

## Development rates, larval survivorship and wing length of *Culex pipiens* (Diptera: Culicidae) at constant temperatures

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In this study, the effects of rearing temperature on survival and development rate of pre-imaginal stages and on wing length of adult *Culex pipiens* (Diptera: Culicidae) were evaluated. Larvae I were reared until adult emergence at seven constant temperatures between 7 and 33°C. The highest survival was found at 25°C. The development rate of the immature stages decreased with increasing temperature until 30°C. The threshold temperature and thermal constant were, respectively, 5.2°C and 186.5 degree-days for males, and 5.5°C and 199.5 degree-days for females. According to a non-linear model, the lower and upper thermal thresholds were, respectively, 8.4°C and 34.4°C for males, and 9.8°C and 34.2°C for females. Wing length decreased with increasing temperature. Wings of females were longer than those of males. This study showed that survival and development of immatures and adult size were affected by rearing temperature. In addition, results suggest that this effect may differ between sexes.

**Keywords:** Diptera; Culicidae; body size; developmental models; mosquito

### Introduction

The widely distributed members of the *Culex pipiens* group have been thoroughly studied because of their role as vectors of pathogens for humans and domestic animals. The immature stages of *C. pipiens* are found at different breeding sites, most of which are closely related to anthropic environments (Forattini 1965).

In Argentina, there are two members of this complex, *C. pipiens* Linnaeus and *Culex quinquefasciatus* Say (Mitchell et al. 1984), as well as hybrids of both species (Brewer et al. 1987; Almirón et al. 1995). The taxonomic status of these two species remains unresolved (Rossi et al. 2002). In Argentina, *C. quinquefasciatus* has been involved as a vector of St Louis encephalitis virus (Mitchell et al. 1985; Díaz et al. 2006), specimens from natural populations of *C. pipiens* infected with the dog heartworm *Dirofilaria immitis* were found (Vezzani et al. 2006). In addition, West Nile virus was recently detected in *C. pipiens* (Morales et al. 2006), which is the bridge vector for this virus (Hamer et al. 2008).

In Culicidae, as in other arthropods, temperature is one of the most important abiotic factors affecting the development and survival of the immature stages (Clements 1992). Furthermore, the thermal rearing conditions may have a variable effect on some

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adult characteristics, such as body size, fecundity, longevity and vector competence (Kay et al. 1989; Briegel 1990ab; Briegel and Timmermann 2001; Kay and Jennings 2002; Oda et al. 2002). The knowledge of the effect of temperature on the pre-imaginal biology of vector mosquito species is essential for understanding their population dynamics to develop effective control strategies and to construct phenological models.

The objective of this study was to evaluate whether different rearing temperatures have an effect on the developmental time and survival of pre-imaginal stages and on adult body size of *C. pipiens*.

## Materials and methods

### *Mosquito collection and rearing*

Egg rafts of *C. pipiens* were obtained from natural populations in Buenos Aires City (34°35' S, 58°29' W). Surveys were conducted between February and March 2002 in potential breeding sites such as temporary and permanent water bodies and water-filled artificial containers (Forattini 1965). They were maintained until eclosion into individual plastic containers with dechlorinated tap water. Within 12 h of eclosion, between 24 and 48 larvae I were randomly selected from each raft and individually placed in labelled plastic cups (3-cm diameter; 5-cm height) containing 28 ml dechlorinated tap water. They were reared until adult emergence in thermal baths (Loetti et al. 2001) at one of the following constant temperatures: 7, 10, 15, 20, 25, 30 and 33°C ( $\pm 1^\circ\text{C}$ ). For the baths at 25, 30 and 33°C, water temperature was kept constant using aquarium heaters controlled with digital thermostats. Water volume was constant, dechlorinated tap water was added as needed. Larvae were fed daily with a mixture of dry brewer's yeast (Levex<sup>®</sup>) and finely triturated dog food (Purina Dog Chow<sup>®</sup>) (3 : 1 by weight) at a rate of 0.3 mg/larva for first and second instars and 0.7 mg/larva for third and fourth instars. Each individual was monitored daily and at the same hour to record larval stage, moulting, mortality or date of imago emergence. The formation of surface layer was checked every day, and if there was any it was removed using a strip of paper towel. Photoperiod regimen was 14 h light : 10 h dark for all treatments.

Adults were killed by freezing ( $-18^\circ\text{C}$ ) after emergence. Afterwards, each specimen was examined under a stereoscopic microscope with an ocular micrometre. Wing length, measured from the base of the costa vein to the distal extreme of the R3 vein excluding the fringe setae, was used as an indicator of imaginal body size (Christophers 1960; Clements 1992).

In Buenos Aires City and Greater Buenos Aires, two members of the *C. pipiens* complex (*C. pipiens sensu stricto* and *C. quinquefasciatus* Say) live in sympatry (Rossi et al. 2002). In this study they will be referred to as *C. pipiens*. The taxonomic determination of the specimens was based on the exuviae of larva IV using the key of Darsie (1985).

### *Data analysis*

Survival was calculated as the number of individuals that reached the adult stage divided by the number of larvae I at the start of each treatment. The results obtained at each rearing temperature were compared using the test of proportions for independent samples (Fleiss 1981).

For each immature stage, differences in developmental time (days) among rearing temperatures were tested by a Kruskal–Wallis non-parametric analysis of variance followed by Dunn’s method for multiple comparisons. For each thermal condition, the Mann–Whitney  $U$ -test was used to compare total time of development (from larvae I to adult emergence) between sexes (Zar 1999). Development rate, expressed as the reciprocal of total developmental time, was modelled as a function of temperature using the three-parameter model proposed by Brière et al. (1999) and the degree-day model (Clements 1992). The expression of the Brière model is:  $r(T) = nT(T - T_0)(T_L - T)^{1/2}$ , where  $r(T)$  is the rate of development (1/developmental time) at temperature  $T$ ,  $T$  is the temperature ( $^{\circ}\text{C}$ ),  $T_0$  is the low-temperature development threshold (lower threshold),  $T_L$  is the lethal temperature (upper threshold), and  $n$  is the empirical constant. The equation for the Degree-day model is:  $r(T) = a + bT$ , where  $a$  and  $b$  are the parameters to estimate;  $-a/b$  is the lower developmental threshold; and  $1/b$  (thermal constant) is the number of degree-days required above the lower developmental threshold for emergence (Clements 1992).

For each thermal condition, wing length differences between sexes were tested using Student’s  $t$ -test. For each sex, wing length differences among rearing temperatures were analysed by one-way analysis of variance followed by Tukey’s post test. The relationship between wing length and temperature was determined by fitting data to three types of curves: linear  $Y_{(\text{wing length})} = b_0 + b_1 * T^{\circ}$  (rearing temperature), quadratic  $Y = b_0 + b_1 * T^{\circ} + b_2 * (T^{\circ})^2$  and logarithmic  $Y = b_0 + b_1 * (\ln T^{\circ})$ . Statistical analyses were performed with STATISTICA 7.1 (Statsoft Inc. 2005)

## Results

### *Pre-imaginal survival and developmental time*

The survival of pre-imaginal stages of *C. pipiens* was significantly ( $P < 0.05$ ) affected by the rearing temperature. The maximum survival was 76% at 25 $^{\circ}\text{C}$ , and almost no adults emerged at the lowest and highest temperatures (7 and 33 $^{\circ}\text{C}$ , respectively) (Table 1). At 33 $^{\circ}\text{C}$  survival decreased similarly in all stages. At 7 $^{\circ}\text{C}$  survival dropped sharply in the pupa stage, a similar pattern was also observed at 15 and 30 $^{\circ}\text{C}$  (Table 2).

Table 1. Effect of constant temperatures on *Culex pipiens* survival from first-instar larvae (L I) to adult emergence.

Temp. ( $^{\circ}\text{C}$ )	Initial number of L I	Number of L I reaching adulthood	Survival (%)
7	48	0	0.0 <sup>a</sup>
10	48	24	50.0 <sup>b</sup>
15	48	19	39.6 <sup>c</sup>
20	158	78	49.4 <sup>b</sup>
25	144	110	76.4 <sup>d</sup>
30	240	69	28.8 <sup>c</sup>
33	128	2	1.6 <sup>a</sup>

Note: Percentages followed by different letters are significantly different ( $P < 0.05$ , test of proportions for independent samples).

Table 2. Mean ( $\pm$  SD) and median (Q1–Q3) duration of developmental stages (days) of *Culex pipiens* at seven constant temperatures.

Development stage	Temp. (°C)	<i>n</i> *	Mean ( $\pm$ SD)	Median (Q1–Q3)
Larva I	7	40	6.3 ( $\pm$ 1.9)	6.0 (5.0–7.0) <sup>a</sup>
	10	42	4.7 ( $\pm$ 0.6)	5.0 (4.0–5.0) <sup>a</sup>
	15	48	4.2 ( $\pm$ 0.6)	4.0 (4.0–4.0) <sup>a</sup>
	20	154	2.8 ( $\pm$ 0.5)	3.0 (3.0–3.0) <sup>b</sup>
	25	143	2.0 ( $\pm$ 0.7)	2.0 (1.0–2.0) <sup>c</sup>
	30	235	1.2 ( $\pm$ 0.4)	1.0 (1.0–1.0) <sup>d</sup>
	33	96	1.3 ( $\pm$ 0.5)	1.0 (1.0–2.0) <sup>d</sup>
Larva II	7	29	12.7 ( $\pm$ 2.5)	12.0 (11.0–13.0) <sup>a</sup>
	10	38	8.6 ( $\pm$ 1.0)	8.0 (8.0–9.0) <sup>a</sup>
	15	47	3.2 ( $\pm$ 0.6)	3.0 (3.0–4.0) <sup>a</sup>
	20	148	1.7 ( $\pm$ 0.9)	1.0 (1.0–2.0) <sup>b</sup>
	25	141	1.5 ( $\pm$ 0.6)	1.0 (1.0–2.0) <sup>b</sup>
	30	232	1.1 ( $\pm$ 0.4)	1.0 (1.0–1.0) <sup>c</sup>
	33	70	1.3 ( $\pm$ 0.5)	1.0 (1.0–2.0) <sup>b,c</sup>
Larva III	7	25	10.1 ( $\pm$ 1.2)	10.0 (10.0–10.0) <sup>a</sup>
	10	36	7.1 ( $\pm$ 0.7)	7.0 (7.0–8.0) <sup>a</sup>
	15	46	3.2 ( $\pm$ 0.5)	3.0 (3.0–3.0) <sup>a</sup>
	20	106	2.1 ( $\pm$ 0.9)	2.0 (2.0–2.0) <sup>b</sup>
	25	133	1.7 ( $\pm$ 0.6)	2.0 (1.0–2.0) <sup>b</sup>
	30	224	1.2 ( $\pm$ 0.5)	1.0 (1.0–1.0) <sup>c</sup>
	33	26	2.3 ( $\pm$ 1.2)	2.0 (1.0–3.0) <sup>b</sup>
Larva IV	7	23	28.1 ( $\pm$ 3.5)	27.0 (25.0–31.0) <sup>a</sup>
	10	25	11.6 ( $\pm$ 0.8)	12.0 (11.0–12.0) <sup>a</sup>
	15	46	6.9 ( $\pm$ 1.1)	7.0 (6.0–8.0) <sup>a</sup>
	20	90	4.3 ( $\pm$ 1.5)	4.0 (3.0–5.0) <sup>b</sup>
	25	126	3.3 ( $\pm$ 1.2)	3.0 (3.0–4.0) <sup>c</sup>
	30	187	2.7 ( $\pm$ 1.0)	3.0 (2.0–3.0) <sup>d</sup>
	33	3	4.3 ( $\pm$ 0.6)	4.0 <i>NT</i>
Pupa	10	24	7.9 ( $\pm$ 0.7)	8.0 (7.5–8.0) <sup>a</sup>
	15	19	4.5 ( $\pm$ 0.5)	4.0 (4.0–5.0) <sup>a</sup>
	20	78	2.7 ( $\pm$ 0.6)	3.0 (2.0–3.0) <sup>b</sup>
	25	110	1.8 ( $\pm$ 0.4)	2.0 (2.0–2.0) <sup>c</sup>
	30	69	1.7 ( $\pm$ 0.5)	2.0 (1.0–2.0) <sup>c</sup>
	33	2	1.0	1.0 <i>NT</i>
	Larva I to adult emergence	7	0	–
10		24	39.8 ( $\pm$ 1.9)	39.0 (38.5–40.5) <sup>a</sup>
15		19	21.6 ( $\pm$ 1.6)	22.0 (20.0–23.0) <sup>a</sup>
20		78	13.3 ( $\pm$ 2.0)	12.5 (12.0–14.0) <sup>b</sup>
25		110	10.2 ( $\pm$ 1.9)	9.0 (9.0–12.0) <sup>c</sup>
30		69	8.0 ( $\pm$ 1.0)	8.0 (7.0–8.0) <sup>d</sup>
33		2	11.5	11.5 <i>NT</i>

Notes: Within each developmental stage, medians followed by different letters are significantly different ( $P < 0.05$ , Dunn test).

*NT*, not tested.

\*Number of individuals that reached the next developmental stage.

The developmental time of all immature stages decreased significantly as temperature was increased until 30°C, after which it increased or remained constant (Table 2). The total developmental time (from larva I until adult emergence) ranged between 39.8 days at 10°C to 8 days at 30°C.

The developmental time of females was similar to that of males only at 10°C; they required a longer time to reach adulthood at the remaining rearing temperatures. This difference was because of the longer developmental time for the larvae IV of females than for those of males (Figure 1).

**Fitting data to the Brière model and degree-day model**

The non-linear relationship between development rate and rearing temperature between 7 and 33°C was described by the three-parameter model of Brière. The percentage of explained variability was 81.7 and 67.0% for males and females, respectively.

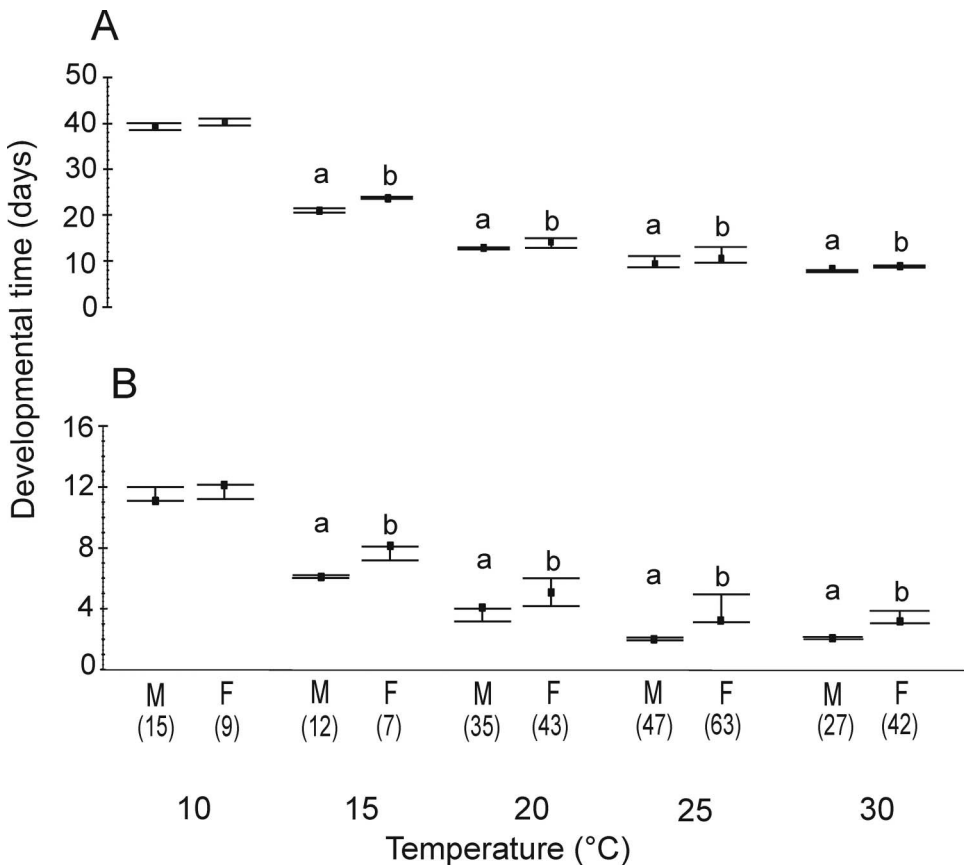


Figure 1. Median (Q1–Q3) developmental time (days) of males (M) and females (F) of *Culex pipiens* at five constant temperatures: (A) from larva I until adult emergence; (B) for larvae IV only. Within each temperature and life stage, medians followed by different letters are significantly different ( $P < 0.05$ , Mann–Whitney  $U$ -test). Numbers of individuals that emerged as male or female are indicated in parentheses.

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The predicted lower and upper thermal thresholds were, respectively, 8.4 and 34.4°C for males, and 9.8 and 34.2°C for females. The estimated optimum rearing temperature for males was 28.5°C, with a maximum development rate of about 0.125 days<sup>-1</sup>. The estimated optimum rearing temperature for females was also 28.5°C, with a maximum development rate of about 0.117 days<sup>-1</sup>. In the linear portion of the curve (15–30°C), the development rate was described by the degree-day model; a good fit was obtained for both sexes (males:  $F_{1,118} = 475.9$ ,  $P < 0.01$ ,  $R^2 = 0.80$ ; females:  $F_{1,153} = 329.51$ ,  $P < 0.01$ ,  $R^2 = 0.68$ ). The estimated lower threshold for male development was 5.2°C and the thermal threshold was 186.5 degree-days; for females, these parameters were 5.5°C and 199.5 degree-days, respectively (Figure 2).

### Wing length

The wing length of males and females was affected by the rearing temperature (Figure 3). For both sexes, the quadratic curve explained a higher percentage of the variability in comparison to the linear and logarithmic curves. The resulting model was  $Y = 3.84 - 0.005 * T^{\circ} - 0.001 * T^{\circ 2}$  ( $n = 75$ ,  $r^2 = 0.840$ ) for males, and  $Y = 4.225 + 0.026 * T^{\circ} - 0.002 * T^{\circ 2}$  ( $n = 86$ ,  $r^2 = 0.838$ ) for females. The linear model explained 82.2 and 79.9%, and the logarithmic model 74.7 and 70% for males and females, respectively.

The wings of females were always significantly longer than those of males within the range of rearing temperatures registered (Figure 3).

### Discussion

The rearing temperature had a significant effect on the survival of pre-imaginal stages of *C. pipiens*. Most of the individuals reached adulthood at 25°C, suggesting that it is the most favourable rearing temperature. This result is consistent with field studies carried out in Buenos Aires City that reported high abundances of *C. pipiens* larvae during the summer, in temporary rain pools at mean weekly temperatures between 20 and 27°C (Fischer and Schweigmann 2004) and in artificial containers filled with water at temperatures between 22 and 25°C, within a cemetery (Vezzani and Albióccco 2009).

The developmental time of *C. pipiens* was also affected by temperature. All stages showed a trend towards decreasing developmental time with increasing temperature. A similar relationship was observed by other authors (Madder et al. 1983; Rueda et al. 1990; Ribeiro et al. 2004). Although the 30°C developmental time in this study was close to those reported in these works, some differences were observed. For example, the total developmental time at 15°C was between 6 and 10 days shorter than those reported by Madder et al. (1983) and Rueda et al. (1990), and approximately one-half that recorded by Ribeiro et al. (2004). These differences may be the result of distinct experimental conditions (i.e. larval density, diet, photoperiod) and the different mosquito populations used by different authors. In addition, the lack of genotypic variability in our work [because within several treatments (7, 10 and 15°C) the larvae came from the same egg rafts] must be considered. A significant effect of genotype (family) on development rate of *C. pipiens*, besides temperature, has been reported Mpho et al. (2002).

Mosquitoes show sexual dimorphism usually involving developmental time and body size, among other characters (Clements 1992). In this study, sex differences in the

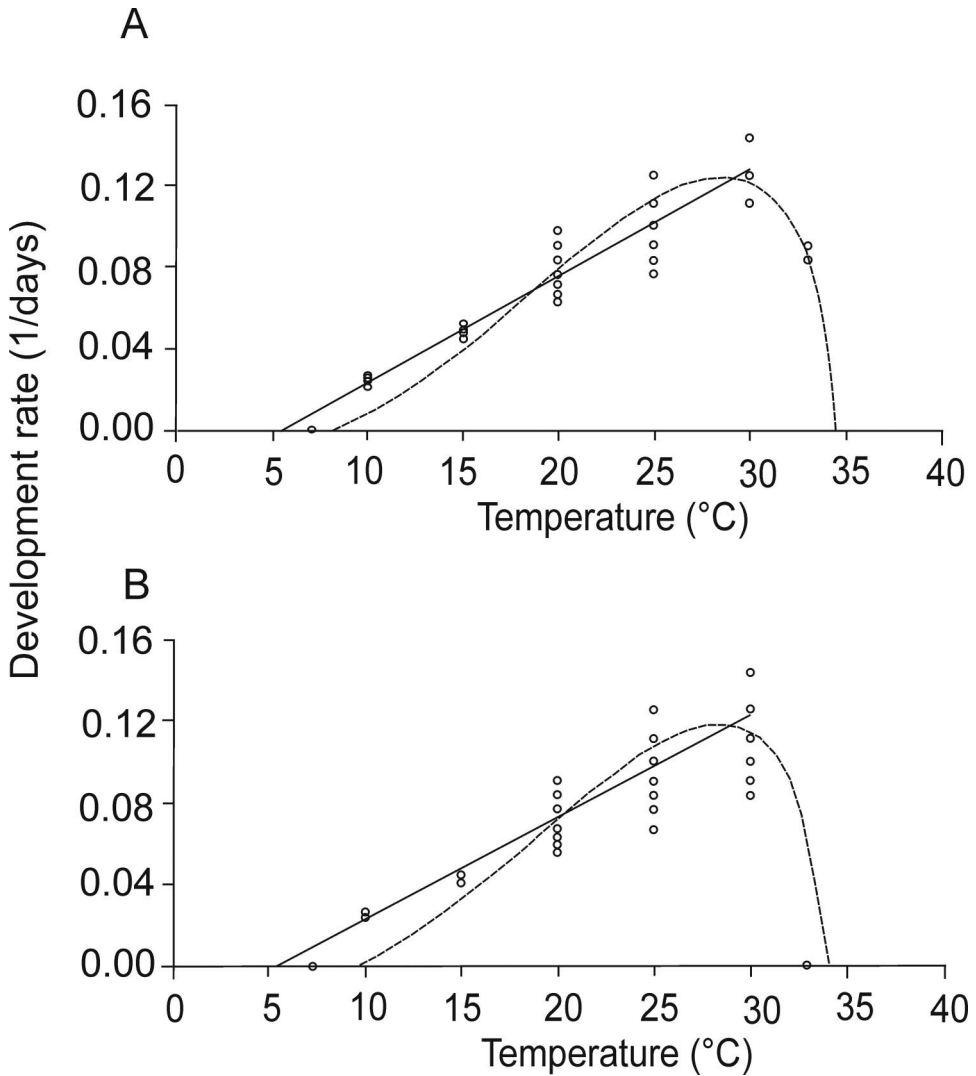


Figure 2. Temperature-dependent rate of development in male (A) and female (B) *Culex pipiens* from larva I until adult emergence: observed data (open circles) fitted to the Briere model (dotted line) and degree-day model (solid line).

developmental time were observed between 15 and 30°C; females required more time to reach the adult stage than males because of a longer larva IV stage. This difference could be related to the fact that many internal structures that originate the adult characters develop at this stage (Clements 1992). The wings of females were longer than those of males even when both sexes had a similar developmental time at 10°C. In culicids, wing length is considered a good estimator of body size (Christophers 1960; Clements 1992) and a direct relation between wing length and female fecundity has been documented in many species (Briegleb 1990ab; Lounibos et al. 1995; Armbruster and Hutchinson 2002). In organisms whose fecundity is directly related to body size,

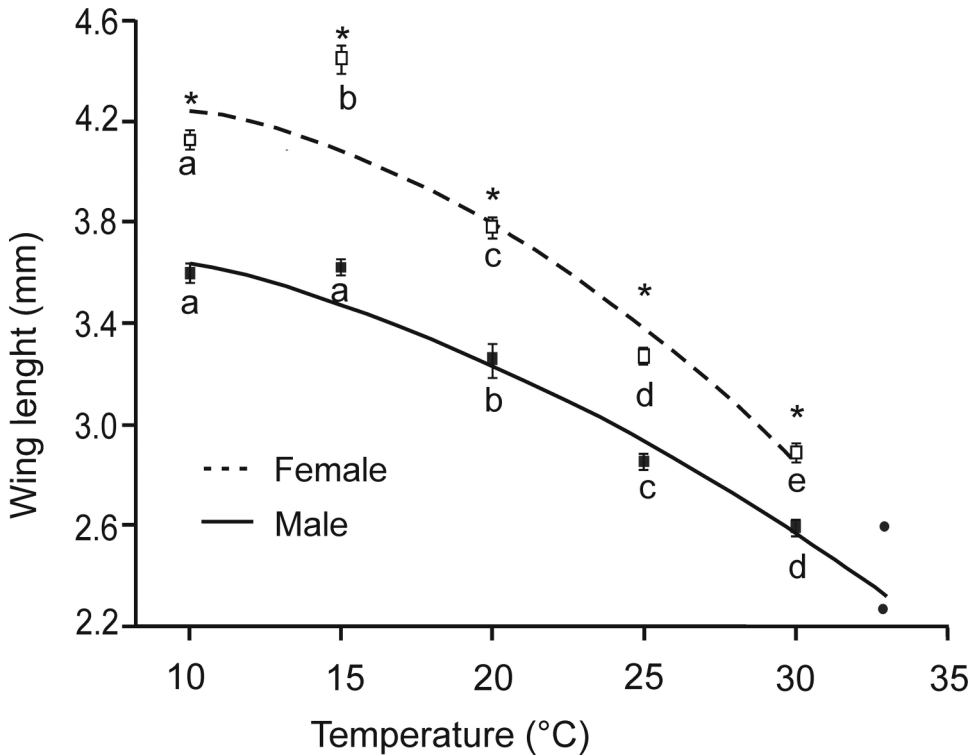


Figure 3. Mean ( $\pm$  SEM) wing length of males (filled squares) and females (open squares) of *Culex pipiens* reared under constant temperature conditions. Asterisks indicate significant differences between sexes (Student's *t*-test,  $P < 0.05$ ). For each sex, means with different letters are significantly different (analysis of variance, Tukey's test,  $P < 0.05$ ).

selection pressure is expected to favour larger females. Results obtained in this study indicate that *C. pipiens* females may be subjected to such selection pressure and that body size may depend on sex rather than on developmental time. On the other hand, if a rearing temperature of 10°C can be considered a stress situation, because females had smaller wings than when reared at 15°C (Figure 3) and males do not emerge earlier than females, then our results might suggest that the effect of temperature on these traits differs between sexes. Sex-specific responses have been reported in other mosquito species (Bradshaw et al. 1997; Bedhomme et al. 2003). Adult size and developmental time are key life-history traits, and the variation of each one usually has important consequences for fitness, as has been emphasized by Nylin and Gotthard (1998) in their review of life-history plasticity.

Results from the non-linear model showed that the estimated optimum temperature and the upper thermal threshold were similar between sexes, the lower thermal threshold was slightly lower in males (8.4°C) than females (9.8°C). Developmental thresholds at low and high temperatures showed values in accordance with the experimental data of the current study. However, although we observed only two individuals that reached the adult stage at 33°C, the upper thresholds (34.4°C in males and 34.2°C in females) could be wrong because other works reported adult emergence at 35°C



(Rueda et al. 1990), and 37°C (Mpho et al. 2002). To more accurately estimate the thresholds, additional data at high and low temperatures, including more experimental temperatures and more individuals, must be collected.

The threshold temperature estimated by the Degree-day model was lower than 7°C, where no individual reached adulthood. This result disagrees with the threshold estimated by the Brière model, probably as a result of the non-linearity of the development at low temperatures close to the threshold (Brière et al. 1999). On the other hand, the threshold temperatures reported by other authors (Madder et al. 1983; Almirón et al. 1996; Ribeiro et al. 2004) are about twice those recorded in this study, whereas the thermal constant obtained (186.5 and 199.5 degree-days, for males and females, respectively) was within that reported by these authors (132–252.5 degree-days). As mentioned before, the greater differences in this study compared with the previous work on developmental time were observed at 15°C, which could explain why the threshold was different. These differences may result from different adaptations to living at latitude 34° S and with an annual mean temperature of 17.6°C (SMN 2009). However, differences in experimental conditions must also be considered.

In mosquitoes, pre-imaginal development time and survival rate determined, in part, their abundance and distribution. In addition, female wing length could be used as an indicator of fecundity (Briegel 1990ab; Lounibos et al. 1995; Armbruster and Hutchinson 2002), and has also been related to biting rates (Strickman and Kittayapong 2003). All of these variables are related to the main parameters that describe the capacity of a mosquito population to act as vectors. In contrast, all components of their capacity to act as vectors are highly sensitive to climate (particularly to temperature), so it seems likely that even small alterations in global climate could cause significant changes in both the overall incidence and duration of transmission and the geographical distribution of disease transmission (Kovats et al. 2001). Hence, the effect of temperature on development time, survival rate and adult size of vectors like *C. pipiens* could be useful information to include in predictive models of capacity to act as vectors, or population dynamics models for the purposes of control. Our data have been obtained in controlled laboratory conditions; future research should involve field trials to validate the experimental results. Finally, this study emphasizes the importance of using local data in models that predict the population dynamics of this mosquito or for designing effective control strategies.

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