Oogenic development and gonotrophic cycle of *Aedes aegypti* and *Aedes albopictus* in laboratory

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Abstract

Objective. To determine the time of oogenic development and the length of the gonotrophic cycle of *Ae. aegypti* and *Ae. albopictus* in laboratory. **Materials and methods.** Bloodfed females of *Ae. aegypti* and *Ae. albopictus* were dissected every 4 h to determine the development status of the follicles according to the Christophers' stages. **Results.** The minimum time of oocyte maturation in *Ae. aegypti* and *Ae. albopictus* was 64-82 h and 52-64 h post-feeding, respectively. We found that the gonotrophic cycle of *Ae. aegypti* (3.7-4.2 d) is longer than that of *Ae. albopictus* (3.2-3.7 d). The follicle length showed significant differences between species at Christophers' stages 2" and 5, whereas follicle amplitude was different between the two mosquitoes at stages 2", 3 and 4. **Conclusions.** The study provided new evidence on the reproductive strategies of *Ae. aegypti* and *Ae. albopictus* females that coexist in the Neotropical region of Mexico.

Keywords: oogenic development; gonotrophic cycle; *Aedes*; vectors; dengue

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Resumen

Objetivo. Determinar el tiempo de desarrollo oogénico y del ciclo gonotrófico de *Aedes aegypti* y *Aedes albopictus* en laboratorio. **Material y métodos.** Hembras de *Ae. aegypti* y *Ae. albopictus* alimentadas con sangre fueron disecadas cada cuatro horas para determinar el estado de desarrollo folicular, según los estadios de Christophers. **Resultados.** El tiempo mínimo de maduración del oocito en *Ae. aegypti* y *Ae. albopictus* fue de 64-82 h y 52-64 h post-alimentación, respectivamente. El ciclo gonotrófico de *Ae. aegypti* (3.7-4.2 d) fue mayor que el de *Ae. albopictus* (3.2-3.7 d). La longitud folicular presentó diferencias significativas entre las especies en los estadios de Christophers 2" y 5, mientras que la amplitud folicular fue diferente entre ambos mosquitos en los estadios 2", 3 y 4. **Conclusiones.** El estudio proporcionó nueva evidencia sobre la estrategia reproductiva de las hembras de *Ae. aegypti* y *Ae. albopictus* que coexisten en la región neotropical de México.

Palabras clave: desarrollo oogénico; ciclo gonotrófico; *Aedes;* vectores; dengue

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The dengue fever is a disease caused by any one of the four related viruses distributed in more than 100 countries. Recently, the emergence and reemergence of other arboviruses such as Chikungunya and Zika have worsened the global epidemiological situation, mainly in the tropical and subtropical regions of the planet.[1,2] These diseases are transmitted by mosquitoes, mainly by two species of the *Stegomyia* group: *Ae. aegypti* (Linnaeus, 1762) and *Ae. albopictus* (Skuse, 1894).[3,4] Several studies have shown some differences between ecological aspects of *Ae. aegypti* and *Ae. albopictus*. For example, the former predominates in highly urbanized and suburban areas, whereas the latter is found in periurban and rural areas.[5,6] Additionally, the former species depends mainly on human blood and tends to bite and rest indoors,[7] whereas the latter feeds on a variety of vertebrate hosts outdoors.[8] These ecological differences have been observed as well in local populations of *Ae. aegypti* and *Ae. albopictus* in villages located along the coastal plain of Chiapas, where the presence of both species was reported since 2002.[9,10] From the perspective of human health, the gonotrophic cycle is one of the most important physiological processes in the life of the mosquito and an essential epidemiological component of the vectorial capacity model. This biological aspect is significant and decisive for the population dynamics of *Ae. aegypti* and *Ae. albopictus* in urban, suburban and rural ecological scenarios with outbreaks of endemic dengue and other arboviral diseases. Although there is information regarding the duration of the reproductive cycle of both species in several states of Mexico, few studies have generated information on the reproduction of both species occurring in the same geographical region and sharing identical climatic conditions and resources. Therefore, it is necessary to investigate whether these ecological differences induce changes in the length of gonotrophic cycle in both species. Several laboratory and field studies on their reproductive capacity have been reported separately for each one.[11,12] The objective of this study was to determine the length of the gonotrophic cycles of two day-biting mosquitoes, *Ae. aegypti* and *Ae. albopictus*. An understanding of reproductive aspects of the natural populations of both species is valuable for assessing and predicting their roles in the ecological dynamics and transmission of arboviruses.[13]

Materials and methods

Collection of mosquitoes and rearing process. Females of *Ae. aegypti* and *Ae. albopictus* were collected with entomological nets and mouth aspirators in houses as well as in cemeteries of Tapachula (14° 54′ 29″ N, 92° 15′ 38″ W and 177 masl), Chiapas, Mexico. The collections were performed from July to October 2010, during the rainy season. All collections were performed between 08:00 and 12:00 h. The captured mosquitoes were transported to the laboratory, where they were identified at species level based on their morphological characteristics.[14] The mosquitoes were separated by species and kept in 45 cm^3 metal cages covered with mesh gauze under laboratory conditions of 26.0±2.0oC, 75.0±5.0% RH, and 12:12 light/dark photoperiod. The field-collected females were fed with rabbit blood and submitted to a synchronized oviposition every three and four days throughout 10 gonotrophic cycles in order to obtain enough eggs of the same generation for the experiments. All *Ae. aegypti* and *Ae. albopictus* eggs were maintained on moist filter paper during three days to ensure the development of the embryos.[15] The eggs were placed in white plastic containers (30 x 40 x 10 cm) with 2.5 l of filtered water at 30°C to induce egg hatching. Immature stages were fed with a powdered rodent diet (raw protein 23%, raw fat 4.5%, raw fiber 6%, ash 8%, and 12% humidity). The pupae were placed in round plastic containers (5.5 cm high x 10.5 cm diameter) with 300 ml of water; the containers were introduced into 45 cm^3 metal cages until the adult emerged. All newly emerged mosquitoes were fed with a 10% sucrose solution during three days before the experiments under constant conditions of temperature and RH.

Vitellogenesis. Christophers' stages and duration of the oogenic development were determined for *Ae. aegypti* and *Ae. albopictus* based on the appearance of the follicles.[16] Thirty unfed females (without traces of blood) of each species were dissected to determine the maturation status of their eggs. The remaining females were allowed to feed on a rabbit. Mosquitoes were supplied with cotton pads soaked with a 10% sucrose solution and maintained in the insectary at 26.5±1.1ºC, 81.3±7.0% RH, and with a 12:12 h photoperiod. Starting at 4 h after bloodfeeding and continuing every 4 h up to 75 h, groups of 30 mosquitoes were dissected to determine their Christophers' stages. Females that did not develop beyond Christophers' stages 2 or 2′ 18 h after feeding were reported as pre-gravid.[17]

Morphometry of the follicles of Ae. aegypti *and* Ae. albopictus. In order to document the maturation process of eggs, we photographed 10 follicles per female to compare the appearance of the yolk sac, oocyte and nurse cells, and the follicular size through time, according the scheme developed by Christophers.[18] The size of the follicles was measured using an ocular micrometer (model Tokyo P7X) mounted in a stereo microscope (model SMZ645). The follicles were observed at 10 and 40 X. The length and width of the follicles, grouped by Christophers' stages and species, were analyzed with a one-way variance analysis. Significantly different treatments were identified using a Tukey test. The follicle size for each Christophers' stage was compared between *Ae. aegypti* y *Ae. Albopictus* using a Student's t-test.

Results

Vitellogenesis. All unfed females of both species dissected at the beginning of the experiment were at Christophers' stages 1 and 2. The *Ae. aegypti* females required 64-82 h (2.7-3.4 d) after feeding to reach Christophers' stage 5, whereas in *Ae. albopictus* females the progression to Christophers' stage 5 occurred at 52-64 h (2.2-2.7 d) after feeding (table I).

Gonotrophic cycle. The length of the gonotrophic cycle could be calculated indirectly, by adding 24 h, which is considered the required time to locate an oviposition site, lay eggs, and seek a new host, to the minimum time required by the eggs to attain Christophers' stage 5.[19] When this criterion was used in our data, the estimation of the length of the gonotrophic cycle was 88-106 h (3.7-4.2 d) for

Table I

Comparison between the oogenic developments of *Ae. aegypti* and *Ae. albopictus* females under laboratory conditions (26.5±1.1°C and 81.3±7.0% relative humidity). Tapachula, Chiapas, México, 2010

Ae. aegypti										*Ae. albopictus*									
Hours post-feeding	No. dissected	Christophers' stages (%)								Hours post-feeding	No. dissected	Christophers' stages (%)							
		1	2	2'	2"	3	4	4-5	5			1	2	2'	2"	3	4	4-5	5
0	30	90	10							0	30	30	57	13					
4	30	33	67							4	30	3	30	54	13				
8	30	7	73	20						8	30		3	57	40				
12	30		47	50	3					12	30			17	83				
16	30		17	66	17					16	30				20	80			
20	30			30	57	13				20	30				3	97			
24	30		7*		60*	33				24	30				3*	97			
28	30		3*	3*	24*	70				28	30					43	57		
32	30		3*		10*	64	23			32	30					20	80		
36	30					60	40			36	30					7	90	3	
40	30			3*		33	64			40	30						80	20	
44	30				3*	7	87	3		44	30						60	40	
48	30						83	17		48	30						13	87	
52	30						77	23		52	30						7	43	50
56	30						47	53		56	30							10	90
60	30					3	24	73		60	30							3	97
64	30						3	54	43	64	30								100
68	30							37	63										
72	30							10	90										
76	30							3	97										
80	30								100										

* Pregravid females

Ae. aegypti and 76-88 h (3.2-3.7 d) for *Ae. albopictus*. Ninety percent (135/150) of those *Aedes aegypti* females that were maintained up to 80 h to complete vitellogenesis developed to Christophers’ 5, whereas only 84.1% (101/120) of the *Ae. albopictus* females completed vitellogenesis. We observed that 23.3% (35/150) of the *Ae. aegypti* females did not develop beyond Christophers’ stage 2 or 2”, *i.e.*, remained pre-gravid, compared to a mere 3.3% (1/30) of *Ae. albopictus* females (table I).

Morphometry of the follicles of Ae. aegypti *and* Ae. albopictus. We observed that the follicles had a semispherical shape throughout the development of the oocyte of both species (figure 1).

Their length and width increased gradually between Christophers’ stages 1 and 3. From Christophers’ stage 4, the follicles of both species grew in length until they reached Christophers’ stage 4-5 (figure 2A and 2B).

Discussion

Aedes aegypti and *Ae. albopictus*, two important vectors of several arboviruses that affect humans worldwide, often interact in their invasive ranges. In these circumstances, a number of factors are thought to influence their population dynamics. The distribution and abundance of both species, which have profound epidemiological implications, are often governed by competitive interactions between sympatric mosquito populations.[20]

The gonotrophic cycle stands out among the most widely researched reproductive aspects of the physiology of vectors. A number of authors argue that the period from blood ingestion to egg deposition is one of the most important factors in the population dynamics of *Ae. aegypti* and *Ae. albopictus*. The duration of the gonotrophic cycle varies between mosquito species because multiple factors are involved.[21,22]

The results reported here correspond to the time of the first gonotrophic cycle of F_1 females of each species, under laboratory conditions. *Aedes aegypti* showed a gonotrophic cycle length of 3.66 d, similar to that reported by MacDonald,[20] who performed laboratory experiments with mosquitoes from the Malayan Peninsula (3 d), while *Ae. albopictus* exhibited a gonotrophic cycle of 3.16 d. The same trends were also observed with Gubler’s and Bhattacharya’s method.[21] We may argue that the observed differences could be due to characteristics intrinsic of each species, such as the temporal genetic divergence of the individuals, the nutritional reserves stored during the larval stages, and the hormonal modulation of the physiological processes,[22-26] rather than the effect of the experimental factors, because our study was performed under controlled conditions of temperature and relative humidity. However, it is known that climatic factors can affect the duration of the gonotrophic cycle of both mosquitoes. For instance, a laboratory experiment showed that wide fluctuations in the daytime and nighttime environmental temperatures (± 7 °C) had an important effect on the gonotrophic cycle of *Ae. albopictus* that resulted in an average duration of more than a week, due to changes in the rates of blood digestion and vitellogenesis. Recently, a field study reported that the duration of the gonotrophic cycle of *Ae. aegypti* was affected by the climatic seasonality.

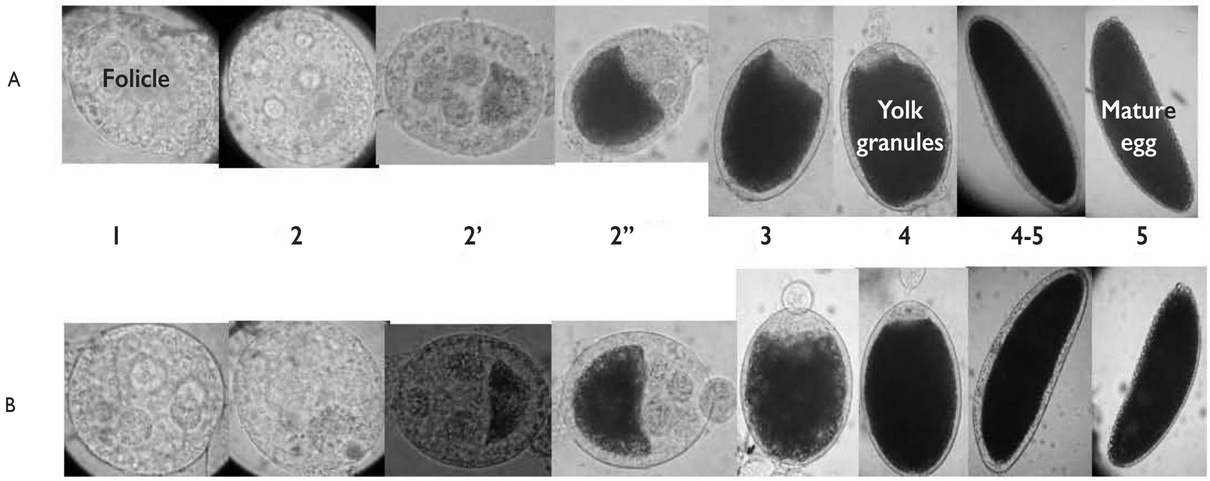

Figure 1. Structural comparison of the follicles of *Ae. aegypti*: (A) and *Ae. albopictus* (B) in different Christophers’ stages. Tapachula, Chiapas, México, 2010

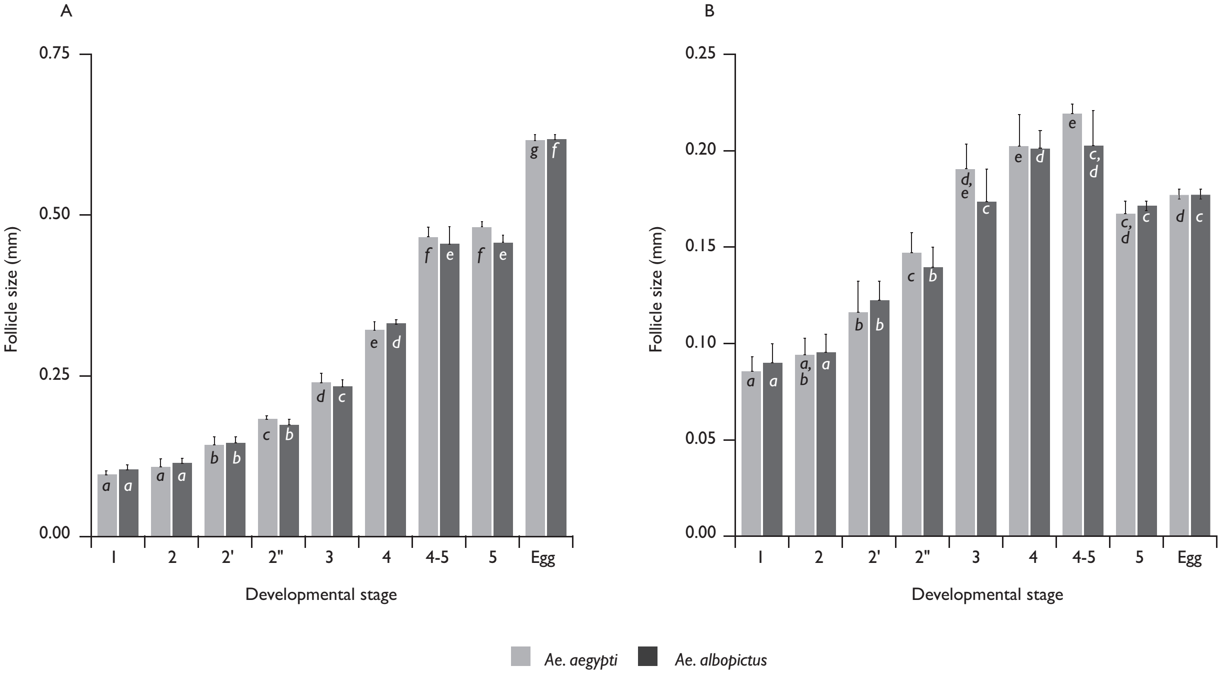

Figure 2. Size of *Ae. aegypti* and *Ae. Albopictus* follicles and eggs: A) length (mean±SD) and B) width (mean±SD). Different letters in the same color bars indicate significant differences (*P*≤0.05). Tapachula, Chiapas, México, 2010

Thus, in the rainy season at 26.7 °C it lasted four days, while in the dry season at 29.8 °C it lasted three days.[27] Consequently, it should be considered that the time of the oogenic development and, therefore, the duration of the gonotrophic cycle of both species in the field may vary due to the heterogeneity in the age structure of the female population, the influence of variations in the ambient temperature and relative humidity during the day and night,[28-30] as well as to the source of blood supply and the presence of bacterial symbionts in the midgut of mosquitoes.[31]

In contrast to other studies,[27] the design of our experiment was more robust because we included a greater number of females dissected per time interval and three repetitions instead of one, and used F1 females of wild mosquitoes. In general, other studies have utilized females that have been kept in the laboratory for more than two years.[27]

In epidemiological terms, the reduction of the time for completing the gonotrophic cycle has direct implications on the vectorial capacity of synanthropic mosquitoes because it increases the biting frequency of gonoactive females on human hosts. Consequently, this physiological aspect constitutes an intrinsic factor that greatly affects the explosive nature of arboviral epidemics.[19,32] In addition, the population structure of the females may become an entomological parameter that might determine the transmission dynamics of arbovirus diseases because older females exhibit more partial feedings than younger females, and therefore older, infected females have more possibilities of contacting a larger number of hosts compared to younger females.[33] Climate change and environmental modifications may be important factors impacting the physiological capacities of females. Thus, it might be expected that the population dynamics of mosquitoes would be affected by the reduction of the duration of the biological cycles and the reproductive processes,[34] while the extrinsic incubation period of the etiological agents would decrease, changing the vectorial competence of the species and the condition of mosquitoes from uninfected to infective in a shorter time.[28,29]

From an ecological point of view, the growth of the *Ae. aegypti* and *Ae. albopictus* populations will depend, in part, on the number of gonotrophic cycles and their length, as well as on their host preferences and fecundity.[30,31] Thus, the physiological time of egg development and the gonotrophic cycle constitute a reproductive competence strategy for the establishment and spatial and temporal permanence of *Ae. aegypti* and *Ae. albopictus* populations in the community of urban, suburban and rural mosquitoes. At the same time, the changes in temperature and humidity may affect the reproductive activity and influence the length of the gonotrophic cycle.[27] Moreover, it is likely that multiple oviposition with a single blood meal is the result of strategic behavior for the proliferation of mosquito populations in altered environments.[35] The pregravid stage in *Ae. aegypti* females indicates their need for more than one feeding to produce their first batch of eggs, which increases the contact and consequently their chances to become infected and later transmit diseases.[36]

On the other hand, the morphometric dimensions and the structural appearance of the follicles of *Ae. aegypti* and *Ae. albopictus* were similar. However, comparison of the interstage longitudinal growth showed differences between the two species, while the interstage transversal growth only exhibited significant changes at the interval between Christophers' stages 4 and 5. In general, the step from one follicular stage to another was determined by the speed of digestion and assimilation of the blood.

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Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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