Temperature Influence on Embryonic Development of *Anopheles albitarsis* and *Anopheles aquasalis*

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Temperature influence on the embryonic development of Anopheles aquasalis *and* An. albitarsis *was investigated. At 26ºC, 75% and 60% of respectively* An. aquasalis *and* An. albitarsis *eggs hatched, with one peak of eclosion, between the 2nd and 3rd day after oviposition. At 20 ± 2ºC, around 66-70% of* An. aquasalis *eggs hatched, with one eclosion peak, on the 5th day. On the other hand,* An. albitarsis *eclosion at 21 ± 2ºC decreased to 10-22%, with two eclosion peaks, on the 4th-5th day and on the 9th-12th day. These data indicate a stronger temperature influence over* An. albitarsis *than over* An. aquasalis *embryos.*

Key words: *Anopheles albitarsis* - *Anopheles aquasalis* - *Anopheles* rearing - malaria vector - temperature influence - embryonic development

Nowadays, despite control efforts, malaria affects around 400 million persons each year. This disease is typical in tropical and subtropical regions, where the elevated relative humidity and temperature, together with poor social and economic conditions, offer the ideal environment for the development and maintenance of its vectors (Butler et al. 1997, Morel 2000).

In Brazil, malaria is one of the major endemic diseases, the Amazon region being responsible for more than 99% of the cases (Passos & Fialho 1998). In this country, *Anopheles* (*Nyssorhynchus*) *darlingi* Root, 1926 and *Anopheles* (*Nyssorhynchus*) *aquasalis* Curry, 1932 are primary vectors. *Anopheles* (*Nyssorhynchus*) *albitarsis* Lynch-Arribálzaga, 1878, a complex of at least four species, is considered a secondary malaria vector (Rosa-Freitas et al. 1990, Consoli & Lourenço-de-Oliveira 1994, Wilkerson et al. 1995).

Although *An. albitarsis* and *An. aquasalis* have been reared in our laboratory as free-mating colonies since 1995, unexpected mortality events and mosquito density fluctuations have been hampering their use as laboratory models. Our present aim is to investigate some basic aspects concerning their biology, in order to optimize *Anopheles* colony maintenance.

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Our previous empirical observations suggested that temperature can greatly influence the developmental kinetics and the longevity of these vectors. Accordingly, there are several reports dealing with temperature influence over the developmental kinetics of larvae and adults of *Aedes aegypti* (Rueda et al. 1990, Tun-Lin et al. 2000), *Culex* spp. (Rae 1990, Rueda et al. 1990, Reisen 1995) and *An. sergentii* (Beier et al. 1987). Influence of temperature on mosquito vectorial competence (Kay et al. 1989), induced cross-tolerance between temperature and an insecticide (Patil et al. 1996) and temperature effect on phenotypic characteristics – and its implication to taxonomy (Le Sueur & Sharp 1991) have also been noted. However, there are few reports dealing specifically with temperature influence over mosquito embryogenesis (Trpiš et al. 1973, Rayah & Groun 1983, Van der Linde et al. 1990).

As a first approach to optimize the maintenance of our colonies, we decided to specifically investigate *An. albitarsis* and *An. aquasalis* embryonic development under two different temperatures.

MATERIALS AND METHODS

Mosquitoes - Free mating colonies of both *An. albitarsis s. s.* and *An. aquasalis*, maintained in the laboratory since 1995, were used. Rearing conditions were 26ºC ± 1ºC and 80% r.h. as described elsewhere (Horosko et al. 1997). Larvae kept in dechlorinated (*An. albitarsis*) or 10% seawater (*An. aquasalis*) were fed with powdered fish food (Tetramin®) twice a day. Adults had continuous access to a 10% sucrose solution, and females were fed on anesthesized guinea pigs in order to produce eggs.

Synchronous egglaying - Since neotropical *Anopheles* females lay eggs preferentially at dawn, an insectary was adapted with a 12 h dark:12 h light cycle (lights turned off from 9:00 a.m to 9:00 pm and turned on from 9:00 pm to 9:00 a.m of the following day). This procedure enabled the collection of synchronized eggs (periods of 1 h) during the day.

Cold anesthesized females were transferred to Petri dishes (6 cm diameter) covered internally with a filter paper. After recovery of the females at 26ºC, the filter paper was wetted with 600 µl of dechlorinated (*An. albitarsis*) or brackish (*An. aquasalis*) water to induce oviposition (Valencia et al. 1996).

Egg hatching monitoring - Oviposition was performed in the insectary at 26ºC and the resulting eggs were either kept in the insectary (control group) or placed in an incubator, at 22ºC (experimental group). Twenty-four hours later the eggs were split in aliquots. A total of 935 and 479 *An. albitarsis* eggs were used in each experimental group divided in, respectively, 15 and 9 replicates, ranging from 48 to 70 eggs. Control *An. albitarsis* group consisted of 160 eggs raised in pool. Each *An. aquasalis* experimental group was divided in seven replicates ranging from 48 to 92, in a total of 544 and 350 eggs, respectively. Control *An. aquasalis* group consisted of 10 replicates of 100 eggs. Hatching was scored daily at each temperature as well as maximal and minimal temperatures in both the insectary and in the incubator. The maximal temperature in the experimental condition did not exceed 22ºC or 23ºC, in the case of *An. aquasalis* and *An. albitarsis* respectively. The minimal temperature for *An. albitarsis* experiments was 20ºC while for *An. aquasalis* it attained 17ºC.

RESULTS

An. aquasalis - Two trials at 20 ± 2ºC have been performed, with a total of 894 eggs. Hatching was monitored up to 10 days after egglaying. Results were similar in both experiments: control eggs, maintained at 26ºC, hatched mainly on the 2nd day after egglaying (Fig. 1A). Total eclosion rate was 75% for the control group. On the other hand, 66-70% of eggs from the experimental groups hatched and the eclosion peak was attained at the 5th day (Fig. 1B). No eclosion was observed from the 7th day after egglaying on.

An. albitarsis - Two trials at 21 ± 2ºC were also performed with this species, with a total of 1,414 eggs. Hatching was monitored up to 20 days after egglaying. Again in this case the results obtained were similar for both experiments. In the control condition (26ºC) the majority of the eggs hatched between the 2nd and the 3rd day and total hatching was 60%. In the experimental groups, however, total eclosion did not exceed 22% (Fig. 2A). In this situation, when daily percent of hatching eggs was plotted against time, two eclosion peaks were noted, the first at the 4th-5th day after oviposition and the second after the 8th day (Fig. 2B). In both trials a period of at least 24 h with no eclosion event at all was observed, around the 6th-7th day. It was also realized that 60-70% of *An. albitarsis* eggs hatched before this period of no eclosion while 30-40% eclosion was obtained after this period. No eclosion was observed from the 17th day after egglaying.

DISCUSSION

Studies regarding temperature influence over the embryonic development of Culicidae can help laboratory rearing. On the other hand, the precise knowledge of mosquito embryonic developmental kinetics would assist the

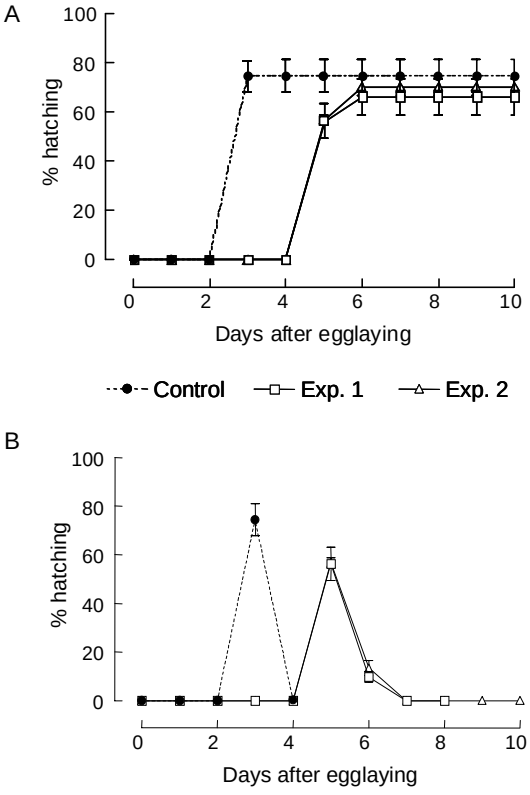

Fig. 1: eclosion rates of *Anopheles aquasalis* embryos at 26ºC (closed circles) or at 20 ± 2ºC (open symbols). A: cumulative eclosion rates; B: daily eclosion rates. Data are represented as means ± standard deviation. Note the presence of a single eclosion peak in all cases.

improvement of transgenic production protocols: generation of stable transformed lineages depends on the injection of exogenous DNA in a precise stage during embryogenesis (Catteruccia et al. 2000), which, in turn, varies greatly with temperature (Clements 1992). However, in spite of its potential importance, little has been done concerning this subject. The great majority of data related to temperature influence over Culicidae development deals mainly with larvae and adults (Rueda et al. 1990, Rae 1990, Tun-Lin et al. 2000).

Analysis of temperature influence over *Aedes sticticus* embryonic development revealed that the minimal and maximal temperature thresholds are, respectively, 6-8°C and 33°C. It was also verified that the time span of embryogenesis is inversely related to temperature: first instar larvae eclosion took 11.3 days (272 h) at 15ºC and only 6 days (120 h) at 30ºC. The authors did not investigate the rate of eggs' viability under different temperatures (Trpiš et al. 1973).

Cx. quinquefasciatus minimal and maximal temperature thresholds for embryonic development are 13ºC and 39ºC, respectively. In this species the hatching rate varies directly with temperature, up to 32ºC, after which eclosion rates drop gradually (Rayah & Groun 1983).

An. sergentii embryos kept at 34ºC do not hatch. Nevertheless, their viability at 17°C and 27ºC is equivalent

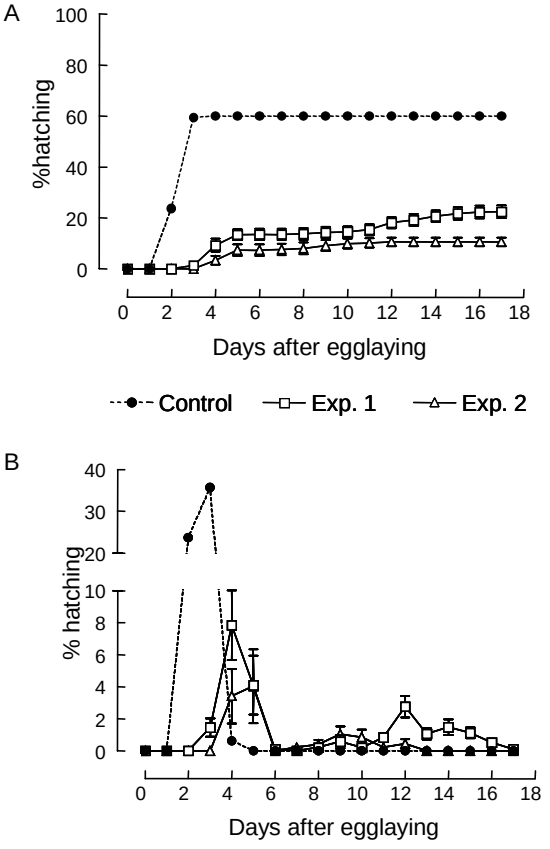

Fig. 2: eclosion rates of *Anopheles albitarsis* embryos at 26ºC (closed circles) or at 21 ± 2ºC (open symbols). A: cumulative eclosion rates; B: daily eclosion rates. Data are represented as means ± standard deviation. Note the presence of two eclosion peaks at the lower temperature.

(around 85%) although in this case, as temperature increases, the duration of embryogenesis is reduced: at 27ºC, 95% of hatching is obtained on the second day, while eggs kept at 17ºC only hatch on the 4th-5th day after egglaying (Beier et al. 1987).

We analyzed the time spent to the completion of embryonic development and the eclosion rates of two neotropical *Anopheles* species under two different temperatures. In both cases, our data point to important differences between *An. aquasalis* and *An. albitarsis*, although both species belong to the same subgenus. Similar to other Culicidae, the duration of embryogenesis varies inversely with temperature for both *Anopheles* species tested. However, a high decrease in viability was only observed for *An. albitarsis*. This is particularly significant if it is taken into account that temperature varied from 20-23ºC for *An. albitarsis* and only 17-22ºC for *An. aquasalis* (Table).

These data indicate that temperature can influence development in a differential manner even when related species are considered. *An. albitarsis* was shown to be much more susceptible to lower temperature than *An. aquasalis*, in terms of both kinetics and rate of eclosion. Additionally, the appearance of two eclosion peaks in *An. albitarsis* at the lower temperature suggests developmental variability in individuals of this species related to the temperature.

TABLE

Eclosion rates of *Anopheles aquasalis* and *An. albitarsis* larvae at different temperatures

		Temperature	% eclosion [a]
An. aquasalis	Control	26ºC	74.7 ± 20.8 (a)
	Experiment 1	20 ± 2ºC	66.1 ± 18.9 (a)
	Experiment 2	20 ± 2ºC	70.3 ± 8.8 (a)
An. albitarsis	Control [b]	26ºC	60 (a)
	Experiment 1	21 ± 2ºC	22.5 ± 9.8 (b)
	Experiment 2	21 ± 2ºC	10.7 ± 5.2 (b)

a: numbers followed by the same letter are not significantly different, according to one-factor analysis of variance ($P < 0.05$, ANOVA/Newman-Keuls multiple comparison test); *b*: *An. albitarsis* control eggs were raised in pool (n = 160 eggs).

We are now analyzing the effect of a broader range of temperatures over the embryonic development of these species. Additionally, the recent description of *An. albitarsis* embryogenesis (Monnerat et al. 2002) will be taken into account to investigate whether temperature influence is exerted preferentially over any given developmental stage or if, alternatively, embryogenesis is affected as a whole.

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