SHORT COMMUNICATION

Improvement of a test-chamber for behavioral studies on adult females of *Aedes aegypti* (Linnaeus) (Diptera, Culicidae)

Isaías Cabrini[1,3] & Carlos Fernando S. Andrade[2]

[1]Instituto de Biologia, Pós-Graduação Departamento de Parasitologia, Universidade Estadual de Campinas, 13084-971, Caixa Postal 6109 Campinas-SP, Brasil. isaias_c04@yahoo.com.br.
[2]Instituto de Biologia, Departamento de Zoologia, Universidade Estadual de Campinas, 13084-971, Caixa Postal 6109 Campinas-SP, Brasil. cfeandra@unicamp.br
[3]Bolsista CAPES

ABSTRACT. Improvement of a test-chamber for behavioral studies on adult females of *Aedes aegypti* (Linnaeus) (Diptera, Culicidae). A test-chamber (K&L-Chamber) made of cardboard and acrylic plastic, and consisting in four sections (A, B, C and D) was developed by Klowden & Lea (1978) for *Aedes aegypti* host-seeking behavior studies. Later, Foster & Lutes (1985) also used an identical chamber to successfully evaluate the efficacy of electronic repellers. It was described here a modified K&L-Chamber for behavioral studies of *Ae. aegypti* adults. The chamber was made in polystyrene, consisting of three sections (A, B and C) and using a human hand and a fluorescent lamp as stimulus to attract the mosquitoes. The suitability of the present test-chamber was validated assaying 80 replicates and releasing 10 *Ae. aegypti* females in each replicate. The females were released in the section A and allowed to fly to the section C. A mean of 96.0% (s.e. 0.213) *Ae. aegypti* females successfully reached section C. The present test-chamber is cheaper and easier to handle and as efficient as K&L-Chamber, when compared to Foster & Lutes (1978) that noticed 93.8% of *Ae. aegypti* reaching the trap section.

KEYWORDS. Attraction; behavior; mosquito; olfactometer.

RESUMO. Aperfeiçoamento de uma câmara-teste para estudos comportamentais com fêmeas adultas de *Aedes aegypti* (Linnaeus) (Diptera, Culicidae). Uma câmara-teste (Câmara K&L) feita de papelão e plástico acrílico, e consistindo em quatro seções (A, B, C e D) foi desenvolvida por Klowden & Lea (1978) para estudos sobre o comportamento de busca pelo hospedeiro por *Aedes aegypti*. Foster & Lutes (1985) também usaram uma câmara idêntica para, com sucesso, avaliar a eficácia de repelentes eletrônicos. Aqui foi descrita uma Câmara K&L modificada (Câmara K&L-Unicamp) para estudos comportamentais com adultos de *Ae. aegypti*. A câmara foi feita com isopor, consistindo em três seções (A, B e C) e usando uma mão humana e uma lâmpada fluorescente para atrair os mosquitos. A adequação da presente câmara-teste foi validada por meio de 80 repetições, cada uma com liberação de 10 fêmeas de *Ae. aegypti*. As fêmeas foram liberadas na seção A e permitidas voar para a seção C. Uma média de 96,0% (e.p. 0,213) de fêmeas de *Ae. aegypti* chegaram a seção C. A Câmara K&L - Unicamp é de menor custo, mais fácil de manusear e é tão eficiente quanto a Câmara K&L, quando comparado a Foster & Lutes (1978) que descreveram que 93,8% de *Ae. aegypti* chegaram a seção armadilha.

PALAVRAS–CHAVE. Atração; comportamento; mosquito; olfatômetro.

The use of olfactometers and test-chambers for adult mosquitoes studies is a convenient way to obtain behavioral data. Some systems models (square or rectangular, two coupled funnels or tubes forming a 'Y') have been used under controlled odors release, temperature and relative humidity conditions (Gouck & Schreck 1965; Mboera *et al.* 1998; Posey *et al.* 1998; Geier & Boeck 1999; Charenviriyaphap *et al.* 2002). In all these systems mosquitoes are allowed to respond to the stimulus of hosts, insecticides or other attractant or repelling substances (Smith *et al.* 1970; Price *et al.* 1979; Carlson *et al.* 1992).

The aim of the present work is the improvement and validation of a previous described test-chamber in order to make it cheaper and easier to handle.

Once developed by Klowden & Lea (1978) (K&L-Chamber) for *Ae. aegypti* (Linnaeus, 1762) host seeking studies, the same test-chamber was also used by Foster & Lutes (1985) to evaluate the efficacy of commercial electronic devices in repelling mosquitoes. K&L-Chamber consists in four sections, described as: a 30 cm^3 mosquito holding cage (A) (*sic*), a 120 cm x 30 cm^2 flight section (B), a 20 cm long x 18 cm diameter mosquito trap (C) and a host compartment (D). A fan sucking the air is coupled to the section A carrying attractants as the odors from a human arm and the breath (section D). The mosquitoes are released in the section A, and allowed to fly through the section B reaching the section C where they are trapped. The mosquitoes are not allowed so to reach the human arm. Foster & Lutes (1985) evaluated the efficacy of electronic repellers switched on and switched off. They released fresh groups of about 50 mosquito females, in five replicates, for

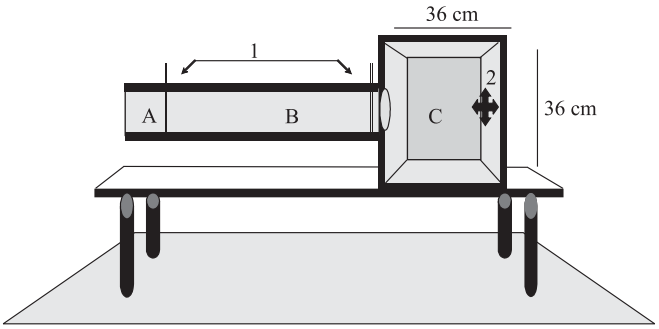

Fig. 1. Lateral view of K&L-Unicamp Chamber showing the sections A, B and C, the removable disks between the sections A/B and B/C (1), the position of fan directed to the tube (2).

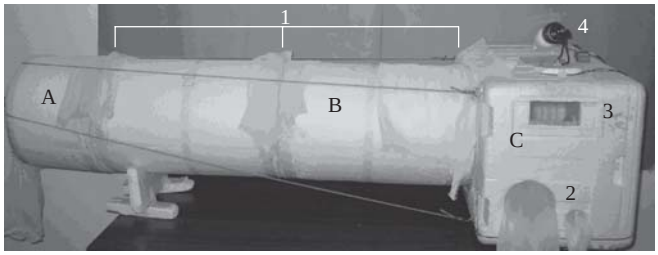

Fig. 2. K&L-Unicamp Chamber showing the sections A, B and C with the lycra fabrics separating A/B and B/C sections (1), the apertures to insert human hand (larger) and the stick with a feather (smaller) (2), the window at section C for observation (3) and the light source above the section C (4).

periods of ten minutes, counting the number of those, which successfully reached section C.

The test chamber presently developed (K&L-Unicamp Chamber) was made of polystyrene. The chamber consists in a tube 1.25 m long x 20 cm diameter connected to a cage measuring 36 x 36 x 57 cm (named section C). The distal part of the tube (section A) measuring 25 cm long and the proximal (section B) measuring 100 cm long (Figure 1, Figure 2).

It was used a personal computer 12V fan (Yate Loon, model DC FAN D80SH-12), turned at 3V current, to gently blow the air from section A to section C. The air velocity produced by the fan was determined using an anemometer (Dwyer®) (Figure 1). An aperture with a glass window of 15 x 15 cm was made at the upper wall of the section C allowing illumination from the light source. Initially it was evaluated a 9W fluorescent day light lamp (FLC®) and later a 24W fluorescent lamp (Ecolume®). The illumination provided by the lamps was measured using a digital luximeter (Lutron LX-107) at a distance of 20 cm from the light source. The distal opening of the tube (section A) was closed with a sleeve of fabric and removable disks were placed between sections A/B and B/C in order to contain the mosquitoes respectively before and after the experiments. Two apertures were installed at the lateral wall of the section C. One of them to allow the insertion of the human hand also as attractive stimulus and the other to insert a stick with a feather at the tip. The stick was used to push the landing mosquitoes avoiding bites. To avoid mosquitoes escaping those apertures were closed by sleeves (Figure 2).

A previous evaluation of 10 replicates with 10 *Ae. aegypti* females each was made to compare two different lamps (9W fluorescent day light lamp - FLC® and 24W fluorescent lamp - Ecolume®).

The validation of the present chamber was proceeded in 80 replicates. Fresh groups of 10 *Ae. aegypti* females were obtained from a colony and maintained in the section A during two minutes. The fan and the light source were than turned on and the human hand was inserted in the section C. After two minutes, the lycra fabrics separating sections A/B was removed allowing the mosquitoes to fly towards the section C. After the 10 min evaluation periods the removable disks separating the sections were positioned in order to confine the mosquitoes in the cage or in the sections A or B, from where they was collected with an electric aspirator.

The analysis of variance test was used to compare attractive efficiency of the lamps and the number of mosquito females attracted to the sections. The association of 9W fluorescent day light lamp and human hand resulted in a mean of only 60.0% (s.e. 0.832) while the association 24W fluorescent lamp and human hand resulted in 96.0% (s.e. 0.213) of females attracted to the section C ($F=51.428$; s.e. 0.522; $p<0,05$). In the comparison between the sections to second association there was significant difference (between A and C, $F= 9733.074$, s.e. 0.188, $p<0.05$; B and C, $F= 11160.797$, s.e. 0.176, $p<0.05$). When assaying *Ae. aegypti* females, Foster & Lutes (1985) obtained 93.8% of mean attraction to the trap section, for the electronic devices switched off. The present results (96.0%) compared to that of Foster & Lutes (1985), with the devices turned off, demonstrated similarity or better responses for *Ae. aegypti* females.

In the present study, the mosquitoes were allowed to reach the human hand, and during the experiments it was possible to study the bite attempts too.

It could be noted that the 4.8 Km/h wind produced by the fan, formed an air current enough to direct the mosquito females through the tube. This air emission seems to appropriate spread the odors through the system, as efficient as the suction current used by Klowden & Lea (1978). It can be concluded therefore that the present chamber (K&L-Unicamp Chamber) showed to be as suitable to assay *Ae. aegypti* as that developed by Klowden & Lea (1978) because the results here obtained demonstrate that the mosquitoes responded to the attractive stimulus (human hand and 24W fluorescent lamp). Thus, evaluations of products against mosquitoes could be done with security in the attainment of data.

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