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The Siren's Song: Exploitation of Female Flight Tones to Passively Capture Male *Aedes aegypti* (Diptera: Culicidae)

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Abstract

The need to capture male mosquitoes has intensified recently as a result of a number of male-based sterile insect technique (SIT) and population-modification programs focused on *Aedes aegypti* (L.) having initiated field releases. Here, we report the results of the successful exploitation of the attraction of male *Ae. aegypti* to female flight tones to enhance male collections in nonmechanical passive (nonbattery powered) Gravid *Aedes* Traps (GAT). Prior to field studies, male attraction to female flight tones of 484 and 560 Hz, as well as to a male flight tone of 715 Hz, were assessed in a series of controlled release–recapture and semifield trials. These trials determined that a pure tone of 484 Hz was significantly more attractive to free-flying males than the other flight tones and enabled their collection in sound-baited GATs (ca. 95% capture rate after 2 h; 484 Hz at 65 dB). In contrast, gravid females were unresponsive to male or female flight tones and were evenly distributed among sound-baited and control GATs. Importantly, under normal field conditions sound-baited GATs (484 Hz at 70 dB) captured significantly more male *Ae. aegypti* per 24-h trap interval (1.3 ± 0.37) than controls (0.2 ± 0.13). Overall, sound-bated GATs captured approximately twice as many *Ae. aegypti* (male and female; 3.0 ± 0.68 per interval, 30 total) than controls (1.5 ± 0.56 per interval, 15 total). These results reveal that sound-baited GATs are a simple and effective surveillance tool for *Ae. aegypti* that would allow current male-based SIT and population-modification programs to effectively monitor males in their target populations.

Key words: *Aedes aegypti*, wing frequency, sound bait, Gravid *Aedes* Trap, surveillance

Males of most mosquito species of medical importance use their superb auditory sense to detect and locate female mosquitoes by recognizing the female's unique flight tone (Charlwood and Jones 1980, Belton 1994, Gopfert et al. 1999). Interestingly, in the case of *Aedes aegypti* (L.), the primary vector of dengue and yellow fever viruses, males do not form large mating swarms around natural or artificial structures like many mosquito species, but instead prefer to mate near bloodmeal hosts (Cabrera and Jaffe 2007). This enables researchers to easily study their mating behavior, and consequently, some of the most recent advances in our understanding of the physiological mechanisms behind the attraction of male mosquitoes to female flight tones have been discovered using *Ae. aegypti* as the model organism (Cator et al. 2009, 2011; Cator and Harrington 2011). Exploiting this attraction to capture male mosquitoes of other species has been the subject of numerous studies, many of which have highlighted the success of capturing free-flying male *Culex tritaeniorhynchus* Giles, *Culex quinquefasciatus* Say, *Culex tarsalis* Coquillett, and *Aedes albopictus* (Skuse) mosquitoes using artificially produced female flight tones (Ikeshoji et al. 1985, 1987; Ikeshoji and Ogawa 1988). Despite these successes, interest in exploiting male attraction to female flight tones to capture males had largely been abandoned by the early 1990 s, particularly because males do not participate in disease transmission and sound traps did not collect females. However, the need to capture male mosquitoes has intensified recently as a result of a number of male-based sterile insect technique (SIT) and population-modification programs focused on *Ae. aegypti* having reached the field testing stage (Harris et al. 2011, Hoffmann et al. 2011). Unfortunately, because the majority of commercially available traps are designed to capture either host-seeking or oviposition-ready females, accurate estimates of male populations are currently unattainable.

Here, we report the results of a series of semifield and field studies (outdoor and indoor) aimed at exploiting the attraction of male *Ae. aegypti* to female flight tones to enhance male collections in nonmechanical passive (nonbattery powered) Gravid *Aedes* Traps (GAT; Eiras et al. 2014, Ritchie et al. 2014). The GAT is an economical (ca. $20 USD) passive trap that attracts gravid females through the use of a simple oviposition attractant; male *Ae. aegypti* commonly comprise <5% of GAT collections (S.A.R, unpublished data). We also report on the attractiveness and repellency of male flight tones to both male and female *Ae. aegypti*.

Materials and Methods

Mosquitoes and Study Sites

All laboratory studies were conducted in large semifield flight cages housed on the campus of James Cook University, Cairns, Australia, and used *w*Mel-infected *Ae. aegypti* fully backcrossed with wild-type *Ae. aegypti* collected from Cairns, QLD. The semifield cages are designed to mimic a typical open-air Queenslander home floor plan common to North Queensland (Ritchie et al. 2011). All mosquitoes were reared according to standard laboratory protocols, and all studies were conducted using GATs that were treated with a pyrethroid-based surface spray (Mortein Barrier Outdoor Surface Spray, Australia). The field and indoor studies were conducted at a single Queenslander style residence located in the Edge Hill suburb of Cairns, QLD.

Selection and Playback of Flight Tones

Two female flight tones, 484 Hz and 560 Hz, and one male flight tone, 715 Hz, were chosen for this study based on reported wing beat frequencies. 484 Hz is the mean female wing beat frequency recorded by Brogdon (1994) and Moore et al. (1986), whereas 560 Hz is closer to the frequency recorded by Cator et al. (2011). A male flight tone of 715 Hz was chosen based on the recordings of Brogdon et al. (1994) and Arthur et al. (2014). All tones were played as pure sine tones generated using the NCH Tone Generator Software for Macintosh (NCH Inc, CO). The audio device consisted of a Clip Mini Mp3 player with 4 GB micro SD card (ebay.com.au, last accessed 15 March 2015), a X-mini II speaker (X-mini II, Singapore), and two 5 V/2600 mAh rechargeable batteries (Favolcano, NJ). This setup allowed for up to 48 h of audio playback at 65–70 dB.

Controlled Tent Study

Two controlled release–recapture studies were conducted to assess the feasibility of exploiting male attraction to female flight tones to enable their capture in GATs and to assess the responsiveness of gravid females to male and female flight tones. The studies followed a 4 by 4 Latin square design and were conducted in 3.4-m^3 nylon tents positioned at the corners of a 5 m square within an enclosed semifield cage. A single GAT baited with hay infusion (8 g per 4 liter, 1-wk incubation at 27°C) and containing an audio device was placed in the center of each tent. Three devices were programed to playback a single tone (i.e., 484 Hz, 560 Hz, and 715 Hz) at 65 dB, and one was set to play no sound to serve as a control. In the first Latin square, 30 virgin males (5–7 d old) were released into each tent at the beginning of each trap interval, and the number captured was assessed after 1 and 2 h. At the end of each interval, the GATs were cleared of any mosquitoes and rotated among the tents. The study protocol was the same during the second Latin square except that 30 gravid females (5 d post blood feeding, blood fed at 7 d of age) were released into each tent.

Large Semifield Cage Study

The feasibility of capturing males in sound-baited GATs while competing with free-flying females was assessed in a semifield cage (Ritchie et al. 2010) that regularly houses a population of ca. 1,000 male and female *w*Mel-infected *Ae. aegypti*. The study protocol and trap positions were similar to that of the release–recapture study. Due to the large number of mosquitoes present, the number of male and female mosquitoes collected in each trap was assessed at 30-min intervals. Four complete replicates were conducted on separate days to minimize confounding effects of sound inundation on male and female response.

Field Efficacy of Sound-Baited Gravid *Aedes* Traps

A field study was conducted over a period of 10 d in June 2015 to assess the effectiveness of sound-baited GATs at capturing male *Ae. aegypti* under field conditions. During the study a single sound-baited and control GAT, both baited with hay infusion (8 g per 4 liter, 1-wk incubation at 27°C), were placed 10 m apart at opposite ends of the patio of the study residence. The number of male and female *Ae. aegypti* captured in each trap was recorded at 24-h intervals from 1700 to 1700 hours the following day, during which a pure tone of 484 Hz, determined to be the most attractive to males under semifield conditions, was constantly played from the sound-baited GAT at 70 dB. At the end of each interval, the traps were cleared of any captured mosquitoes and exchanged among the trap positions.

Indoor Use of Sound-Baited Gravid *Aedes* Traps

To assess the feasibility of using sound-baited traps to collect male *Ae. aegypti* within a residence, we conducted a series ($n = 8$) of 48-h collections within the same residence used in the above field trials during early July 2015. To minimize disturbance to the residents, a pure tone of 484 Hz was played back continuously at a level of 60 dB.

Statistical Analysis

Differences in the percentage of released or total male and female *Ae. aegypti* captured among the different treatments and tent and trap positions in the release–recapture and semi-field cage studies were analyzed by two-way ANOVA on arcsine-transformed data followed by Tukey's HSD post hoc analysis. Differences in the total number of male and female *Ae. aegypti* captured during the field efficacy study in sound-baited and control GATs, as well as at each trap position, were analyzed by two-way ANOVA followed by Tukey's HSD post hoc analysis. All statistical analyses were performed using Prism 6 (Graphpad Software Inc., CA).

Results

Controlled Tent Study

The male capture rate was the greatest in GATs in which a pure tone of 484 Hz was being emitted compared with the other flight tones and the control (Fig. 1A), with significant ($F_{3,8} = 36.6$, $P < 0.001$) differences occurring after 2 h of playback. Overall, the capture rate of 484 Hz was 67.1 ± 19.3% and 97.3 ± 2.3% after 1 and 2 h, respectively, whereas the capture rate of 560 Hz was 44.1 ± 12.3% and 63.3 ± 12.3% after 1 and 2 h, respectively. In contrast, the capture rate of 715 Hz and control GATs was 5.2 ± 1.2% and 1.5 ± 1.0%, respectively, after 2 h.

Large Semifield Cage Study

Similar to the tent studies, the playback of 484 Hz captured significantly ($F_{3,24} = 5.04$, $P = 0.01$) more (53.5 ± 16.3% of total collected) males than any of the other flight tones and the control (Fig. 1B). No significant difference in the number of gravid females collected per trap was observed, and although the flight cage contained nongravid females, none were collected among any of the traps.

Field Efficacy of Sound-Baited Gravid *Aedes* Traps

The 484 Hz sound-baited GAT captured significantly ($t_8 = 4.01$, $P = 0.01$) more male *Ae. aegypti* (1.3 ± 0.37 per 24-h interval, 26 total) than the control (0.2 ± 0.13 per 24-h interval, 2 total; Fig. 1C). No significant difference in the number of gravid females collected in sound-baited (1.7 ± 0.45 per interval, 21 total) and control traps (1.3 ± 0.56 per interval, 15 total) was observed.

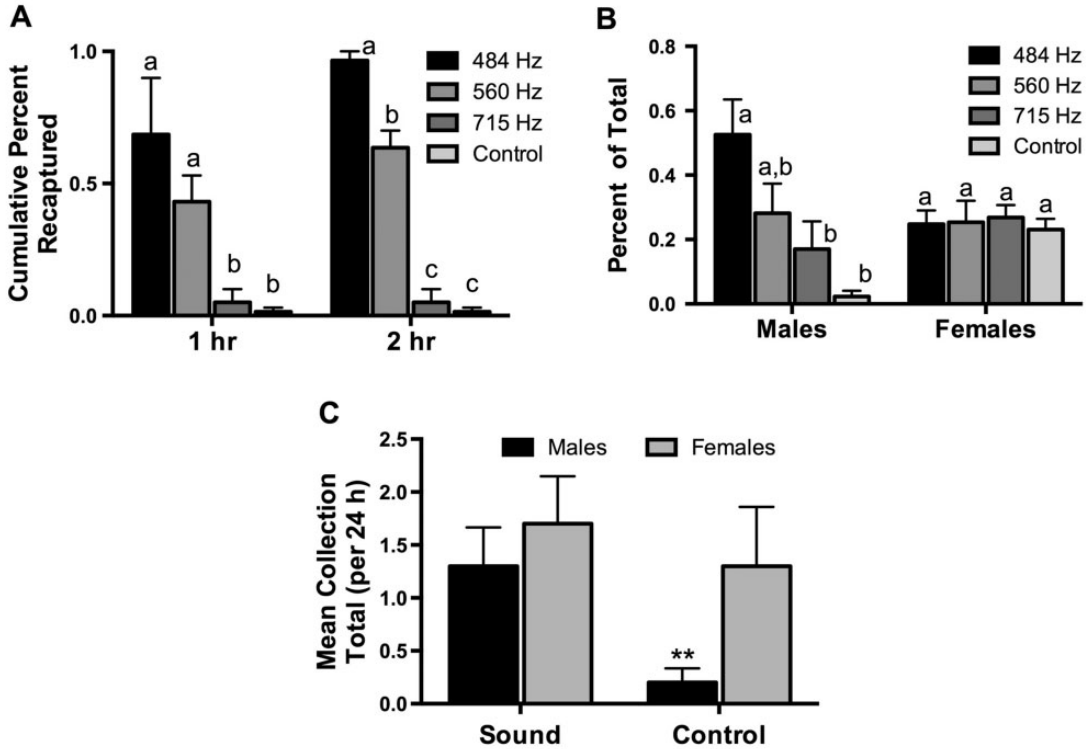

Fig. 1. **(A)** Mean (±SE) percent of released males recaptured during release–recapture tent trials and **(B)** percentage of total free-flying male and gravid female *Ae. aegypti* captured during semifield trials for each tested flight tone. **(C)** Mean (±SE) number of male and female *Ae. aegypti* captured in sound-baited (484 Hz) and unmodified control GATs during a series ($n = 10$) of 24-h field observations. Different letters indicate a statistically significant difference ($P < 0.05$) within groups. The double asterisk represents a statistically significant difference between male collections in the sound-baited (484 Hz) and control GAT as well as between male and female collections in the control GAT.

The sound-baited GAT captured approximately twice as many male and female *Ae. aegypti* per trap interval (3.0 ± 0.68 per interval, 30 total) compared with the control (1.5 ± 0.56 per interval, 15 total).

Indoor Use of Sound-Baited Gravid *Aedes* Traps

The attractiveness of 484 Hz extended indoors as the sound-baited GAT captured a mean of 1.2 ± 0.17 male *Ae. aegypti* (total = 9) per 48-h trap interval. Overall, males comprised 58.3 ± 9.3% of the total number of *Ae. aegypti* captured resulting in a mean male to female collection ratio of 1.2:1.

Discussion

The results presented here demonstrate the successful exploitation of the attraction of male *Ae. aegypti* mosquitoes to female flight tones to capture male *Ae. aegypti* in a simple passive trap that is equally attractive to gravid female *Ae. aegypti.* Our results reaffirm the strong attraction of male *Ae. aegypti* to female flight tones (Stone et al. 2013) and highlight the indifference of females to either male or female flight tones as well as the indifference of males to their own flight tones. These latter observations are supported by the fact that the male antennae, which behave like harmonic oscillators when acoustically simulated, are finely tuned to the wing frequencies of conspecific females, whereas the resonant frequency of the female antennae does not match the flight tones of males or females (Gopfert et al. 1999). Because these vibrations are then detected by the Johnston's organ to induce "hearing," female *Ae. aegypti* were presumed to be unable to respond to male or female flight tones; however, recent evidence suggests that female *Ae. aegypti* can respond and alter their flight tone to match that of males during copulation, but at a shared harmonic of 1,200 Hz (Cator et al. 2009). Importantly, male attraction to female flight tones was found to be strong enough to enable their collection in passive traps, particularly those previously determined to be unattractive to them, but which maintained their attractiveness to gravid females. In contrast to previous sound-baited traps that involved modifications to battery or mains powered light and fan traps (Stone et al. 2013), which are often unpractical for use in developing countries, the passive sound-baited GAT more than doubled collection totals by attracting males without reduction in female capture. These results indicate that the deployment of sound-baited GATs will allow current male-based SIT and population management programs to effectively monitor their target populations, particularly once economical, long-lasting sound devices are developed. Furthermore, the practicality of sound-baited GATs may extend to other day active, container-utilizing members of the *Stegomyia* subgenus, such as *Ae. albopictus* and *Aedes polynesiensis* Marks.

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