

Short Communication

Wingbeat Frequency-Sweep and Visual Stimuli for Trapping Male *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Combinations of female wingbeat acoustic cues and visual cues were evaluated to determine their potential for use in male *Aedes aegypti* (L.) traps in peridomestic environments. A modified Centers for Disease control (CDC) light trap using a 350–500 Hz frequency-sweep broadcast from a speaker as an acoustic stimulus, combined with a black poster-board half-cylinder behind the trap as a visual stimulus, captured a significantly greater proportion of males in a laboratory arena during daylight than a CDC trap with the visual stimulus alone or a CDC trap alone without stimuli. Traps of each treatment type captured relatively more males when they were placed at darker positions in the arena. Potential applications are discussed for the incorporation of these findings into trapping programs to reduce transmission of human pathogens vectored by *Ae. aegypti*.

Key words: attraction, ZIKV, dengue, chikungunya, yellow fever

Observations of male mosquito attraction to sound captured scientific interest in the late 1800s (Belton 1994) and were followed later by development of acoustic traps (Kahn and Offenhauser 1949, Offenhauser and Kahn 1949) to reduce populations of important disease vectors. Until recently, however, mosquito acoustic trapping failed to elicit strong practical interest (Silver 2008) because males respond only over short distances, and male trapping has little immediate effect on rates of female biting and disease transmission (Wishart and Riordan 1959). Acoustic trapping efforts therefore focused on particular research questions such as the surveillance of male escapees in insectaries (Fay 1968), the potential for reduction of small, isolated mosquito populations (Ikeshoji et al. 1985, Ikeshoji and Yap 1990), optimization of trapping methodology (Ikeshoji and Ogawa 1988, Kerdpibule et al. 1989), monitoring of species isolation or mating dynamics (Duhrkopf and Hartberg 1992, Gibson and Russell 2006, Cator et al. 2011), or optimal timing of management efforts (Mankin 1994, Raman et al. 2007). Interest in acoustic trapping of male mosquitoes (Stone et al. 2013, Johnson and Ritchie 2016, Balestrino et al. 2016) has been rejuvenated, however, by innovative control methods that involve genetic or biological modifications of males (Harris et al. 2011, Hoffmann et al. 2011, Alphey et al. 2013, Dutra et al. 2016), or employ males to

target transfer of pesticides or biological control agents to females or breeding sites (Mains et al. 2015, Garza-Hernández et al. 2015).

In addition, the trapping technology has decreased in size and cost and has improved in the capability to manipulate the spectral and temporal patterns of signals easily (Ikeshoji and Ogawa, 1988, Mankin 2012, Mankin et al. 2016). Johnson and Ritchie (2016) inexpensively produced a continuous 484-Hz tone to trap male *Ae. aegypti*. Wishart and Riordan (1959), studying *Ae. aegypti*, and Ikeshoji et al. (1985), studying *Culex* spp., reported increased trap captures when the signals were intermittent rather than continuous. Until recently, however, it has been difficult to produce chirps (frequency-sweeps) inexpensively, and there have been few reports of the use of chirps to trap male mosquitoes in field studies.

To consider whether the improved sound production technology might enable improved trapping capability, a study was conducted to optimize effectiveness of acoustic stimuli in *Aedes aegypti* (L.) traps in combination with other known stimuli in peridomestic environments, potentially to reduce the threat of globally spreading pathogens (Powell 2016), including Zika (Petersen et al. 2016) and dengue (Rodhain and Rosen 2001), as well as chikungunya and yellow fever arboviruses (van den Hurk et al. 2012).

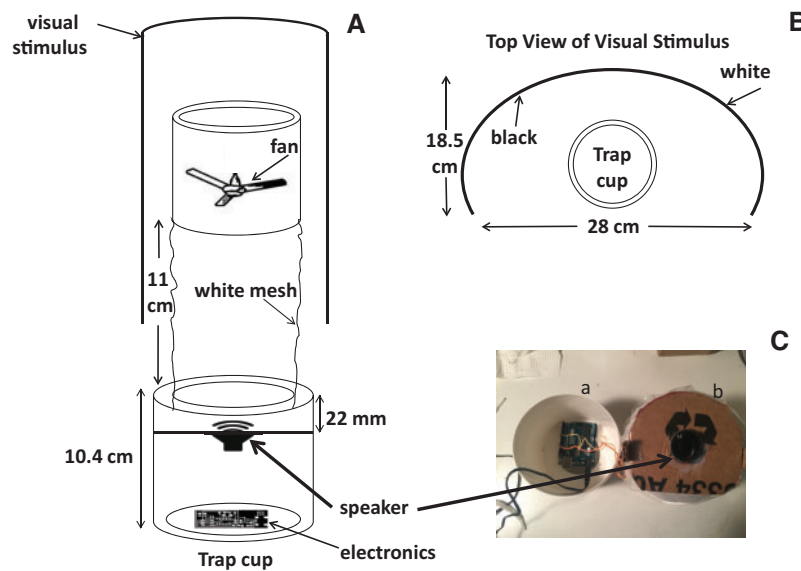


Fig. 1. Diagrams of (A) side view of trap assembly, (B) top view of visual stimulus section of trap assembly, and (C) picture of trap assembly bottom section showing (a) microcontroller platform at bottom and (b) speaker on cardboard insert removed from top of trap cup.

Materials and Methods

Insects

Aedes aegypti pupae were obtained from a colony at the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE), Gainesville, FL. The colony (origin, Orlando, FL 1952) is maintained under procedures described in Gerberg et al. (1994). Pupae were kept in 30- by 30- by 30-cm screened cages in the laboratory kept on a photoperiod of 14:10 (L:D) h schedule with light ending at 8:00 PM. For bioassay replicates, groups of 100 virgin males (2–7 d old) were collected into 946-ml paper containers (Solo Cup Co., Lake Forest, IL) with screened lids on the morning after emergence. Adults were provided with a 10% sucrose solution on cotton balls before and during testing.

Traps

Two bioassays were conducted separately with different sets of stimuli: 1) single-tone versus chirp, which compared trap captures of males by 484-Hz tones and 350–500-Hz chirps, and 2) control versus visual versus chirp + visual, which compared captures of males by controls, visual stimuli, and chirps combined with visual stimuli. Both bioassays employed US Centers for Disease Control and Prevention miniature light traps (Model 512, John W. Hock Co., Gainesville, FL), each of which included a downdraft fan (positioned at top) and a screened, trap collection cup (at bottom). Lids, fan guard, and light bulbs were removed from the traps for ease of access. The traps were powered by rechargeable 6-V, 13 Amp-h batteries. A ring stand on a 13-cm-height, white platform suspended the trap so that the fan was ~30 cm above the platform floor.

Tones or chirps were generated by microcontroller platforms (Arduino Uno, Arduino Inc., Ivrea, Italy), with each microcontroller operating a 3-cm-diameter, 1-W 8- Ω speaker (Laptop internal speaker, Gino Inc., Tokyo, Japan) facing up, 8 cm from the bottom of the trap cup (Fig. 1). One set of platforms produced the continuous 484-Hz tone used in Johnson and Ritchie (2016). The second set produced a 350–500-Hz chirp that stepped 1.2 Hz (up or down) every 0.1 s over a 25-s loop. Only part of the chirp lay within the 421–578 Hz range of *Ae. aegypti* female wingbeats typically encountered

(Arthurs et al. 2014), and preliminary studies indicated that males were attracted during speaker broadcasts only for brief, 5–10-s intervals of each chirp. Signal levels 1.5 cm from the speaker were calibrated using a microphone (Model 4145, Bruel and Kjaer [B&K], Nærum, Denmark) and preamplifier (Model 2639, B&K) connected to an amplifier (Model 2610, B&K), and the chirp patterns were visualized using Raven Pro sound analysis software (Charif et al. 2008).

Acoustic attraction by insects that detect particle velocity instead of pressure variation is necessarily short-range (Tautz 1979, Towne and Kirchner 1989, Mankin et al. 2004); consequently, to increase its range, the acoustic trap included a visual stimulus, a 30- by 45-cm sheet of black matt finish poster board curved (lengthwise) into a half-cylinder, centered at the height of the trap entrance. The visual stimulus was similar to visual cues of the Fay–Prince trap (Fay 1968, Fay and Prince 1970, Kloter et al. 1983) that captured both male and female *Ae. aegypti*. The inner side facing the trap was black and the outer side white. The visual stimulus also was tested alone (poster board and trap) to determine whether the increased attraction to the combined stimuli was statistically significant.

Bioassay Arena and Test Procedures

Trapping was conducted between 10:00 a.m. and 4:30 p.m. (4–10.5 h after beginning of photophase) in a 1.75-m cubical white screened cage (Model 1406B, BioQuip Products, Rancho Dominguez, CA) in a 3.5-m square laboratory room with white walls and six 32-W fluorescent lights providing a minimum light level of ~470 lux at the trapping positions. Trapping occurred from the beginning to the middle of a period of increased male activity (Cabrera and Jaffe 2007). To consider the effects of light on trapping during this daylight period, a 33- by 18-cm window 1.14 m from the cage edge was left uncovered on the east side of the room, providing variable levels of increasing and then decreasing light throughout the trapping period. Light levels at each trapping position were checked with a light meter (Model LX-103, Lutron Electronics Co., Coopersburg, PA) at the beginning and end of each 1-h replicate. A trap with a platform producing a single tone and a trap with a platform producing chirps were set up in two opposite corners in the (tone vs. chirp) bioassay. Traps with one of three stimuli: control, visual, or chirp + visual, were set up in three

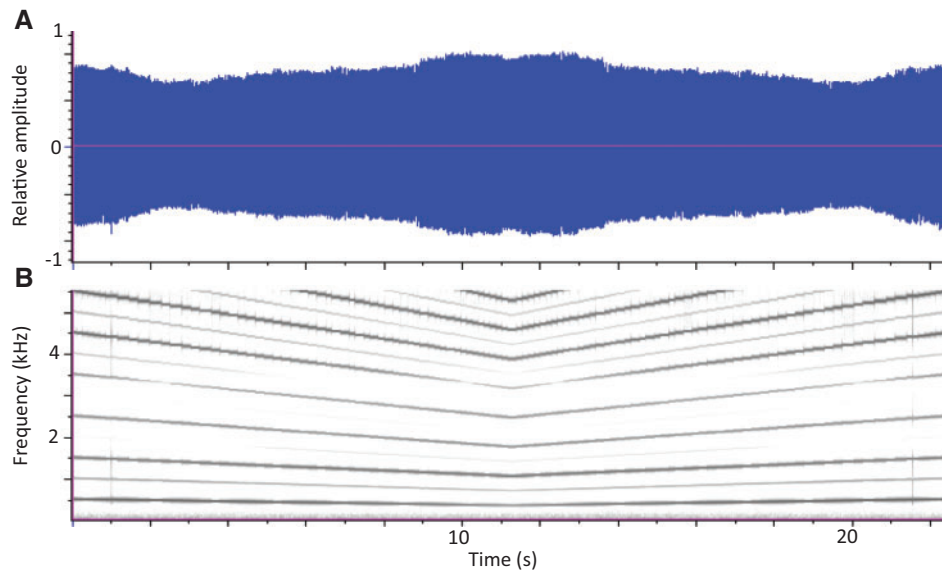


Fig. 2. (A) Oscillogram and (B) spectrogram of chirp used for Tone versus Chirp and Control versus Visual versus Chirp + Visual Stimuli bioassays. Darker shade in spectrogram indicates greater energy at specified frequency and time.

different corners in the (control vs. visual vs. chirp + visual) bioassay. In each replicate, 100–200 virgin males were released from a container into the center of the arena. After 1 h, the trap collection cups were removed and the numbers of males collected in the cups and remaining in the arena were counted. For each trap, proportions of total released were calculated as $P_{tr} = \text{number captured in trap} / \text{total released into arena}$, and proportions of total captured were $P_{tc} = \text{number captured} / \text{total captured in all traps}$. Traps were rotated among positions, and mosquitoes remaining in the arena were removed after each test.

Statistical Analyses

Shapiro–Wilks tests (Proc Univariate, SAS Institute Inc. 2012) were conducted for each bioassay to consider whether the proportions, P_{tc} , were normally distributed, enabling the use of parametric statistical tests. Seven replicates were obtained in the tone versus chirp bioassay to estimate whether significant differences in P_{tc} occurred between treatments. A paired-difference, two-tail Student's *t*-test was conducted to compare mean P_{tc} values in the tone versus chirp bioassay.

Thirty replicates were obtained over 25 d in the control versus visual versus chirp + visual bioassay. Analysis of variance with a Tukey Honest Significant Difference (HSD) test and an analysis of covariance (Proc GLM, SAS Institute Inc. 2012) were performed to compare effects of trap type on mean P_{tc} , with and without inclusion of light level effects. The covariance model was—

$$P_{tc} = \text{stimulus} + \text{meanlightlevel},$$

where *stimulus* was chirp + visual, visual, or control, and *meanlightlevel* was the mean of the light level readings taken from each trap position at the beginning and end of each test.

Results

Tone Versus Chirp Stimuli

The sound pressure levels of the tones and chirps produced by the Arduino platforms were similar, ranging from 87.5–92.5 dB at 1.5 cm, or 70–74 dB at 10 cm from the speaker. The chirp pattern is

shown in Fig. 2. In the seven tone versus chirp bioassay replicates, 31 males were captured by the tone and 65 by the chirp, with $P_{tr} = 0.044$ and 0.093, respectively. The mean P_{tc} for the tone trap was 0.29 ± 0.04 (standard error [SEM]), and 0.71 ± 0.04 for the chirp.

The values of P_{tc} in the tone versus chirp bioassay were normally distributed ($W = 0.953$, $n = 14$, $P = 0.61$), so a paired-difference, two-tail Student's *t*-test was performed. The means were significantly different ($t = 5.08$, $df = 6$, $P = 0.002$). Overall, the effectiveness of traps emitting the chirp stimulus was greater than that of traps emitting the tone, with a mean of 71% of males captured using chirps compared with a mean of 29% captured using the tone. This result suggested a series of tests to compare among controls, visual stimuli, and chirps combined with visual stimuli.

Control Versus Visual Versus Chirp + Visual Stimuli

In the 30 replicates comparing control, visual, and chirp combined with visual stimuli, 346 males were captured in the control, 676 in the visual, and 1,124 in the chirp + visual trap, with $P_{tr} = 0.082$, 0.161, and 0.267, respectively. The values of P_{tc} were normally distributed ($W = 0.953$, $n = 70$, $P = 0.0552$), and analysis of variance indicated statistically significant differences among means across stimulus types ($F_{2, 67} = 43.74$, $P < 0.0001$). The mean values of P_{tc} were significantly different from each other in ascending order of control, visual, and chirp + visual stimuli, 0.155 ± 0.039 , 0.342 ± 0.027 , and 0.519 ± 0.039 , respectively.

Effects of Light Level on Response to Attractive Stimuli

Preliminary observations of males released into the bioassay arena suggested that they preferentially oriented toward or rested in darker areas, possibly searching for shelter or cover (Paz-Soldan et al. 2011). Greater attractiveness of traps in relative darkness was confirmed by the analysis of covariance (Fig. 3). The mean values of the intercepts for the regressions of P_{tc} on mean light levels were significantly different among the three stimulus types and the slope of the mean light level was significantly different from zero ($F_{3, 66} = 46.66$, $P < 0.001$; Table 1).

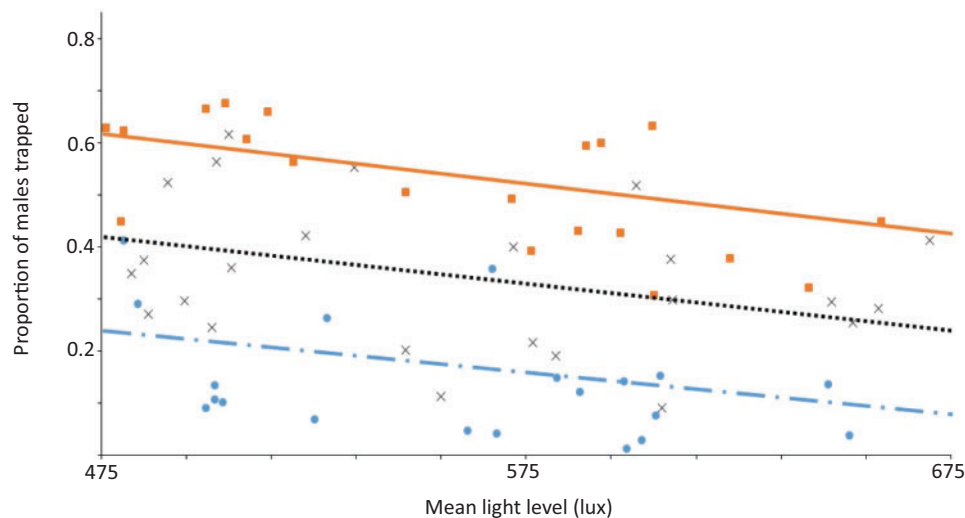


Fig. 3. Proportions of males captured, P_{tr} , in traps with control (dots, dot-dashed line), visual (\times , dotted line), and chirps combined with visual (square, solid line) stimuli at different mean light levels.

Table 1. Trap type and light level (\pm SEM) estimates in analysis of covariance of mean proportions of total males captured at different mean light levels in chirp + visual, visual, and control traps

Parameter	Estimate	t
Chirp + visual trap	1.0564 \pm 0.0335	5.70
Visual trap	0.8656 \pm 0.1108	7.81
Control trap	0.6782 \pm 0.0333	-5.62
Light level (lux)	-0.0009374 \pm 0.0001941	-4.83

For each estimate, the value of $P > t$ is statistically significant ($P < 0.001$).

Discussion

The goal of developing an inexpensive trapping system with significantly improved capability for attracting and capturing *Ae. aegypti* males was successful, and new information was obtained with respect to male attraction to acoustic and visual stimuli at different light levels. Although it is known that *Ae. aegypti* males are diurnal, with an activity peak just after dawn and a second peak beginning within 3–4 h of dusk (Cabrera and Jaffe 2007), their shelter- and mate-seeking activities in variable lighting during daylight are not well understood. Our results confirm that males (Fay 1968), like females (Christophers 1960, Allan et al. 1987, Bidlingmayer 1994) orient toward dark-colored objects or toward relatively lower light levels during the 10:00 a.m. - 4:30 p.m. time period. In this study, the mean values of P_{tr} averaged over all light levels in the Control versus Visual versus Chirp + Visual bioassay were found to be significantly different from each other.

The results suggest that long-distance, visual cues combined with short-distance attraction of males to chirp stimuli are useful for monitoring of *Ae. aegypti* male populations in peridomestic environments. By themselves, traps with tones or chirps captured proportions, $P_{tr} < 0.1$, but combinations of chirps and visual cues yielded $P_{tr} > 0.25$. Part of the trapping success of the acoustic stimuli may have resulted from the multiple harmonics of the wingbeat frequency that appear in Fig. 2, produced by the speaker and its trap-cup support structure (Mankin et al. 2013), given that similar harmonics are produced by wings of both sexes in flight (Cator et al. 2011).

The significantly increased P_{tr} in darker areas of the arena suggests that positioning of traps in dark or covered areas may increase

their effectiveness. Further improvements may result from using lids of different sizes or including shrouds that co-opt preferences of both sexes of *Ae. aegypti* for darkened areas or cover. Also, traps that incorporate olfactory stimuli especially attractive to *Ae. aegypti* (Williams et al. 2006), may benefit by addition of chirp stimuli.

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