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Temperature Effects on the Immature Development Time of *Culex eduardoi* Casal & García (Diptera: Culicidae)

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Abstract

The effect of constant temperatures on the development time from first instar to adult emergence was studied in *Culex eduardoi* Casal & García reared at 7, 10, 15, 20, 25, 30 or 33°C. Data were adjusted to the linear degree-day model and the nonlinear Briére model. According to the linear model, the development time was inversely related to the rearing temperatures between 7°C and 25°C. Maximum mortality (100%) was recorded at temperatures ≥ 30°C. According to the linear model, the development threshold temperature and thermal constant were 5.7°C and 188.8 degree days, respectively. The lower and upper threshold temperatures and the optimum temperature for the nonlinear model were -2.3, 30.0 and 28.1°C, respectively.

Introduction

Temperature is one of the most important abiotic factors affecting the preimaginal biology of culicids (Clements 1992). Although the development time of mosquito immatures is generally shortened by warm temperatures and lengthened by the cool ones, adult emergence is successfully achieved within a species-specific thermal range. The rearing temperature influences some characteristics of the adult (e.g., Briegel 1990, Briegel & Timmermann 2001, Oda *et al* 2002), which in turn may affect species fitness. On the other hand, temperature limits the geographic distribution of the species (Krebs 1994). Therefore, the knowledge of the effect of temperature on the preimaginal development is essential to understand the biology and ecology of a mosquito species.

Culex (*Culex*) *eduardoi* Casal & García is found in south of Brazil (Forattini *et al* 1993, Lopes 1997a) and in Argentina. The southern most limit of its distribution was extended from 34°S (Mitchell & Darsie 1985) to 45°S (Burroni *et al*2007), with records either on natural or artificial habitats (Forattini *et al* 1993, Fischer *et al* 2000, Vezzani *et al* 2001, Lopes 2002). *Culex eduardoi* was also reported as the most abundant mosquito species and its presence was documented throughout the year in Buenos Aires, Argentina (34° 33'S, 58° 29'W) (Fischer *et al* 2000) and Londrina, Parana State, Brazil (23°23'S, 51°11'W) (Lopes 1997a). Annual mean temperatures are 17.6ºC and 20.6°C, respectively for Buenos Aires and Londrina. The highest mean temperatures are recorded in January (24.5°C – Buenos Aires; 23.8ºC - Londrina), and the lowest in July (11.0ºC – Buenos Aires; 16.8ºC - Londrina) (Maack 1981, SMN 2009). The thermal amplitude from the coolest and warmest seasons in the regions where this species was shown to be abundant may reflect a tendency of immatures to tolerate different thermal conditions. However, most of the preimaginal biology of *C. eduardoi* still remains unknown and no information is available on the immature thermal requirements. Therefore, the objective of this work was to investigate the effects of the rearing temperature on the preimaginal development time of *C. eduardoi* under laboratory conditions.

Material and Methods

Mosquito eggs were collected from rain pools in Buenos Aires from October 2001 to June 2002. Egg rafts were maintained until hatching in individual plastic containers with tap water. Within 12h of eclosion, between 10 and 15 first instars were randomly selected from each egg raft and individually placed in labeled plastic cups (3 cm in diameter, 5 cm in height) with tap water. They remained in water baths at one of the constant temperatures investigated (7, 10, 15, 20, 25, 30 and 33ºC) until adult emergence. The total number of first instars tested in each one of the temperatures tested ranged from 92 to 220 for temperatures between 7 ºC and 30ºC, while only 48 larvae were observed at 33ºC. Larvae were kept under a photoperiod of 14:10 h light-dark and fed on a daily basis with 0.6mg/larva of brewer´s yeast (Levex®).

The presence of exuviae, mortality and date of imago emergence were recorded daily at the same hour to estimate the development time of each immature stage (ecdysis to ecdysis). The adults were killed by freezing at -18ºC.

The taxonomic determination of the larvae species was based on the fourth-instar exuviae using the key of Darsie (1985).

Mathematical models

Development time in degree days was estimated by regressing the development rate (1/development time) as a function of temperature (Clements 1992). In the degree-day model, the relationship between rate of development and temperature is described by the regression equation $y = a + b*T$, where y is the rate of development, T = temperature (°C), and a and b are the model parameters. The lower developmental threshold is given by $–a/b$; and the degree-day requirement above the lower developmental threshold (i.e. the thermal constant k) is given by 1/b. Development rate was also modeled as a function of temperature using the Briére model (Briére et al 1999), given by the following equation: $r(T) = a*T*(T - T_0)*(T_L - T)^{1/m}$, where r(T) is the rate of development (1/development time) at temperature T, T = temperature (°C), T_0 = low-temperature development threshold (lower threshold), T_L = lethal temperature (upper threshold), a = empirical constant, and m = shape parameter.

Results and Discussion

The development time of each immature stadium or stage of *Culex eduardoi* was differently affected in the range of temperature analyzed (Table 1). As expected, the total development time (from first instar larva to adult emergence) was inversely related to the rearing temperature in the range of 7ºC to 25ºC, as observed for several mosquito species (e.g., Rueda *et al* 1990, Mahamood & Crans 1998, Ribeiro *et al* 2004, Monteiro *et al* 2007). The time required by the immatures maintained at the lowest temperatures to reach adulthood was extended between six to eight orders of magnitude as compared for those maintained at 25°C. The different immature stages also showed a decrease in the development time with increasing rearing temperatures, with a high standard deviation of about 50% of the mean for the first, second and third instars at 7, 25 and 30°C, respectively. The proportion of time spent in each stage varied between 7ºC and 25°C with respect to the total development time. However, like in *Aedes albopictus* (Skuse) (Calado & Navarro-Silva 2002, Monteiro *et al* 2007), the fourth instar was the longest stage, accounting for 25-36% of the total development time, what is probably related with the nature of the larval growth process, since most of the growth occurs during the last instar (Clements 1992).

The first instar was the least susceptible to the lowest and highest temperatures tested as similarly reported for *Culex hepperi* Casal & García (Loetti *et al* 2008), in which first and second instars seemed to be more tolerant to nonoptimal temperatures. High preimaginal mortality at warmer temperatures has been found in other *Culex* species, such as *Culex restuans* Theobald (> 29ºC) (Shelton 1973), *Culex quinquefasciatus* Say (> 30ºC) (Shelton 1973, Rueda *et al* 1990) or *Culex sitiens* Wiedemann (35°C) (Mottram *et al* 1994). Pupal survivorship ranged from 5% (5/92) at 7°C up to 13% (12/92) at 15°C, and no larvae completed their development in temperatures ≥ 30ºC (Table 1). Nevertheless, other culicids common to the south of Brazil and Argentina are able to complete their development in temperatures between 30ºC and 35ºC (Calado & Navarro-Silva 2002, Ribeiro *et al* 2004, Monteiro *et al* 2007). However, none of them have been detected on latitudes higher than 40ºS. Thus, the high mortality rate observed for *C. eduardoi* at 30ºC and its presence in high latitudes could be an indication of its higher tolerance to colder than to warmer temperatures.

The simple linear regression showed a good fit ($R^2 = 0.90$; df = 47; $P < 0.001$) and the total development rate (first instar to adult) of *C. eduardoi* was described by the regression equation $y = -0.030 + 0.005x$ between 7ºC and 25°C (Fig 1). Based on this regression, the linear degree-day model estimated a threshold development temperature of 5.7°C and a thermal constant of 188.8 degree-days. According to these parameters, *C. eduardoi* may have 29 generations/year in the northern extreme of this species distribution range (23ºS), and 14.5 generations/year in the southern extreme range (45ºS) where annual mean temperature is approximately 13.2°C. At this latitude, this species would not be expected to develop during the winter season, since the mean temperature is 6.6°C, with the minimum temperatures averaging 0.8°C (INTA 2009), which are close to its

Table 1 Duration of the development stages (days) and mortality of immature stages of *Culex eduardoi* at seven constant temperatures.

Temperature (°C)	n[1]	Mean (days)	SD	Mortality (%)	Temperature (°C)	n[1]	Mean (days)	SD	Mortality (%)
		Larva I					Larva IV		
7	87	8.1	3.91	5.4	7	17	27.8	5.96	60.5
10	72	7.4	1.74	21.7	10	10	18.7	2.67	45.0
15	89	3.6	1.14	3.3	15	26	6.6	2.14	48.0
20	214	2.8	0.49	2.7	20	41	4.3	1.15	64.4
25	91	1.8	0.43	5.2	25	13	3.0	1.00	68.3
30	87	1.6	0.50	9.4	30	1	3.0	-	90.0
33	44	2.0	0.00	8.3	33	0	-	-	-
		Larva II					Pupa		
7	40	12.5	2.96	54.0	7	5	14.2	0.84	66.7
10	49	9.1	2.02	31.9	10	6	10.3	0.82	45.5
15	76	3.4	0.84	14.6	15	12	4.5	0.67	53.8
20	199	2.1	0.46	7.0	20	16	3.0	0.37	61.9
25	71	1.5	0.67	22.0	25	10	2.0	0.47	23.1
30	54	1.3	0.49	37.9	30	0	-	-	100
33	0	-	-	100.0	33	0	-	-	-
		Larva III					Larva I to adult emergence		
7	38	13.0	1.95	5.0	7	5	78.2	5.89	94.6
10	20	9.8	1.24	59.2	10	6	54.0	7.07	93.5
15	50	3.7	0.99	34.2	15	12	21.3	4.43	87.0
20	118	2.4	0.90	40.7	20	16	13.8	1.61	92.7
25	41	2.7	1.15	42.3	25	10	9.8	1.99	89.6
30	10	1.4	0.70	81.5	30	0	-	-	-
33	0	-	-	-	33	0	-	-	-

[1]Number of individuals surviving to the next stage.

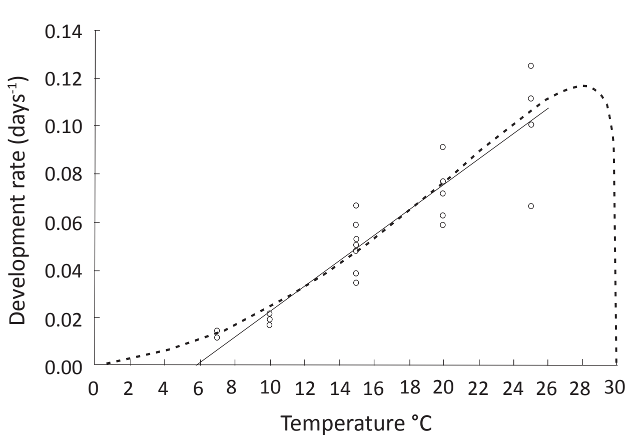

Fig 1 Temperature-dependent rate of development of *Culex eduardoi* from first-instar larva to adult emergence: Observed data (o) fitted to degree-day model (solid line) and the Briére model (dotted line).

thermal limit (but see discussion below).

The nonlinear Briére model (Fig 1) also satisfactorily described the relationship between the development rate and the rearing temperature (R^2 = 0.91). The lower and upper threshold temperatures for *C. eduardoi* development were -2.3°C and 30°C, respectively. The estimated optimum temperature was 28.1°C, with a development rate of about 0.1153 days^{-1}. The parameters *a* and *m* were 1.24^{-04} and 7.63, respectively. The parameter *m* is interpreted as the potential of an insect species to develop and survive at high temperatures, with this ability being higher when $m < 2$ (Briére *et al* 1999). In the present case *m* was 7.63, suggesting that high temperatures would be unfavourable for the immature development of *C. eduardoi*. The number of generations per year estimated by the Briére model was similar to the estimated by the degree-day model. So, *C. eduardoi* may have 28.7 generations/year in Londrina

(23ºS), and 13.4 generations/year in Chubut (45ºS). In addition, at this latitude, this species may be able to continue development during winter.

The difference in the low-temperature development threshold estimated by the degree-day model (5.7°C) and the Briére model (-2.3°C) is possibly explained by the fact that the relationship between the development rate and temperature deviates from a linear function at 7°C, and this non-linearity is not considered in the degree-day model. Thus, the lower threshold would be better estimated by the Briére model than by the degree-day model, although it is clearly impossible for the immature stage to survive in frozen aquatic habitat. Nevertheless, the parameters fitted by both models may indicate that *C. eduardoi* immatures can tolerate low temperatures (even close to 0°C). These experimental results are consistent with ecological studies reporting the occurrence of *C. eduardoi* immatures throughout the year at middle latitudes (Lopes 1997a, Fischer *et al* 2000) and during summer at high latitudes (45ºS) (Burroni *et al* 2007). At present, whether this species survive as an immature or if it hibernates as an adult during winter at high latitudes is unknown. This information would aid to decide which of these models better represents the lower threshold.

The breeding sites of *C. eduardoi* are often found next to human settlements (Lopes 1997b, 1999, Vezzani *et al* 2001) and some authors emphasize the ability of this mosquito to adapt to anthropogenic environments (Lopes 1997a, Zequi *et al* 2005), with both of these features having important epidemiologically implications. Our study contributes to the limited knowledge of the biology of *C. eduardoi* and could be useful to understand its population dynamic. However, caution is necessary in drawing conclusions from our results since other factors, like the daily temperature and photoperiod cycles, food quality and larval crowding may also affect mosquito developmental processes (Clements 1992).

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