1st and 2nd gonotrophic cycles. When temperatures controlled at 21˚C, 26˚C, and 29˚C, clear photoperiodic responses of dormancy were demonstrated by 14 strains of *Ae. albopictus* (Pumpuni et al. 1992). Our results on the *Ae. albopictus* Gainesville strain differed from the above reports regarding the photoperiodic response of dormancy as we did not observe any significant variation with all photoperiodic regimens at 25˚C.

There are different definitions for gonotrophic development duration and gonotrophic cycle. Gonotrophic cycle is the interval between two successive oviposition, including the time from teneral period after emergence to the first host-seeking / blood feeding, and oviposition (Clements 1992). The most used term of the duration of gonotrophic development is from blood meal to oviposition only (Clements 1992). There are reports concerning effects of temperature and photoperiod on gonotrophic cycle and duration of gonotrophic development in other strains of *Ae. albopictus* mosquitoes (Hawley 1988, Fu 1990). The immature development, survival, longevity, fecundity, and gonotrophic cycles of *Ae. albopictus* were controlled at 5˚ C, 10˚ C, 15˚ C, 20˚ C, 25˚ C, 30˚ C, 35˚ C, and 40 ˚C, all parameters were influenced by the variations. Also, the gonotrophic cycles were shortest at 30˚C (Delatte et al. 2009). Our results show that temperatures 25˚C, 30˚C, and 33˚C shorten the gonotrophic development duration of *Ae. albopictus* Gainesville strain in the 1st cycle at the laboratory chambers. The results were similar with other reports (Li et al. 2015, Casas-Martinez et al. 2020).

The study concluded that the fecundity of the *Ae. albopictus* Gainesville strain was significantly affected by temperature variations favoring temperatures 25˚C to 33˚C, but not by photoperiod variations. However, the gonotrophic development duration was significantly affected by variations of both temperature and photoperiod with shorter duration at 25˚C to 33˚C and longer photoperiods up to 12L:12D. This information may benefit to mass rearing and further study on biology and population of *Aedes albopictus*.

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