SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Development, Longevity, Gonotrophic Cycle and Oviposition of *Aedes albopictus* Skuse (Diptera: Culicidae) under Cyclic Temperatures

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Desenvolvimento, Longevidade, Ciclo Gonadotrófico e Oviposição de *Aedes albopictus* Skuse (Diptera: Culicidae) sob Temperaturas Cíclicas

RESUMO - Os efeitos de temperaturas cíclicas no desenvolvimento, longevidade, ciclo gonadotrófico e número de ovos postos de *Aedes albopictus* Skuse foram analisados por meio de experimentos laboratoriais. Os experimentos foram conduzidos com mosquitos oriundos de Registro, SP e mantidos em insetário por dois anos. O desenvolvimento do inseto foi acompanhado desde o ovo até a emergência do adulto, a 25/18ºC e 27/20ºC e o adulto a 27/20ºC, ambos associados com fotoperíodo de 12h. Os ovos receberam dois tratamentos: (A) manutenção do volume da água; (B) troca periódica e completa da água. A alimentação sangüínea era oferecida diariamente aos mosquitos com interrupção quando o repasto era realizado, e retomada após a primeira oviposição. O desenvolvimento dos imaturos foi significativamente diferente sob os diferentes regimes de temperatura ($P < 0,05$), e a velocidade de desenvolvimento foi afetada positivamente pelo aumento da temperatura. A combinação do regime de temperaturas mais altas com a troca periódica e completa da água permitiu maior viabilidade dos ovos e encurtou o período de incubação. A longevidade dos adultos não foi diferente entre machos e fêmeas e a mortalidade foi regular ao passar do tempo. A comparação da longevidade do mosquito sob temperaturas constantes e cíclicas sugere que a menor temperatura do regime cíclico seja um fator limitante para a sobrevivência do mosquito. Esse fato pode ser um importante limitador da distribuição de *A. albopictus* por sugerir que o mosquito está restrito a ocupar áreas com temperaturas mínimas não muito inferiores a 20ºC.

PALAVRAS-CHAVE: Insecta, biologia, mosquito, vetor

ABSTRACT - The effects of cyclic temperatures on *Aedes albopictus* Skuse development, longevity, gonotrophic cycle and the number of oviposited eggs were assessed by means of laboratory experiments. The experiments were carried out with mosquitoes from Registro, São Paulo, Brazil, kept in insectary for two years. The development of the insect was followed from egg to adult emergence under 25/18ºC and 27/20ºC and adult stage under 27/20ºC, both associated with LD 12:12h. The eggs received two treatments: (A) maintenance of water volume; (B) periodical and complete change of water. Blood meal was offered daily and it was interrupted after haematophagy and restarted after first oviposition. The immature development was significantly different under the temperature regimes ($P < 0.05$) and the increased temperatures positively affected the development speed. The combination of higher temperatures regime and periodical and complete change of water increased the eggs viability and shortened the incubation time. Adult longevity was not different between males and females and the mortality was regular through the time. Comparing the mosquito longevity under constant and cyclic temperatures, it is suggested that the lowest temperature of the cyclic regime is a limiting factor for mosquito survival. This fact may limit the *A. albopictus* distribution range to areas where the minimal temperatures are not much bellow 20ºC.

KEY WORDS: Insecta, biology, vector, mosquito

Aedes albopictus Skuse, also known as "Asian-Tiger", reached the Neotropical region in the mid 80`s of the last century (Forattini 1986). Rai (1991) proposed three hypotheses for the mosquito entry in Brazil: first one suggests that *A. albopictus* came from North America through continental spread. A second one suggests that mosquitoes have came in imported used auto tires from Japan and the last one proposes that bamboo stumps traded with Asian countries have brought the mosquito to Brazil.

As other species of the *Stegomyia* group, *A. albopictus* has an important epidemiological role as an arboviruses vector (Mitchell *et al*, 1992, Savage *et al.* 1994). Although *A. albopictus* larvae naturally infected with dengue virus types 2 and 3 were found in Reynosa, Mexico (Ibañez-Bernal *et al.* 1997) and larvae naturally infected by dengue virus type 1 were found in State of Minas Gerais, Brazil (SERUFO *et al.* 1993), *A. albopictus* still has not been hold responsible for dengue infestations in the Americas.

The mosquito has a narrow interspecific relationship with *Aedes* (*Stegomyia*) *aegypti* L. Nasci *et al.* (1989) observed insemination by *A. albopictus* males on *A. aegypti* female and Forattini (1998) alerted that *A. albopictus* is able to replace *A. aegypti* ecological niche at any moment.

The mosquito has an eclectic preference by breeders such as containers made of metal, glass, stone, earthenware, plastic, wood or rubber and tree holes, bamboo stumps, rock pools, leaf axils (Hawley 1988).

Experiments with *A. albopictus* in laboratory under constant temperatures have shown that higher temperatures and precipitation directly affected mosquito growth (Alto & Juliano 2001a, 2001b). Eggs are very sensitively affected by temperature and dissecation. The diapause phenomenon can be observed in eggs submitted to lower temperatures in laboratory and in the field (Mori *et al.* 1981, Focks *et al.* 1994, Hanson & Craig Jr. 1995). Developmental time from hatching to pupation is negatively related with temperature (Briegel & Timmermann 2001) and female longevity, fecundity and haematophagic activity are directly affected by increased temperatures (Calado & Navarro-Silva 2002).

Joshi (1996) has reported the effects of cyclic and constant temperatures on *Aedes krombeini* adults. The cyclic and constant temperatures induced different mosquito responses. *A. krombeini* development time was shorter and longevity was longer under cyclic than under respective average constant temperatures.

Experiments about *A. albopictus* biology have been made under constant temperatures and there is little information on the impact of cyclic conditions of temperature on life cycle of the "Asian-Tiger". Besides that, the new conditions *A. albopictus* is subject in Neotropical region propel deeper studies about this vector and the abiotic factors that affect its survivorship and reproduction.

In the current manuscript, the experiments were performed aiming to describe the possible effects of cyclic temperatures on *A. albopictus* life cycle. Egg viability and incubation period, larva and pupa development period and adult longevity, pre-blood meal period, gonotrophic cycle and fecundity were observed under cyclic temperatures.

Material and Methods

Mosquitoes Source. *A. albopictus* used in this study were obtained from a laboratory colony established for two years from field collected mosquitoes. The mosquitoes were captured in artificial containers in Registro, São Paulo, Brazil (24º20'S and 47º51'W) and kept in insectary. The insectary was maintained under approximately 25ºC, 60% relative air humidity and natural photoperiod. Human blood meals were offered to mosquitoes for the colony maintenance.

Experiment Conditions. Two environmental chambers were used in the experiments. One programmed for 12h dark under 18ºC ± 0.2ºC and 12h light under 25ºC ± 0.2ºC and a second chamber programmed for 12h dark under 20ºC ± 0.2ºC and 12h light under 27ºC ± 0.2ºC. The changes of temperatures took 12 min. to stabilize. In these experiments the relative air humidity was not controlled, but a daily monitoring frequency indicated that the relative air humidity was 75-85% in the light phase (photophase) and 90-99% in the dark phase (scotophase). Water dishes were placed inside the chambers to raise the relative air humidity since the beginning of the experiments. The cyclic temperature simulated the rising of the temperature during the day (thermophase) and the lowering of the temperature during the night (cryophase). It was related with photoperiod in a geographic region nearby latitude 0º where the light and the dark phases are about 12h.

Experiment Protocol. The egg viability and incubation period experiments were performed using 100 eggs for each chamber displayed in ten white plastic containers of 120 ml filled with aired water and 0,002g of fish food (TetraMin®). The fish food was added into the egg containers because, as organic matter in natural breeders and mouse pellets in laboratory, it stimulates the hatching process (Hawley 1988). Half of the egg containers received treatment A, maintenance of water volume, and the other half, received treatment B, 2-3 days periodical and complete change of water. These treatments were design to simulate precipitation renewal of water. Treatment A allowed a low renewal regime and treatment B a high one. After egg hatching the larva was immediately isolated in small container of 50 ml filled with aired water plus 0,005 g of fish food. The instar periods were verified by the appearance of the exuviae. Adults for the experiment were acquired from immature forms subjected to 27/20ºC. After emergence, adults were arranged in couples inside a cylindrical PVC recipient (7.5-cm diameter and 10 cm height) with a lateral hole where sugar solution (10%) was offered. Human blood was daily offered until the day haematophagy was performed and restarted after the first oviposition. The pre-blood meal period was considered as the period from adult emergence until haematophagy and the successive pre-blood meal period as the period from oviposition until next heamatophagy. For the oviposition, black recipients (3 cm diameter and 3 cm height) with cotton and filter paper were available and flooded when females had blood meal.

Procedure, Data Collection and Analyses. Every day, the egg containers were checked for early instar larvae. The treatments A and B were performed on the randomly pre-chosen containers. Early hatched larvae were transferred into the 50ml white container and the larval and pupal exuviae were searched and removed daily. The data were analyzed by using parametric statistic, ANOVA. Adults' data were analyzed by means of ANOVA for longevity and Chi-square test for survivorship linearity.

Results and Discussion

Egg Viability and Incubation Period. Under cyclic regimes, the temperatures affected the egg viability in different ways. The regime of lower temperatures allowed 26% and 22% of the eggs to develop, from treatments A and B respectively. However, under 27/20ºC eggs were highly viable and for both temperature regimes the treatments were different (Table 1). Treatment A, maintenance of water volume, low water renewal regime, simulated a static and undisturbed environment. This condition promoted egg hatching for a longer period (Table 1). On the other hand, treatment B, periodically and complete change of water, high renew regime, simulated conditions of an unstable microhabitat and an accelerated development was observed. This is probably a reproductive strategy to promote survival in disturbed microhabitats, as seen in Alto & Juliano's (2001b) precipitation experiment.

Immature Post-Hatch. The larvae and pupae response to the temperature increase was a higher developmental rate. Duration of all immature stages reared at 25/18ºC significantly differed from those reared at 27/20ºC. Fourth instar and pupa stage were the longest period among the immature post-hatch stages as seen in the field (Gomes *et al.* 1995) and in laboratory, under constant temperatures (Calado & Silva 2002). Joshi (1996) showed that the immature mean period for the males were shorter than for the females, not only under constant temperatures but also under cyclic temperatures, as observed for immature forms under 25/18ºC and 27/20ºC (Fig. 1).

Adult Longevity and Survival. The cyclic regime of 27/20ºC allowed the development of *A. albopictus* adults. Longevity was not different between males and females (Table 2). However, Joshi (1996) found that females lived longer than males and Calado & Navarro-Silva (2002) observed females living 1.5 time longer than males under constant temperatures. Also, mean longevity was similar between male mosquitoes under 20ºC (Calado & Navarro-Silva 2002) and under cyclic 27/20ºC. This fact suggests that the lower temperature of a cyclic regime is a limiting factor in mosquito development. The survival frequency was linear with a constant mortality rate through the time ($\chi^2_{14, (0.05), males} = 1.726$ and $\chi^2_{14, (0.05), females} = 2.969$). This slope pattern suggests that mosquito age did not affect the survival frequency (Fig. 2).

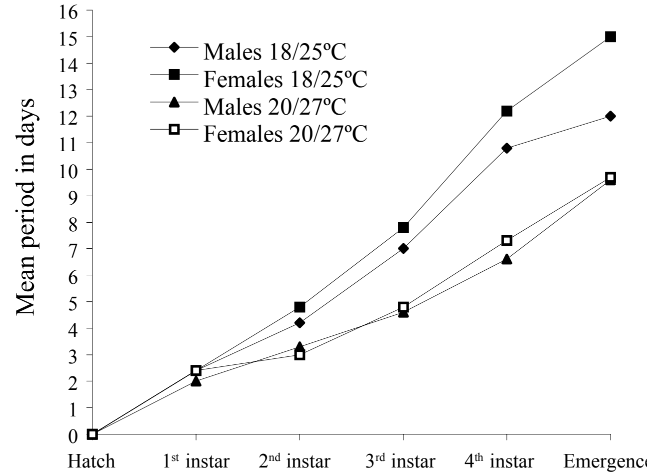

Figure 1. *A. albopictus* male and female immature periods (days) since hatch until adult emergence, under cyclic temperatures and 12h photoperiod.

Pre-Blood Meal Period. Pre-blood mean period under 27/20ºC was 8.0 (S.D.= 0.83) days and it was longer than in experiments with constant temperatures between 24-29ºC (Gubler & Bhattacharya 1971, Mori & Wada 1977, Hawley 1988). Under constant temperatures, 15, 20, 25 and 30ºC, the frequency of haematophagic activity was directly affected by the increase of temperatures. The highest frequency of haematophagic activity was observed under 25ºC and the lowest, under 15ºC and it is believed that the higher temperatures decrease the buccal esclerotinization period (Calado & Navarro-Silva 2002).

Gonotrophic Cycle. The first gonotrophic cycle under 27/20ºC was 11.2 (S.D.= 6.9) days and the second cycle was 7.4 (S.D.= 3.0) days (Table 2). Mori & Wada (1977), using capture-recapture method, observed that *A. albopictus* gonotrophic cycle was five days under 25ºC, LD 16:8h in the field. Furthermore, Briegel & Timmermann (2001) verified that with increased temperatures all the gonotrophic cycles became faster.

Oviposition. *A. albopictus* needed no more than one blood meal for oviposition activity, as seen by other authors (Mori

Table 1. *A. albopictus* egg incubation period (days), viability (%) and immature development period (days) under cyclic temperatures and 12h photoperiod.

	Eggs[1]						Immature[2]	
	Treatment A			Treatment B				
	Mean	SD[3]	Viab.	Mean	SD	Viab.	Mean	SD
25/18ºC	17.4 a	17.8	26%	8.1 b	2.3	22%	13.7 c	2.3
27/20ºC	16.8 a	14.3	52%	7.1 b	3.0	94%	9.60 d	1.3

[1]Anova between treatments of the same regimen and between regimes of the same treatment
[2]Anova between temperature regimes; [3]Standard Deviation
ANOVA at a 5% level of significance

Table 2. Longevity, pre-blood meal period, gonotrophic period in days and number of eggs of *A. albopictus* under 27/20ºC, 12h photoperiod and 75-99% R.H.

	Mean	SD[1]	Min-max
Mosquito longevity	35.0	20.3	4 - 72 days
Males longevity	35.3 b	22.9	4 - 72 days
Females longevity	35.9 b	18.5	10 - 72 days
Pre-blood meal period	8.0	3.9	2 - 18 days
From blood meal until oviposition	11.2	6.9	3 - 29 days
Number of eggs from 1st oviposition until next blood meal	33.1 c	21.6	6 - 71 eggs
From 1st oviposition until 2nd blood meal	10.0	6.8	0 - 18 days
From 2nd blood meal until oviposition	7.4	3.0	4 - 11 days
Number of eggs from 2nd oviposition period	38.7 a	21.5	9 - 59 eggs

[1]Standard Deviation
ANOVA at a 5% level of significance

& Wada 1977) (Klowden & Briegel 1994). Also the females were able to oviposit more than once (Hawley 1988). This behavior suggests that female seek more than one breeder for oviposition. This proliferation strategy was observed by Rozeboom *et al.* (1973) when breeders in field were found containing eggs from different females. The number of eggs produced in successive batches in the different gonotrophic cycles varied at random (Table 2). Gubler & Bhattacharya (1971) have also detected this fact in experiments with *A. albopictus* under 26ºC.

Higher temperatures allow *A. albopictus* to grow faster and reach the adult stage earlier. The combination of higher temperatures and water renewal increased egg viability and shortened the incubation period. Larvae and pupae development also became quicker under higher temperatures. Adult longevity was very similar under cyclic temperatures and constant temperature of 20ºC, and this suggests that the lowest temperature of the cyclic regime is a limiting factor for survival and distribution. This fact may determinate the probable areas where *A. albopictus* can inhabit. Male and females longevity did not differ under cyclic temperature condition of 27/20ºC. The mortality rate of adults was constant through the time. The pre-blood mean period was longer than in other experiments (Gubler & Bhattacharya 1971, Mori & Wada 1977, Hawley 1988), probably due to abrupt changes in temperature that affected the buccal esclerotinization time. It is believed that the changes of temperature have affected the reproduction activity by elongating the gonotrophic cycles. Many ovipositions with only one blood meal suggest a strategic proliferation behavior under disturbed conditions.

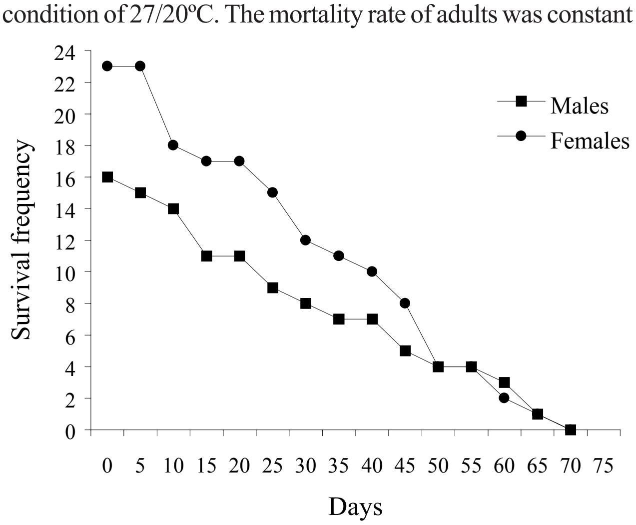

Figure 2. Survival frequency of *A. albopictus* adults under 27/20ºC, 12h photoperiod, and 75-99% R.H.

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