Influence of Temperature on Immature Development, Survival, Longevity, Fecundity, and Gonotrophic Cycles of *Aedes albopictus*, Vector of Chikungunya and Dengue in the Indian Ocean

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ABSTRACT *Aedes albopictus* is a mosquito originating from Asia, which has extended its range worldwide the last decades. It is a competent vector for several arboviruses. It was first described in La Réunion (an island of the South West part of the Indian Ocean) in 1913. Since then, it has become the dominant *Aedes* species and a serious threat to public health, especially during the two last arboviruses outbreaks of dengue (1977) and chikungunya (2005–2006). Despite its pest status, data on the biology of this vector are scarce, especially the population present in the Indian Ocean (IO), which has never been studied in detail. Therefore, the immature development, survival, longevity, fecundity, and gonotrophic cycles of *Ae. albopictus* were studied for an F_2 population of the IO. These biological parameters were studied in controlled conditions at eight constant temperatures (5, 10, 15, 20, 25, 30, 35, and 40°C). The minimal threshold of immature stages development was found at 10.4°C and its optimum at 29.7°C. The shortest periods for immature development were found at 30°C, with in average of 8.8 d. The optimum intrinsic rate of growth (r) was observed between 25 and 30°C. The gonotrophic cycles were also evaluated, and the shortest cycles were found at 30°C (mean, 3.5 d). Those results are according to the field repartition of this species in La Réunion, allowing *Ae. albopictus* survival at a large range of temperatures.

KEY WORDS *Aedes albopictus*, arbovirus, chikungunya epidemic, demographic parameters, biology

The mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) was first described as "the banded mosquito of Bengal" by Skuse (1894) from India. It is considered to be originally indigenous to Southeast Asia (Smith 1956) and is supposed to have been introduced in the Indian Ocean during the last centuries. One of the hypotheses is that the immigrants who came from Asia in the 17th and 18th centuries were responsible for the introduction of *Ae. albopictus*. *Ae. albopictus* has recently spread during the last decades to Africa, the Mideast, Europe, and the Americas (north and south) after extending its range eastward across the Pacific islands during the early 20th century (Gratz 2004, Benedict et al. 2007). Because of its worldwide spread, public health authorities are aware of the presence of *Ae. albopictus* as a potential threat in generating serious outbreaks of arbovirus diseases. Indeed, *Ae. albopictus* has been proven to be a competent vector for at least 22 arboviruses (Shroyer 1987, Turrell 1988, Mitchell 1995, Gratz 2004), notably dengue (all four serotypes), which is more commonly transmitted by *Aedes* (*Stegomyia*) *aegypti* L.

La Réunion is an island (2,500 km^2, 770,000 inhabitants) in the Indian Ocean, situated 700 km east of Madagascar. La Réunion is a subtropical island with mild winters and warm summers; it has a peculiar geography with mountains in the center of the island up to 3,000 m high generating a temperature gradient. This mountainous topography allows freezing temperatures above 2,000 m during the winter season. Twelve species of *Culicidae* are present on the island, among which *Ae. albopictus* has been the dominant *Aedes* species since 1953 (Hamon 1953). *Ae. albopictus* was responsible for the two last arboviruses outbreaks: dengue 2 (DENV) in 1977, with 35–38% of people affected (Coulanges et al. 1979), and chikungunya (CHIKV) in 2005–2006 (with 38% of the population affected; Perrau et al. 2006). For both epidemics, the highest rates of infection were described during the months of February and March (Coulanges et al. 1979). In some cases, fluctuation of the vector population might be linked to adaptation of population biotic parameters that are linked to abiotic factors; these parameters need to be determined under controlled conditions to better understand the dynamic aspect of vector populations, especially in countries

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with such a range of temperatures. Despite its pest status, data on the biology of this vector are scarce, especially the population present in the Indian Ocean, which has never been studied in detail.

Therefore, we present biological data from a laboratory study of an Indian Ocean population of *Ae. albopictus.* Our goal was to evaluate the life history traits of this population under controlled conditions to obtain insights on its vectorial capacity. In-depth data on mosquito biology are a prerequisite for improving vector control, including potential genetic methods such as sterile insect technique, and for modeling previous and potential outbreaks.

Materials and Methods

Mosquito Source

Aedes albopictus used in this study were obtained from a laboratory colony established for 3 mo from field-collected mosquitoes. The mosquitoes were captured in artificial containers in Saint Pierre, La Réunion Island, France (55°29′ S and 47°51′ W) and kept in an insectarium. The insectarium was maintained under ≈27.5 ± 1°C, 80 ± 20% RH, and a natural photoperiod. Mouse blood meals were offered to mosquitoes twice a week for colony maintenance. Mice were anesthetized with a penthobarbituric before introducing them to the colony rearing and were left for 1 h. Mosquitoes of generations F_2 and F_3 were used for the different experiments.

This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of laboratory animals and was approved by the Direction des Services Vétérinaires de la Réunion.

Experimental Conditions

Environmental chambers (Sanyo MLR-350; Sanyo, Osaka, Japan) were used in the experiments. They were programmed with a photoperiod of 12:12 (L:D) h under eight constant temperatures (5 ± 1, 10 ± 1, 15 ± 1, 20 ± 1, 25 ± 1, 30 ± 1, 35 ± 1, and 40 ± 1°C). In these experiments, the relative air humidity was controlled and was 80 ± 10% RH (checked by a thermo-hygrometer HOBO H08–004-02; Onset, Pocasset, MA). Water dishes were placed inside the chambers to raise the relative air humidity from the beginning of the experiments.

Development of Immature Stages

Egg Stage. The freshly laid eggs on wet pieces of paper were incubated at 25°C and 80% RH for 5 d until the experiment. The egg viability and incubation period experiments were performed using 100–190 eggs for each of the eight temperatures tested and placed in groups of 10 in white plastic containers filled with 30 ml dechlorine water. Ten to 19 repetitions (of 10 eggs each) were made according to the temperatures tested. The 10-egg papers were left in water for 5 d, and the number of hatched eggs was recorded daily. Every 5 d, the eggs were taken out of the water and dried for 2 d. The eggs were left again in the water for 5 d. This procedure was followed three times; after the end of the third 5-d period, unhatched eggs were regarded as nonviable. These treatments had for objective to cause a hydrous stress and stimulate egg hatching by simulating precipitation renewal of water in breeding sites (Hawley 1988).

Immature Stages. The larval development was followed at eight temperature (5–40°C), and for each temperature, eight repetitions of 10 larvae each were monitored. After egg hatching, larvae <2 h old were isolated in groups of 10 using small pipettes in containers filled with 120 ml dechlorine water plus 2 mg of brewers yeast (Agros Organics, NJ). Daily, the instar period was identified by larvae observation and confirmed by the appearance of exuviates at each stage. Larvae were fed daily, with the quantity of food increasing according to the stage of development (0.2 mg of yeast per L1, 0.4 mg per L2, 0.6 mg per L3, and 0.8 mg per L4).

Statistical Analysis for Immature Stages. Temperature influences on egg hatching and larval development were compared with nonparametric tests. A Wilcoxon test was used to compare duration of immature stage development and a binomial test for the survival rate at 5%. Another Wilcoxon test was used to test whether survival was different between temperatures. Larval developmental rate was modeled by the model of Logan et al. (1976) corrected by Lactin et al. (1995). This model is a nonlinear model used to describe the relation that exists between the rate of development and temperature. Binomial tests were performed on the sex ratios obtained between the different temperatures (at $\alpha = 5\%$). All statistical tests and models were performed with R software (R Development Core Team 2004).

Adult Stage

Longevity and Fecundity Tests. Eggs of generation F_3 were used for this experiment. Egg hatching and larval rearing were performed in environmental chambers at 30°C. After emergence, females and males of the same age (<6 h) were individualized and placed as a couple inside a small cage (length: 15 cm, width: 7 cm, height: 5 cm) covered with white polyester tulle with a 0.4-mm grid. In each cage, a piece of cotton soaked with 10% sugar solution and a round recipient (4.5 cm diameter and 1.5 cm height) filled with water in which an egg-laying paper was placed, were provided. Every day, the cotton soaked with 10% sugar solution was removed, and an anesthetized mouse was offered for 1 h until the death of the female mosquito. After the mouse exposure, a new piece of cotton soaked with 10% sugar was placed, and every female was observed to see if they took a blood meal. Every day, the egg containers were checked, and eggs were counted for each couple at each temperature. After each count, new egg-laying paper was replaced in each container. Two experiments with 12 couples each at 15, 20, and 30°C and 15 couples at 25 and 35°C were carried out.

Table 1. Survival rate from hatching to emergence of *Ae. albopictus* maintained at eight constant temperatures: 5, 10, 15, 20, 25, 30, 35, and 40°C

T (°C)	Eggs (*n*)	Egg—L1	L1 (*n*)	L1—L2	L2—L3	L3—L4	L4—Pupae	Pupae—adult	Adults (*n*)	L1—adult
5	180	4.4 a	80	0.0						
10	100	4.0 a	80	0.0						
15	110	8.2 a	80	88.8 a	93.0 a	86.4 ac	84.2 a	83.3 a	40	50.0 a
20	130	66.9 b	80	96.3 b	100.0 b	92.2 ab	97.2 b	89.9 ab	62	77.5 b
25	130	49.2 c	80	92.5 ab	94.6 a	95.7 b	97.0 b	93.8 b	61	76.3 b
30	140	51.4 c	80	87.5 a	98.6 b	95.7 b	90.9 ab	90.0 ab	54	67.5 b
35	190	10.0 a	120	92.5 ab	83.8 c	77.4 c	50.0 c	8.3 c	3	2.5 c
40	100	0.0	80	0.0						

A Wilcoxon test was carried out with the threshold of 5% to compare the survival rate. Means in the same columns with the same letter are not significantly different at the $\alpha = 5\%$ level.

Statistical Analysis for Adult Stages. The survivals of females and males adult stage were modeled two ways: Gompertz (Gompertz 1825) and Weibull (Weibull 1951). The Weibull model best fitted our data set and was used. The Weibull model is a classical nonlinear parametric model to describe the relation between death rate and time. This model is generally applied with a mortality rate increasing roughly exponentially with increasing age at senescence. All statistical tests and model were performed with R software (R Development Core Team 2004).

Pre–Blood Meal and Gonotrophic Cycles. The pre–blood meal period was considered the period from adult emergence until the first blood meal, and the gonotrophic cycle was considered as starting with a blood meal, including the succession of physiological phenomena of oocyte maturation, and ending with oviposition (Clements 1992).

Statistical Analysis. A Wilcoxon test adjusted by Bonferroni correction was used to compare the mean duration of pre–blood meal periods (error $\alpha = 5\%$). Thereafter, the same test was used to compare the duration of gonotrophic cycles and oviposition durations (error $\alpha = 5\%$).

Calculation of the Demographic Growth Parameters

Demographic parameters were computed using standard methods (Carey 1982, Ebert 1999). Immature age-specific survivorship rates were interpolated as in Carey (1982). Confidence intervals for demographic parameters were estimated as the 2.5 and 97.5 percentiles of a bootstrap distribution resampled 1,000 times (Efron and Tibshirani 1993, Caswell 2001). For the demographic parameters, the assumption of a 1:1 sex ratio was used (according to our results, see Table 2).

Results

Life Table, Survivorship, Length of Development of Eggs, and Immature Stages

Life tables were constructed from the number of each larval stage of *Ae. albopictus* entering each stage (Tables 1 and 2). The lowest average time for egg hatching was observed for the temperature of 20°C (2.9 d), with the highest rate of egg hatching (66.9%; $P < 0.5$). On the contrary, temperatures of 30, 35, and 15°C were less favorable, with an average egg hatching period of ≈7 d, except for 10°C, where it was 2 d, but only 4% egg hatching was recorded (Tables 1 and 2). Immatures were not able to survive further than the first instars at 5 and 10°C, and no egg hatching or immature development was recorded at 40°C. Only 2.5% of L1 studied reached the adult stage at 35°C. Time from L1 to emergence was significantly different between temperatures ($P < 0.05$). Length of development from L1 to adult varyied on average from 8.8 (at 30°C) to 35 d (at 15°C) (Table 1). Despite the longest development rate observed for 15°C, 50% of the immatures reached the adult stage. The highest immature survival rates were recorded between 20 and 30°C (Table 2).

Table 2. Length of time between immersion of eggs in water and hatching response and duration of development (d) of each stage of *Ae. albopictus* at eight constant temperatures: 5, 10, 15, 20, 25, 30, 35, and 40°C

T (°C)	Egg	L1	L2	L3	L4	Pupae	L1-adult	Sex ratio
	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	
5	11 ± 1.3							
10	2 ± 0							
15	7.4 ± 1.8 a	5.6 ± 0.3 a	3.3 ± 0.2 a	4.6 ± 0.2 a	13.4 ± 0.8 a	8.7 ± 0.6 a	35.0 ± 0.9 a	47.5%
20	2.9 ± 0.4 b	3.0 ± 0.2 b	1.4 ± 0.2 b	2.1 ± 0.3 b	4.1 ± 0.3 b	4.1 ± 0.2 b	14.4 ± 0.4 b	43.5%
25	4.5 ± 0.7 c	2.1 ± 0.2 c	1.2 ± 0.2 b	1.2 ± 0.1 c	3.3 ± 0.2 c	2.7 ± 0.1 c	10.4 ± 0.7 c	41.0%
30	6.7 ± 0.7 a	1.4 ± 0.1 d	1.3 ± 0.1 b	1.4 ± 0.2 c	3.0 ± 0.3 c	1.9 ± 0.1 d	8.8 ± 0.6 d	46.3%
35	7.1 ± 0.8 a	1.7 ± 0.1 c	1.2 ± 0.1 b	2.4 ± 0.4 b	6.8 ± 1.1 d	1.7 ± 0.7	12.3 ± 0.7	66.6%

A binomial test was carried out with the threshold of 5% to compare duration of development. Mean in the same columns with the same letter are not significantly different at the 5% level.

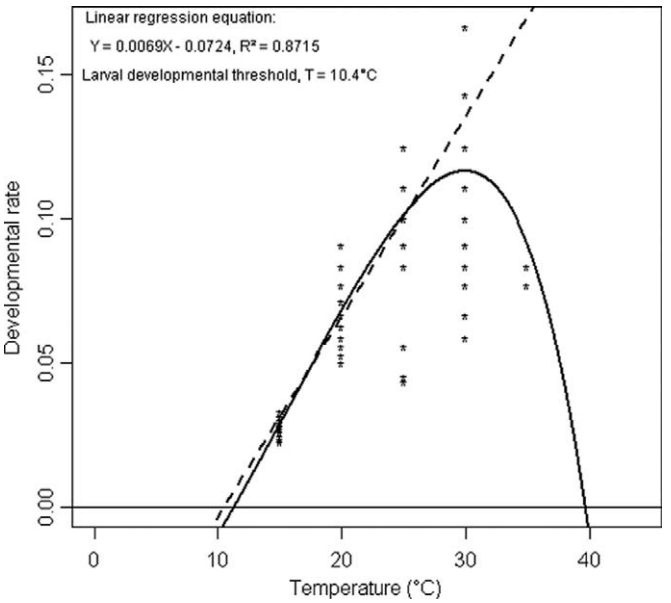

Fig. 1. Developmental rate of *Ae. albopictus* estimated by the Logan model corrected by Lactin.

Sex ratios observed ranged from 41 to 47.5%, except at 35°C, where 66.6% of females were recoded. The ratios found were not statistically different from 50%, and there was no effect of temperature on adult sex ratio except at 35°C where the value was not taken into account regarding to the very low number of individuals obtained ($n = 3$).

Temperature Thresholds

Using a nonlinear model (Logan et al. 1976, Lactin et al. 1995) fitted to the data across the whole range of experimental temperatures, the optimum development temperature was found to be $T_{opt} = 29.74$°C (Fig. 1).

A linear relationship between larval stage survival and temperatures in the range 15–40°C was used to estimate the lower temperature threshold for development (according to this study data) and was 10.4°C (Fig. 1).

Adult Life Expectancies

A Weibull model (Weibull 1951) was used and fitted to the data across the whole range of experimental temperatures for adults females and males

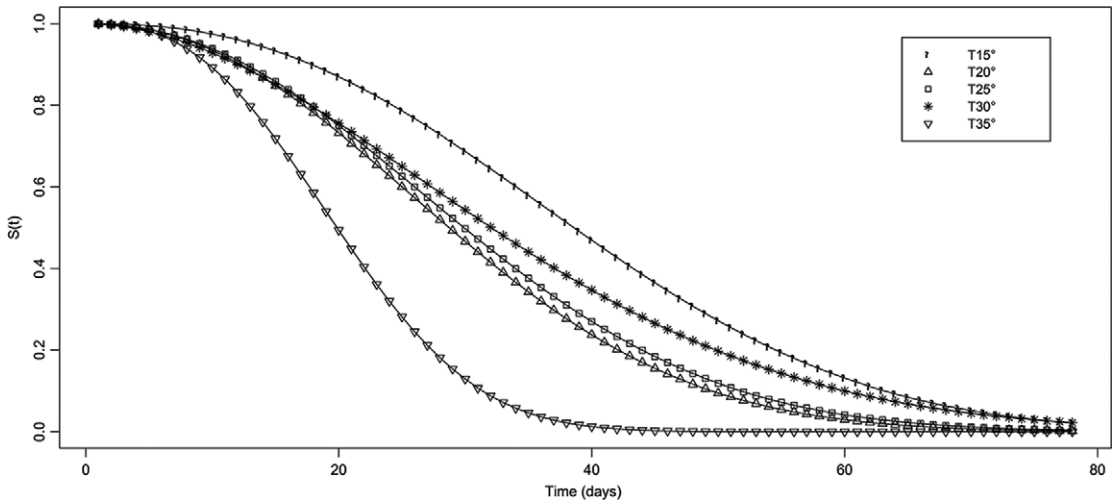

Fig. 2. Survival rate of females *Ae. albopictus* adjusted to the Weibull's model at temperatures of 15, 20, 25, 30, and 35°C.

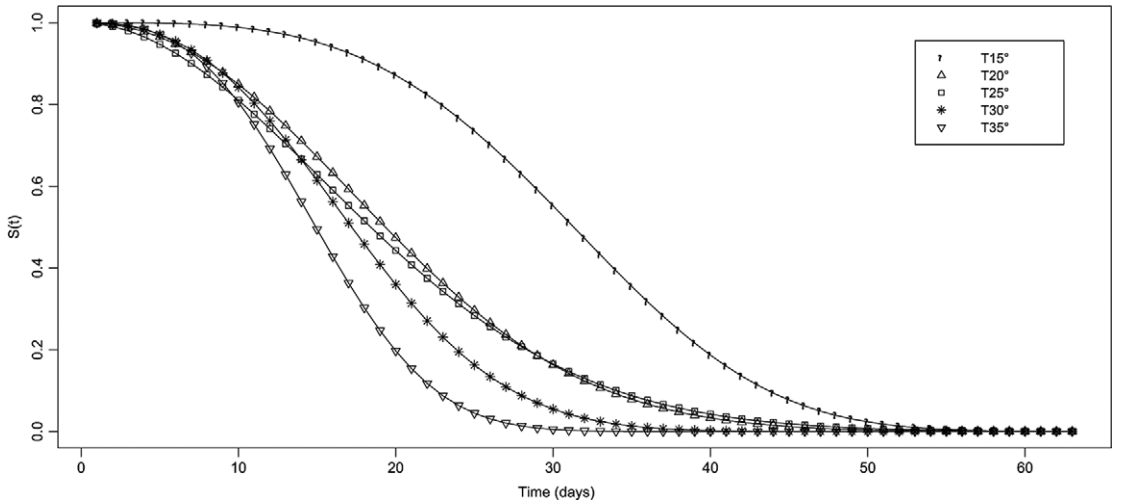

Fig. 3. Survival rate of males *Ae. albopictus* adjusted to the Weibull's model at temperatures of 15, 20, 25, 30, and 35°C.

(Figs. 2 and 3). The model gave the estimation of the average life expectancy for both sexes. The estimated average adult life expectancies were the longest at 15°C for females and males (38.59 and 31.31 d, respectively) and the lowest at 35°C for females and males (19.86 and 14.9 d, respectively). As a whole, adult female life expectancies were longer than males (Figs. 2 and 3).

Demographic Parameters

Demographic parameters were not calculated for temperatures <20°C and >35°C beause no egg laying was observed at 15°C and below and no complete larval development was recorded from 5 to 10°C and 40°C. The sex ratio used for the demographic parameters was 1:1 (see above).

The highest net reproductive rates (R_o) were recorded between 25 and 30°C ($R_{o(25°C)}$ = 33.08; $R_{o(30°C)}$ = 43.29). The lowest mean generation time (T) was observed at 25 and 30°C (25 and 24.2 d, respectively; Table 3).

The analysis of life tables, combining developmental rates, reproduction, and mortality, suggested maximum population growth (r) between 25 and 30°C (r = 0.140 and 0.155, respectively, according to confidence intervals). The lowest r observed had a negative value at 35°C, suggesting a decrease in population at this constant temperature (Table 3).

Gonotrophic Cycles

Pre–Blood Meal Periods. In the range of temperatures studied from 15 to 35°C, a blood feeding activity was recorded. The pre–blood meal period was significantly similar at 20, 25, and 35°C, with values ranging from 4.17 to 5.54 d (Table 4). The minimal pre–blood meal period observed was 2 d for all temperatures studied except at 15°C. The longest pre–blood meal periods were observed at the extreme temperatures of 15 and 35°C (15 and 10.83 d, respectively; Table 4).

Gonotrophic Cycles Numbers, Lengths, and Egg Laying. The shortest average gonotrophic period was observed for 30°C (3.5 d), with the highest number of cycles recorded (3.9) (Table 5). The shortest gonotrophic periods (2 d) were observed for 30 and 35°C (Table 5). At 20, 25, and 35°C, the gonotrophic cycle lengths were not significantly different within each temperature studied. Similarly, the number of eggs was not significantly different at each gonotrophic cycle for any temperature tested (ranging in average from 48.7 egg at 35°C/gonotrophic cycle to 74.2 eggs at 30°C/gonotrophic cycle). For each temperature, females laid their eggs during several days (ranging from 4.3 to 2.9 from 20 to 35°C, respectively, Table 6). No significant variations of number of eggs laid were observed between egg laying cycles at any temperature.

Discussion

Mosquitoes, being poïkilothermes organisms, are susceptible to external temperature variations that directly influence their body temperature (Hawley 1988). Our results clearly showed that temperature affects the population bionomics at larval and adults stages of *Ae. albopictus.* Adult survival was inversely correlated with temperature, with the highest survival rates found at 15°C and the lowest at 35°C. In the literature, very different longevity values are available for populations from different regions of the world. The closest average longevity compared with this study was found for females and males of a population from China with 24 and 17 d, respectively, recorded at 25°C (Liu et al. 1985); in this study, average longevity results for females and males populations tested at 25°C were 30 and 18 d, respectively. Longevities of females were longer than longevities of males at all temperatures observed; similar results were found in other studies with constant temperatures (Joshi 1996, Calado and Navarro-Silva 2002).

Table 3. Population demographic parameters of *Ae. albopictus* at four constants temperatures 20, 25, 30, and 35°C

Parameters	Units	20°C		25°C		30°C		35°C	
		Value	[95% CI]	Value	[95% CI]	Value	[95% CI]	Value	[95% CI]
Gross reproductive rate (GRR)	Eggs/♀	60.39	[12.93, 116.62]	150.80	[109.638, 191.879]	195.04	[154.66, 224.01]	20.20	[11.03, 30.16]
Net reproductive rate (R 0)	Eggs/♀	11.05	[3.88, 19.94]	33.08	[23.94, 43.05]	43.29	[31.84, 54.52]	0.04	[0.02, 0.07]
Intrinsic rate of increase (r)		0.072	[0.043, 0.090]	0.140	[0.123, 0.152]	0.155	[0.141, 0.167]	−0.103	[−0.122, −0.089]
Intrinsic birth rate (b)		0.115	[0.084, 0.133]	0.230	[0.213, 0.243]	0.217	[0.204, 0.226]	0.105	[0.081, 0.121]
Intrinsic death rate (d)		−0.043	[−0.045, −0.040]	−0.090	[−0.091, −0.089]	−0.061	[−0.063, −0.059]	−0.208	[−0.214, −0.199]
Multiplication rate (λ)		1.075	[1.044, 1.094]	1.150	[1.131, 1.165]	1.168	[1.152, 1.181]	0.902	[0.885, 0.915]
Mean generation time (T)	Days	33.4	[28.8, 39.3]	25.0	[23.2, 26.6]	24.2	[22.9, 25.7]	30.3	[27.9, 33.5]
Doubling time (DT)	Days	9.6	[7.7, 16.0]	5.0	[4.5, 5.6]	4.5	[4.2, 4.9]	6.8	[5.7, 7.8]
Average age in stable pop (ā)	Days	9.6	[8.3, 12.8]	4.7	[4.3, 5.3]	4.0	[3.7, 4.4]	11.6	[8.9, 16.5]
	Days	25.9	[22.8, 29.3]	18.0	[16.1, 19.8]	19.5	[17.1, 22.1]	5.0	[5.02, 5.04]

95% confidence intervals were obtained by 1,000 bootstraps.

Table 4. Average time (d) observed between emergence and the first blood meal (pre–blood meal periods) for *Ae. albopictus*

T (°C)	Emergence to first blood meal			
	n	Mean	±SE	Min
15	15	15.00	1.94 a	6
20	23	5.39	0.62 b	2
25	27	5.54	0.82 b	2
30	23	4.17	0.64 b	2
35	18	10.83	2.92 ab	2

Means in the same columns with the same letter are not significantly different at the 5% level.

Min, minimal period of time recorded (d).

Temperatures between 25 and 30°C were the most suitable for the development of *Ae. albopictus.* Indeed the greatest intrinsic rate of increase (*r*) was recorded at those temperatures. A negative *r* was found at 35°C ($r = -0.103$), suggesting a population decline at this constant temperature. Unfortunately, no references were available on demographic parameters for *Ae. albopictus;* only fecundity data are available and can be compared with the raw fecundity calculated in this study (GRR). At 25°C, several studies showed similar results to our data ($GRR_{25°C} = 150.80$ egg/female), with numbers of female eggs per female ranging from 150 to 175 for populations from Florida (Braks et al. 2006) and Asia (Gubler 1970, 1971).

The optimum temperature of development for immature stages was estimated at 29.74°C with the Logan model corrected by Lactin (Lactin et al. 1995). The minimum development threshold temperature was estimated at 10.4°C and the maximum at 40°C; however, if no development was recorded at <10.4°C, 4.4% of egg hatching was observed at 5°C. Few studies are available on this point; for *Aedes krombeni (Stegomyia)* Huang, the critical low temperature for egg hatching was 16°C (1996). Egg hatching is known to be triggered by several criteria, such as oxygen concentration in the water (Imai and Maeda 1976), light regimens (Clements 1992), several periods of desiccation (Hawley 1988), or fluctuating temperatures (Monteiro et al. 2007). In this study, the hatching stimuli chosen were the desiccation and rehumidification process. This process might have been improved by fluctuating temperature exposure regarding to the higher hatching rates observed at 20°C compared with 25°C (66.9% and 49.2%, respectively) in our study. Indeed, all the eggs used in this study were stored at 25°C for 5-d embryonic development before the experiment. Chang et al. (2007) in a recent study showed that *Ae. albopictus* was more cold tolerant than *Ae. aegypti*, with L1 larvae surviving several hours at 2.5 [lethal time (LT_{50}) taken to kill 50% of the population = 31.03 h] and 5°C ($LT_{50} = 64.20$ h). Similar results were found at 5 and 10°C in our experiment, with no development observed further than the L1 stage.

At 35°C, discrepancies are observed between populations of *Ae. albopictus* from Brazil on the mortality rates and length of larval development (Monteiro et al. 2007). In their study, no complete development was

Table 5. Duration of gonotrophic cycle for *Ae. albopictus* for 20, 25, 30, and 35°C

	20°C				25°C				30°C				35°C			
	n	Mean	SE	min	*n*	Mean	SE	min	*n*	Mean	SE	min	*n*	Mean	SE	min
Cycle 1	11	6.7	0.8	3 a	26	4.0	0.3	3 a	21	2.9	0.1	2 a	15	4.7	0.8	2 a
Cycle 2	5	8.4	1.6	5 a	21	4.4	0.6	3 a	19	2.9	0.1	2 a	4	3.3	0.3	3 a
Cycle 3	2	13.5	4.5	9 a	13	5.2	1.0	3 a	16	3.9	0.4	2 ab				
Cycle 4	1	10.0		10	9	5.1	0.6	3 a	11	3.9	0.4	2 ab				
Cycle 5					6	4.2	0.4	3 a	9	4.7	0.6	2 b				
Cycle 6					2	4.5	1.5	3 a	4	4.8	1.4	3 ab				
Cycle 7					2	5.5	1.5	4 a	2	3.0	0.0	3 ab				
Cycle 8					1	4.0		4								
Mean		8.1	a			4.5	b			3.5	c			4.4	b	
Cycle/female		1.7 a	0.2			3.1 b	0.2			3.9 b	0.2			1.3 a	0.1	

A Wilcoxon test was carried out with a threshold of 5% to compare the durations of egg laying per gonotrophic cycle. Means in the same columns with the same letter are not significantly different at the 5% level.

Min, minimal period of time recorded (d).

observed for 35°C, whereas in the La Réunion population tested, 2.5% of adults emerged, with a lower number of larvae observed. Most of the mortality in their study was recorded at the pupa stage (99.7%) at 35°C, whereas, in our study, we observed the highest mortality rates at both L4 and pupa stages (50 and 91.7%, respectively).

Furthermore, shorter development times were observed with La Réunion populations compared with the Brazilian ones, especially at 30°C, with the complete development from L1 to adult of 8.8 ± 0.6 and 10.6 d, respectively. Those results enhance the adaptability of the *Ae. albopictus* population of La Réunion to a large range of temperatures and its susceptibility of temperatures >30°C, with a higher tolerance to elevated temperatures compared with the *Ae. albopictus* Brazilian strains (Monteiro et al. 2007). The cold tolerance found for *Ae. albopictus* in the laboratory matches the results of a recent survey done by Delatte et al. (2007), showing the presence of *Ae. albopictus* up to 1,200 m during the winter in La Réunion (with average temperatures registered at 12.6°C at those altitudes) and >1,200 m during the summer in all the anthropized areas of the island. Fifty years ago, the presence of *Ae. albopictus* was documented in La Réunion to be up to 1,200 m only in the summer (Hamon 1953). The cold tolerance observed in the laboratory, such as the ability of the larvae to develop at 15°C, is likely to have enhanced the spread of *Ae. albopictus* at higher altitudes in La Réunion during the last 50 yr. Compared with *Ae. aegypti* larval survivorship at low temperatures (Chang et al. 2007), *Ae. albopictus* populations from La Réunion have a higher cold tolerance, despite the fact that they are tropical (nondiapausing) populations. Indeed, temperate populations of *Ae. albopictus* are known to be more cold hardy than tropical ones and has they have the ability to overwinter as diapausing eggs; tropical populations cannot (Hanson and Craig 1994). Nowadays, the presence of a nondiapausing tropical strain of *Ae. albopictus* at different altitudes reflects the adaptation of *Ae. albopictus* to a wide range of temperature conditions and its high ecological plasticity, which are important characteristics of populations persisting in seasonally changing environments (Tsuda and Takagi 2001).

The average length of each gonotrophic cycle for each temperature was similar during the life of females, and the average number of eggs did not differ significantly either between females and temperatures. These results did not show any significant age influence on the length of gonotrophic cycles and number of eggs laid per cycle (ranging in average from 35 to 101.5). Similar results were found in the literature with an average of oviposited eggs ranging from 42 to 88 after a blood meal (Hawley 1988), and the average fecundity per gonotrophic cycle was found independent of gonotrophic age for an *Ae. albopictus* strain from Calcutta over seven gonotrophic cycles (Gubler and Bhattacharya 1971). Females were found to lay their eggs during several days; this behavior

Table 6. Average duration of oviposition and the no. of eggs laid by gonotrophic cycles for *Ae. albopictus* at 20, 25, 30, and 35°C

Oviposition by cycle	20°C					25°C					30°C					35°C				
	n	Mean	±SE	Eggs	±SE	*n*	Mean	±SE	Eggs	±SE	*n*	Mean	±SE	Eggs	±SE	*n*	Mean	±SE	Eggs	±SE
Cycle 1	11	3.6	0.8	42.6	8.1	26	2.4 a	0.4	68.6 a	6.2	21	1.9a	0.3	77.2 a	9.6	15	3.1	0.8	47.9	10.0
Cycle 2	5	5.8	2.2	51.8	12.1	21	2.7 a	0.4	70.9 a	5.3	19	4.7a	0.8	98.6 a	9.3	4	2.3	0.8	51.5	15.2
Cycle 3	2	2.5	1.5	80.0	34.0	13	4.1 a	1.2	74.3 a	9.1	16	4.3a	0.8	60.3 a	5.8					
Cycle 4	1	8.0		77.0		9	5.3 a	1.3	53.7 a	7.1	11	4.3a	1.5	67.9 a	5.0					
Cycle 5						6	3.2	1.4	39.7	12.8	9	5.3	2.2	67.2	9.5					
Cycle 6						2	2.5	1.2	101.5	81.5	4	7.8	5.5	41.8	10.1					
Cycle 7						2	1.0		35.0	23.0	2	1.0	0.0	14.5	10.5					
Cycle 8						1	4.0		18											
Mean		4.3 a	0.8	50.8 a	6.76		3.1 a	0.3	65.3 a	3.62		3.9a	0.5	74.2 a	4.12		2.9a	0.6	48.7 a	8.32

A Wilcoxon test was carried out with a threshold of 5% to compare the durations of egg laying per gonotrophic cycle. Means in the same columns with the same letter are not significantly different at the 5% level.

observed in the laboratory might suggest that eggs from a single gonotrophic cycle are laid in more than one oviposition site.

Pre–blood meal periods were not significantly different in the range of temperatures from 20 to 30°C, with a minimum observed of 2 d. The length between a blood meal and egg laying was in average between 3.5 and 4.5 d at 30 and 25°C, and up to seven and eight blood meals were taken for a female at 30 and 25°C, respectively. These results point out the ability of the female mosquito population for taking a blood meal, this being possible at a minimum 2 d after its emergence. Therefore, this ability, its good vectorial competence for CHIKV and DENV (Paupy et al. 2001, Vazeille et al. 2007), ideal temperatures of the subtropical summer of February/March (in average between 25 and 30°C), and the presence of enough viremic travelers with an arbovirus can partly explain the two explosive epidemics transmitted by *Ae. albopictus* observed for DENV and CHIKV in the Indian Ocean in 1997–1998 and 2005–2006, respectively (Coulanges et al. 1979, Metselaar et al. 1980, Calisher et al. 1981, Perrau et al. 2006, Mavalankar et al. 2007).

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