Effect of different thermal conditions on the pre-imaginal biology of *Culex apicinus* (Philippi, 1865) (Diptera: Culicidae)

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ABSTRACT: The time of development and survival of the pre-imaginal period and the adult body size of *Culex apicinus* were analyzed in individuals reared from the 1st instar larva to adult emergence under laboratory and field conditions. In the laboratory, insects were exposed to three constant temperatures (15, 20, and 25° C) and a photoperiod of 14:10 (L:D). In the field, temperature and photoperiod were not manipulated; during the study period water temperature ranged between 15.5 and 24.2° C, and photoperiod changed from 13:11 to 14:10 (L:D). Survival to the imaginal stage at 15° C (85.4%) and in the field (88.8%) was higher than that at 20° C (45.8%) and 25° C (8.3%). Based on femur and wing length measurements, the mosquitoes reared under field conditions (mean water temperature = 20.5° C) were smaller than those laboratory-reared, even when the time of development in the field (29.5 – 36 days) was longer than that observed at 15° C (24 days), 20° C (15 days), and 25° C (11 days). Male emergence was earlier than that of females (protandry) only in the field. Although laboratory conditions included a photoperiod and temperature range comparable with the observed values in the field, the disagreements between field and laboratory results suggest that the characteristics examined in this work could be affected by the joint variation of several factors not controlled in field conditions, such as photoperiod, temperature regime, and/or food quality of larval habitat. ***Journal of Vector Ecology* 32 (1): 106-111. 2007.**

Keyword Index: Body size, culicids, developmental time, survival, water temperature.

INTRODUCTION

In most ectotherms, decreased temperatures reduce growth rate and usually result in delayed maturity at a larger size (Ray 1960, Atkinson 1994). Among insects, particularly mosquitoes, the temperature to which larvae are exposed during their development is one of the more important extrinsic factors affecting this period and may have major or minor consequences for the adult stage (Clements 1992). Several studies have shown the influence of the rearing temperature on the developmental rate and survival of mosquito larvae (Brust 1967, Trpis and Shemanchuk 1970, Madder et al. 1983, Buth et al.1990, Rueda et al. 1990, Reisen 1995, Mahmood and Crans 1998, Su and Mulla 2001, Briegel and Timmermann 2001), as well as on the body size, fecundity, and longevity of adults (Reisen 1995, Oda et al. 2002, Briegel and Timmermann 2001, Briegel 1990a, 1990b). Data obtained are frequently used to fit temperature-dependent development models (Madder et al. 1983, Rueda et al. 1990, Reisen 1995, Mahmood and Crans 1998, Briére et al. 1999) for epidemiological studies. Information involving the above mentioned variables has mainly been collected from studies conducted in the laboratory that occasionally included field trials (Stewart 1974, Madder et al. 1983, Focks et al. 1993, Buth et al. 1990) in order to evaluate the degree of agreement between laboratory and field results.

Culex mosquitoes have been incriminated in the transmission of several arboviruses both to humans and domestic animals (Horsfall 1955), and some of them are considered potential vectors of encephalitis viruses (Humeres et al. 1990). Some of these culicids have evolved highly domiciliary habits that enable them to act as a potential link between the natural environment and humans. On the other hand, the ornithophilic tendency of these mosquitoes makes them potential vectors of a variety of arboviruses that would be subsequently transmitted to humans (Forattini 2002).

Culex apicinus (Philippi, 1865) (Diptera: Culicidae) has been reported from Chile, Perú, Bolivia, and Argentina. In Argentina, its distribution includes the provinces of Catamarca, La Rioja, Jujuy, Córdoba, Santa Fe (Mitchell and Darsie 1985), and Buenos Aires (Ronderos et al. 1992). *Cx. apicinus* is exposed to a wide range of temperatures (and climatic regime) because of its wide geographic distribution, but the effect of this variable upon its life history is still unknown. This is an ornithophilic species (Almirón and Brewer 1995b), which is present during the four seasons (Almirón and Brewer 1995a), and whose larvae usually breed in highlands in small stream bed pools (Forattini 1965) and in artificial domestic or peri-domestic water bodies, such as abandoned swimming pools, water tanks, etc. (Almirón and Brewer 1994, 1996). All these factors reveal the potential sanitary importance of this mosquito and indicate the necessity for studies on unknown aspects of its biology.

The aims of the present study were to determine the time of development, the pre-imaginal survivorship, and the adult body size of *Cx. apicinus* reared in field and laboratory conditions. In addition, we assessed the extent to which laboratory research reflects what happens in natural habitats. Our results will contribute to the knowledge of the basic biology of this species, allowing the understanding of some aspects of its ecology that are useful for further epidemiological studies.

MATERIALS AND METHODS

Egg rafts of *Cx. apicinus* were collected at a house in a Buenos Aires, Argentina suburb (34° 33' S, 58° 33' W), in a cement pool of 5,000 liters capacity, which was not in use but contained approximately 1,450 liters of water. Egg rafts were maintained until the eclosion in individual plastic containers with dechlorinated tap water. Within 12 h of eclosion, between 30 and 48 1st instar larvae were randomly removed from each raft and assigned to one of the two experimental groups: field or laboratory.

Field conditions

The research took place from October 8 to November 21, 2002 in three enclosures located in the same site where the eggs were collected. Water level in the pool was approximately 60 cm and remained constant during the experiment. The photoperiod changed from 13:11 to 14:10 (L:D) during the study period.

The enclosures consisted of cylinders 23 cm in diameter by 60 cm in length, with a net of 350 μm mesh size. The lower end was closed and weights were put inside to prevent the cage from moving. The upper end was attached to a float with a thin clear net that allowed the passage of light but prevented the escape of emerging adults. The available food was organic matter filtered from the water contained in the same pool. Thirty 1st instar larvae belonging to the same raft were put in a different enclosure. Previous laboratory experiments showed that at this larval density, competition has no noticeable effect upon the analyzed variables. The number of emerged adults was counted daily. Water temperature was monitored using a maximum/minimum digital thermometer that was reset daily. The mean rearing temperature was calculated from the average of the maximum and minimum temperatures of the water registered daily from the beginning of the trial (day 1) until the emergence of the last adult.

Laboratory conditions

Larvae were reared in incubators maintained at three constant temperatures: 15, 20, and 25° C. The photoperiod was 14:10 (L:D). Each 1st instar larva was placed in a plastic cup (3 cm diameter by 5 cm length) that contained dechlorinated tap water and was exposed to one of the three thermal baths until adult emergence. Larvae were fed according to Gerber (1970). Each individual was monitored daily, at the same hour, to record larval stage, molting, mortality, or date of imago emergence. Individuals that died in the pupal stage were sexed under a stereoscopic microscope.

In both types of experiments, the adults were killed by freezing (-18° C). Later, each specimen was examined under stereoscopic microscope with an ocular micrometer. Measurements of forefemur and wing lengths (from the base of costa vein to distal extreme of R3 vein, excluding the fringe setae), were used as indicators of imaginal body size (Christophers 1960, Clements 1992).

Statistical analyses

Survivorship from 1st instar larvae to adult emergence was calculated as the total number of adults divided by the number of 1st instars at the start of each experiment. The results obtained in each type of trial were compared using the test of proportions for independent samples (Fleiss 1981). Differences in pre-imaginal development time (in days) among rearing temperatures were tested by a Kruskal-Wallis non-parametric analysis of variance. Dunn's method was used to obtain the multiple comparisons.

For each sex, differences in wing and femur lengths were analyzed by means of one-way analysis of variance and Tukey test for unequal samples. For each thermal condition, wing length and forefemur length within sex were compared by means of the Student's *t*-test. To evaluate the distribution of frequency of the emergence of males and females, the Kolmolgorov-Smirnov test for two samples was used (Zar 1984).

RESULTS

In the field experiments, the maximum, minimum, and mean (± SE) daily water temperatures were 24.2, 15.5, and 20.5 ± 0.36° C, respectively.

The proportion of individuals that reached the adult stage in the laboratory at 20° C (22/48) and 25° C (4/48) was significantly lower than that observed at 15° C (41/48) and in the field (80/90) ($\chi^2 = 89.01$, $p < 0.001$). In the field study, survival among rafts was not homogeneous, the proportion of individuals that reached the adult stage from Rafts 2 (27/30) and 3 (30/30) was greater than from Raft 1 (23/30) ($\chi^2 = 8.32$, $p < 0.025$).

The sex ratio of the emerged adults was not significantly different under any of the study conditions. Males and females emerged in approximately equal numbers (1:1 sex ratio) in both laboratory and field. In the laboratory, this proportion was maintained even when the sex of dead pupae was considered.

In all trials where both sexes were present, the females had significantly larger wings and femurs than the males (Table 1). Within each sex the wing length (Table 1a) showed significant differences. Males reared at 20° C had larger wings than those raised in the field and shorter wings

Table 1. Mean wing (a) and femur (b) lengths separated by sex of *Culex apicinus* reared in field conditions (F) and in laboratory under three constant temperatures (L). Temp.= rearing temperature, N = number of individuals, CV = coefficient of variation.

a.

		Mean wing length (mm)^a				
Condition	Temp. (ºC)	Males (n)	CV (%)	Females (n)	CV (%)	p^b
L	15	4.42 (21)a	2.1	5.32 (20)a	2.3	< 0.001
L	20	3.83 (9)b	1.5	4.57 (12)b	3.0	< 0.001
L	25	ND	ND	4.34 (3)bc	3.5	NT
F	20.5 ± 0.36	3.50 (36)c	5.26	4.19 (44)c	4.6	< 0.001
		(*) $F_{2, 63} = 315.92$; $p < 0.001$		$F_{3, 75} = 204.73$; $p < 0.001$		

b.

		Mean femur length (mm)^a				
Condition	Temp.(ºC)	Males (n)	CV (%)	Females (n)	CV (%)	p^b
L	15	2.65 (21)a	3.8	2.88 (20)a	7.0	< 0.001
L	20	2.38 (10)b	3.0	2.51 (12)b	5.7	< 0.001
L	25	ND	ND	2.36 (4)bc	2.9	NT
F	20.5 ± 0.36	2,06 (36)c	5.8	2.27 (44)c	5.0	< 0.001
		(*) $F_{2, 64} = 305.48$; $p < 0.001$		$F_{3, 76} = 182.52$; $p < 0.001$		

[a] Means followed by the same letter within each sex are not significantly different (p > 0,05; Tukey's test).

[b] Probabilities from Student's t-test comparing male and female mean lengths.

(*) Results of one-way ANOVA between 15ºC, 20ºC, 25ºC and variable temperature.

ND: no data, NT: not tested

than those reared at 15° C, and the same result was observed for the wings of females. Femur length (Table 1b) showed a similar pattern. In males raised at 20° C it was longer than those from the field and shorter than those reared at 15° C. The same result was obtained for female femur size. Higher wing length CVs were observed in the field-reared mosquitoes, like male femur length CVs, while the larger female femur length CV was observed at 15° C (Table 1).

Males developed faster at 20° C than at 15° C, and in both cases were faster than those developing in the field. Among the females, the time of development at 15, 20, and 25° C was similar, but they required fewer days to complete the pre-imaginal period than under natural conditions. Development time for males was similar to those of females at 15° C, while at 20° C and in the field the males developed faster (Table 2). At 25° C no males emerged. In the field, development time varied among the rafts. The individuals of Raft 1 reached the adult stage in less time. Nevertheless, within each raft the males were always faster (Raft 1: $H(1, N_{♂}: 11, N_{♀}: 13) = 16.404$, $p < 0.001$; Raft 2: $H(1, N_{♂}: 11, N_{♀}: 16) = 12.068$, $p < 0.001$; Raft 3: $H(1, N_{♂}: 15, N_{♀}: 15) = 6.446$, $p < 0.05$).

The time between the first and the last emergence was one and two days for males and females, respectively, at 20° C. This interval increased to four days for both sexes at 15° C, while that in field oscillated between seven and 15 days. The distribution of emergence frequency (Figure 1) was similar in both sexes in our laboratory experiments (KS tests, 15° C: $p > 0.1$, 20° C: $p > 0.1$). However, in the field, males emerged significantly earlier than females (protandry) (KS test, Field: $p > 0.001$), and each raft tested maintained this difference (KS tests, Raft 1: $p < 0.001$, Raft 2: $p < 0.001$, Raft 3: $p < 0.05$).

DISCUSSION

We found that different rearing conditions can significantly affect different aspects of *Cx. apicinus* biology. The high survival of *Cx. apicinus* registered in our field experiments (> 80%) may be related to the season of the year. Almirón and Brewer (1995a) have observed peaks of abundance of *Cx. apicinus* in Córdoba Province during September and October, and the Spring may provide favorable conditions for the proliferation of this species. It is also possible that the heterogeneous survival observed among rafts could be due to individual variability.

In the laboratory, the exposure to constant temperatures throughout development affected survival in different ways. While at 15° C the emergence success was as high as in natural conditions, at 20° C it decreased by almost half and declined nearly 10-fold at 25° C. In this way, a difference of 5-10° C would affect in a negative way the survival of *Cx. apicinus*. Since this species is present throughout the year (Almirón and Brewer 1995a), it is likely that immatures are exposed to temperatures of 20° C during the summer months, but only during a few hours per day. It is possible

Table 2. Median development time separated by sex of *Culex apicinus* reared in field conditions (F) and in laboratory under three constant temperatures (L). Temp.= rearing temperature, N = number of individuals.

Condition	Temp. (ºC)	Median development time (days)[a] Males (n)	Females (n)	p[b]
L	15	24.0 (23)a	24.0 (18)a	0.27
L	20	15.0 (10)b	15.0 (12)a	0.018
L	25	ND	11.0 (4)a	NT
F	20.5 ± 0.36	29.5 (36)c	36.0 (44)b	< 0.001

[a] Medians followed by the same letter within each sex are not significantly different ($p > 0.05$; Dunn's test).
[b] Probabilities from the Kruskal-Wallis non-parametric test comparing male and female development time.
ND: no data, NT: not tested.

that a trial at a constant temperature of 20° or 25° C does not adequately reflect the tolerance of this species to seasons or periods with temperatures near this value. This would explain, at least in part, why the field survival was high, even when the temperature in this condition reached peaks of 24.2° C. It has been observed that the sex ratio can vary with the temperature (Fava et al. 2001, Oda et al. 2002). In our study, the temperature, within the analyzed range, does not seem to affect the sex ratio, because this was approximately 1:1 in all experiments even when the sex of the dead pupae was considered. Although the sex of this stage could only be recorded in the laboratory, our observations suggest there is no differential mortality between the sexes, at least during this moment of the pre-imaginal life.

The inverse relationship between temperature and development time and adult body size obtained in the laboratory has already been observed in other species of mosquitoes (Brust 1967, Madder et al. 1983, Rueda et al. 1990, Reisen 1995, Briegel and Timmermann 2001, Su and Mulla 2001). As a result, at higher temperatures the individuals would develop in less time and would be smaller. In this work we observed a contrary result, since in field conditions the larvae were exposed at a mean daily temperature only 0.5° C above what they experienced at 20° C, however the pre-imaginal period was prolonged and body size decreased. Similar results were obtained when

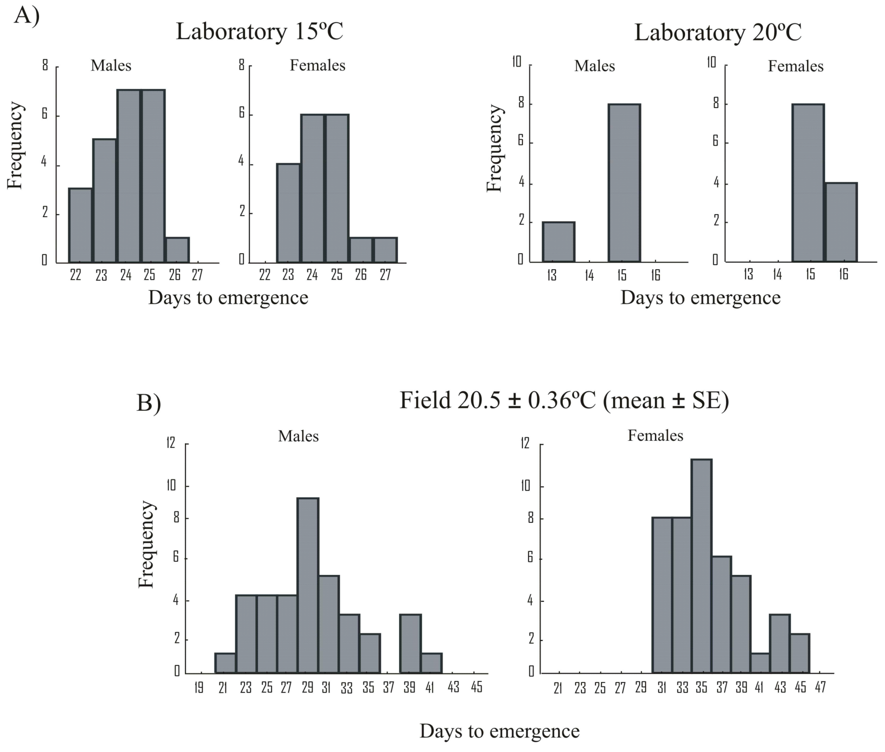

Figure 1. Distribution of emergence times of males and females of *Cx. apicinus* reared in laboratory at two constant temperatures (A) and in the field (B). Protandry (males emerge first) is significant only under field conditions (Kolmolgorov-Smirnov test; $p < 0.05$).

comparing field conditions with experiments at 25° C.

This result could be linked to daily fluctuations of the temperature, among other factors. When the immatures are exposed to constant temperatures, the development rates differ from those observed when water temperatures fluctuate (Clements 1992). The constant temperature may be stressful to the organism (e.g., Blanckenhorn 1997) or daily fluctuation of temperature may trigger physiological processes that influence insect development. On the other hand, field-reared mosquitoes ate the natural food present in the breeding site. Therefore, food is an uncontrolled factor. In fact, decreased food quality (or availability) generally leads to later maturation at smaller sizes (Berrigan and Charnov 1994).

In most species of mosquitoes, sexual dimorphism also included differences in the size and time of development. Typically, males are smaller than females and emerge earlier (Christophers 1960, Clements 1992). In all the trials males were smaller than the females, but this phenomenon of protandry was observed only under natural conditions (Figure 1). In the laboratory, despite the overlaping emergence of both sexes, males were always smaller. A similar result was reported by Brust (1967) in *Culiseta inornata* and *Aedes nigromaculis*, and proposed genetic differences were linked to sex to explain this size inequality. Studies of other species of mosquitoes have shown that the size of the females is positively correlated with fecundity (Briegel 1990a, Briegel 1990b, Blackmore and Lord 2000, Armbruster and Hutchinson 2002), which suggests the existence of a selection pressure favoring bigger females. Protandry is a very common phenomenon in insects with discrete generations (Thornhill and Alcock 1983). This temporal adjustment at the moment of the emergence is usually explained in terms of reproductive strategy allowing males to maximize the number of matings (Wiklund and Fagerström 1977, Carvalho et al. 1998). In addition, male mosquitoes do not reach sexual maturity at emergence, for example, the genitalia acquires the correct orientation for copulation one or two days after emergence (Snodgrass 1957, Clements 1992). It is possible that an earlier emergence could be a more important factor than body size for reproductive success of males. As mentioned before, protandry was only observed in the field trials. Clearly, other studies are necessary to understand the mechanism involved in this phenomenon and analyze its effect upon mating success.

Because the mean temperature in the field (20.5° C) varied from the laboratory temperatures, we would have expected intermediate values of the biological variables, which would be close to those at the similar constant temperature of 20° C. However, this was not observed for the body size and developmental time. In brief, the comparison between laboratory and field results suggest that the studied variables are affected by daily temperature variation and/or food quality (or availability) of the larval habitat and/or photoperiod change. The disagreement between both data types shows the importance of considering the influence of field or laboratory conditions on mosquito biological features, particularly when these are used in epidemiological models.

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