Swarming mechanisms in the yellow fever mosquito: aggregation pheromones are involved in the mating behavior of *Aedes aegypti*

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ABSTRACT: Mosquitoes of various species mate in swarms comprised of tens of thousands of flying males. In this study, we examined *Aedes aegypti* swarming behavior and identified associated chemical cues. Novel evidence is provided that *Ae. aegypti* females aggregate by means of olfactory cues, such as aggregation pheromones. Isolation of *Ae. aegypti* aggregation pheromones was achieved by aeration of confined mosquitoes and collection of associated volatiles by glass filters. The collected volatiles were identified through gas chromatography mass spectrometry (GCMS). Three aggregation pheromones were collected and identified as 2,6,6-trimethylcyclohex-2-ene-1,4-dione (ketoisophorone) (CAS# 1125-21-9, t_R = 18.75), 2,2,6-trimethylcyclohexane-1,4-dione (the saturated analog of ketoisophorone) (CAS# 20547-99-3, t_R = 20.05), and 1-(4-ethylphenyl) ethanone (CAS# 937-30-4, t_R = 24.22). Our biological studies revealed that the identified compounds stimulated mosquito behavior under laboratory conditions. The mechanism of mosquito swarm formation is discussed in light of our behavioral study findings. A preliminary field trial demonstrated the potential application of the isolated aggregation pheromones in controlling *Ae. aegypti.* ***Journal of Vector Ecology* 39 (2): 347-354. 2014.**

Keyword Index: *Aedes aegypti*, swarm formation, mating behavior, aggregation pheromones.

INTRODUCTION

Mosquitoes of various species are reported to mate in swarms comprised of tens of thousands of flying males (Yuval 2006, Cabrera and Jaffe 2007, Ng'habi et al. 2008). The great majority of researchers consider mosquito swarms as a means to facilitate mating (Yuval et al. 1993). The female response to these swarms was proportionally related to the number of males participating in the swarm (Cabrera and Jaffe 2007). Acoustic waves produced by female wing beats are believed to be the primary signal for attracting males for copulation (Roth 1984, Belton 1994, Cator et al. 2009 and Gibson et al. 2010). Such acoustic love songs are species characteristic and are considered the main criterion in mate selection (Edwards 1920, Duhrkopf and Hartberg 1992, Klowden 1999, Hoy 2006). However, other cues might be involved in mosquito mating behavior.

Mosquito electrophysiological analysis indicated that odor molecules are detected by olfactory receptor neurons housed in antennal trichoid and grooved peg sensilla (Davis and Bowen 1994). Presence of CO_2 receptor neurons was first reported by Kellogg (1970). Later it was found that the mechanisms by which mosquitoes locate their human hosts, nectar sources, and oviposition sites, are primarily olfactory driven (Takken and Knols 1999) and many attempts to identify compounds associated with different mosquito biological aspects have been made (Snow 1970, De Jong and Knols 1995, Dekker et al. 2005). Contact pheromones have been suggested to play a role in sexual recognition for *Deinocerites cancer* (Downes 1966, Provost and Haeger 1967), *Aedes albopictus* (Nijhot and Craig 1971), *Culiseta inornata* (Kliewer et al. 1966, Lang and Foster 1976), and some *Culex* species (Gjullin et al. 1967). Previous studies suggested that chemical cues such as pheromones could mediate swarm formation in some species of Diptera (Edwards 1920, Klowden 1999). However, definite information on the cues involved in mosquito swarming has remained unclear.

Aedes aegypti Linnaeus is one of the most medically important mosquitoes as the main vector of dengue, chikungunya, and yellow fever viruses, in addition to its wide geographical distribution. More than 60% of human populations are at risk of diseases transmitted by *Ae. aegypti*. The accrued medical importance of *Ae. aegypti* made this species a center of mosquito biological researches for centuries. Earlier behavioral studies with *Ae. aegypti* concluded that aggregation pheromones stimulate female flight patterns at a distance and chemical cues might be involved in its swarming mechanism (Cabrera and Jaffe 2007). Yet, the aggregation pheromones of *Ae. aegypti* remain unidentified. Revealing the mechanism of swarm formation in mosquitoes and discovering the involved chemical cues will lead to better adaptation of vector control interventions. This study was designed to identify the swarming mechanism and the associated chemical cues involved in the aggregation behavior of *Ae. aegypti*.

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14. ABSTRACT

Mosquitoes of various species mate in swarms comprised of tens of thousands of flying males. In this study, we examined Aedes aegypti swarming behavior and identified associated chemical cues. Novel evidence is provided that Ae. aegypti females aggregate by means of olfactory cues, such as aggregation pheromones. Isolation of Ae. aegypti aggregation pheromones was achieved by aeration of confined mosquitoes and collection of associated volatiles by glass filters. The collected volatiles were identified through gas chromatography mass spectrometry (GCMS). Three aggregation pheromones were collected and identified as 2,6,6-trimethylcyclohex-2-ene-1,4-dione (ketoisophorone) (CAS# 1125-21-9, tR = 18.75), 2,2,6-trimethylcyclohexane-1,4-dione (the saturated analog of ketoisophorone) (CAS# 20547-99-3, tR = 20.05), and 1-(4-ethylphenyl) ethanone (CAS# 937-30-4, tR = 24.22). Our biological studies revealed that the identified compounds stimulated mosquito behavior under laboratory conditions. The mechanism of mosquito swarm formation is discussed in light of our behavioral study findings. A preliminary field trial demonstrated the potential application of the isolated aggregation pheromones in controlling Ae. aegypti.

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MATERIALS AND METHODS

Mosquitoes

Aedes aegypti mosquitoes were reared in the insectary of the Mosquito and Fly Research Unit at the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE-USDA-ARS, Gainesville, FL, U.S.A). This strain of *Ae. aegypti* was colonized in 1952 from Orlando, FL, and provides a standard colony for many attractant and repellent studies. Mosquitoes were reared using standard procedures (Gerberg et al. 1994). Adult mosquitoes were maintained in an environmental chamber at 27° C with 80% RH to simulate a summer day regime. Fluorescent lighting was set to a crepuscular profile with a photoperiod of 14:10 (L:D), including 2 h of simulated dawn and 2 h of simulated dusk. After pupation, mosquito sexes were separated using a pupal separator (Focks 1980). Each sex was placed separately in screened stock cages for emergence. Emerged adult mosquitoes in stock cages were provided with 10% sucrose solution three times per week. Adult mosquitoes were used for the bioassays and field trials.

Olfactometer

A triple-cage, dual-port olfactometer (Posey et al. 1998) was used to conduct the behavioral study of *Ae. aegypti* swarming activity. Each cage unit consisted of a rectangular clear acrylic test chamber with two openings leading to two ports arranged horizontally, left and right. Each port had a trapping tube opening to the test chamber and a treatment chamber opening to the outside. The opening to the outside allowed for easily placing and removing treatments under testing. A sliding door separated the test chamber from the two ports. External air was charcoal-filtered, humidified, and warmed (27 ±_1° C, 80 ±_2% RH). When opening the sliding door, air flowed through the ports first, then to the test chamber. Each test unit was used at a time for bioassay in order to maintain the optimum air circulation (Allan et al. 2006). The flow rate was adjusted at 5 cm/s. At the start of the test trial, approximately 100 mosquitoes were transferred to the test chamber and allowed to acclimate for 30 min. The sliding door opened to allow air circulation from the ports to the test chamber. The air flow carried the volatiles from the ports to the test chamber. Mosquitoes were allowed to fly freely to the test ports during the trial. The sliding door trapped the attracted mosquitoes to the treatments in the port in the trapping tube at the end of the trial where trapped mosquitoes were counted. The bioassays were conducted under high light conditions (2,220-2,400 lux) between 10:00 and 15:00 (Posey et al. 1998). The olfactometer sides were covered with a white sheet to eliminate visual stimulation of mosquitoes during the test.

Bioassay

The behavioral experiments were to observe the response of the confined mosquitoes in the test chamber to mosquitoes confined in one of the two ports. A small (10 x10 x 10 cm) acrylic plastic cage with two opposite screened sides was used to confine mosquitoes. The two opposite mesh sides faced the direction of air flow, allowing air current to carry volatiles produced by the confined mosquitoes downwind into the test chamber. Approximately 600 mosquitoes (up to 1,000) of the same sex were confined in the small cage and placed in the test port of the olfactometer. Confined mosquitoes were reused up to eight trials in the same day.

Mosquitoes were collected from the stock cages and transferred into test chambers using a mechanical aspirator. Mosquitoes were allowed to acclimate in the olfactometer for 30 min before testing. Each trial consisted of 100 mosquitoes of the same sex. The sliding door was opened to allow the air current to carry the volatiles produced by the confined mosquitoes in the port to the test chamber. Mosquitoes in the chamber were allowed 20 min to respond. A small empty cage was placed in the control port. At the end of the trial, the sliding door was closed trapping the responding mosquitoes inside the port. The percentages of mosquitoes trapped in the treatment port and the control port in relation to total number of mosquitoes loaded in the test chamber were calculated, respectively, for each trial. Treatments and controls were randomly assigned to the left or right ports in all experiments to eliminate instrumental bias. All materials placed in the treatment or control ports for testing were handled with gloves to avoid contamination with skin compounds. The response of female *Ae. aegypti* to dead groups of males and females was evaluated separately, using the same procedure described above. Approximately 1,000 mosquitoes were killed by freezing. Frozen mosquitoes were then allowed to thaw at room temperature for 30 min and placed in the olfactometer test port. The percentage of females responding to dead males and females was counted and evaluated in comparison to an empty control port as described earlier.

Collection of volatiles associated with *Aedes aegypti*

Volatiles associated with *Ae. aegypti* were isolated through aeration of a group of unisex mosquitoes in an aeration chamber following the instructions described by Rohrig et al. (2008). Virgin mosquitoes, seven to 14 days old, were placed in a clean, glass, volatile, collection chamber consisting of a central chamber (30 cm long by 4 cm deep) with two hose openings at each side to allow air to flow. Each trial used approximately 500 virgin mosquitoes of the same sex. Mosquitoes were transferred from stock cage to the aeration chamber by mechanical aspiration. Air passing through the chamber was charcoal-filtered using activated charcoal (Analytical Research Systems, Inc., Gainesville, FL). A clean screen was placed at the upwind end of the aeration chamber to prevent mosquitoes from flying upwind. A glass volatile collection filter containing 50 mg of Haye-Sep (Analytical Research Services, Gainesville, FL) was placed at the end port of the aeration chamber to collect volatiles produced off mosquitoes confined in the aeration chamber. The air flow was adjusted at 400 µl/min. This low flow rate was used to prevent blowing off mosquito setae and carrying unneeded mosquito body parts to the glass filter, which might cause an interference with the chemical analysis. A control aeration chamber was run in parallel with each sample. Volatile collections were conducted overnight (17:00 to 09:00) and then the collection filters were eluted in glass ampoules using

200 µl of hexane (HPLC grade, Sigma). Samples were sealed and stored at 4° C until analyzed by gas chromatography mass spectrometry (GCMS).

Identification of volatile chemicals associated with *Aedes aegypti* using GCMS

A ThermoFinnigan DSQII GCMS single quadrupole system (Thermo Fisher Scientific, Waltham, MA) was used. The GC column was a 30 m x 0.25 mm id (df= 0.25 µm) DB-Waxetr column (Agilent Technologies, Wilmington, DE). Prior to analysis of samples, the instrument was tuned and calibrated with perflurotributylamine. The split/splitless injector was set at 260° C and operated in splitless mode (1 min) with surge pressure of 4.35 psi for 1 min. The injection port operated in constant flow mode at an initial rate of 1.20 ml/min He carrier gas and dropped to 1.0 ml/min after 1 min. The GC oven was programmed at 35° C for 6 min after injection, then ramped at 10° C/min to 260° C, and held at that final temperature for 5 min.

Bioassays of identified aggregation pheromones

The identified volatile chemicals associated with *Ae. aegypti* mosquitoes were isolated and identified as described earlier. Identified chemicals were synthesized and provided by Sigma-Aldrich. Responses of *Ae. aegypti* to identified compounds were evaluated following the methodologies described by Bernier et al. (2003). Each tested chemical was placed in a 4 cm diameter concave watchglass. The watchglass carrying the tested chemical was introduced inside one of the two ports of the olfactometer described earlier. Contamination was minimized by wearing plastic gloves and changing gloves between different trials. For each chemical, different concentrations were used to scan the optimal concentration for mosquito response. Pure compounds were diluted 1:9 using acetone (Sigma-Aldrich) to form a working solution. Working solutions were vortexed prior to each usage. The amount of acetone equivalent to the needed concentration was transferred to the glass watch using a mechanical pipette. The acetone vaporized, spreading the tested chemicals on an equivalent area of the watchglass, with a radius of approximately 0.5 cm. The scanned concentrations started at 300 µg/test and went down to 100, 10, 5, and 1 µg/test. Acetone is known to induce attraction in mosquitoes (Bernier et al. 2003). Prior to each test, the equivalent amount of the working solution that contains the needed concentration was applied on the glass watch and left for 3 min to allow the acetone to volatize before placing it in the olfactometer. The control port contained the same volume of pure acetone applied to a glass watch and handled the same way. Similar to the previous trials in bioassays, approximately 100 mosquitoes were placed in the olfactometer cage for each trial, allowed to acclimate for 30 min, and the test ran for 20 min. At the end of the test, the percentage of mosquitoes responded to each test port was calculated in relation to the total number of mosquitoes used in the test.

Large cage and preliminary field trial

Large outdoor screened cages were used to determine whether *Ae. aegypti* could locate Biogents (BG) trap (Biogents AG., Regensburg, Germany) baited with proposed pheromones as outlined by Kline et al. (2003). The large cage was 9.2 m in width x 18.3 m in length x 4.9 m in height on the sides and 6.1 m in height at the gabled peak. Four treatments were compared in a Latin 4 x 4 square design. Each proposed pheromone formed one of the treatments: compounds A, B, and C. The fourth treatment was left empty as a control. A dose of 1 ml of each tested compound was placed in a plastic tube and placed in the treatment pocket of the BG trap with an unsealed cap. An empty tube was placed in the control treatment. Each BG trap used was assigned to a specific treatment through the whole study to avoid cross-contamination. The traps were placed at the corners of the cage, 2 m apart from the cage sides. The four treatments were rotated between the four corners. Each test day, approximately 1,000 *Ae. aegypti* were introduced into each cage. Mosquitoes were released in the center of the cage and at the same distance from all traps. Uncollected mosquitoes in the field cage were retained until the next trapping day.

Statistical analysis

Mosquito response was measured as the percentage of mosquitoes responding to each port in relation to the total mosquitoes used in the test. Treatments were randomized between the left and right ports of the olfactometer to eliminate position bias. The SPSS package (IBM® SPSS® Statistics version 19) was used for statistical analysis. An independent t-test was used to evaluate the significance of responses between mosquitoes attracted to the treated port or a control port. An independent t-test was used to analyze the significant difference of the comparative preferences between both sexes. For all analyses, differences were considered significant when $\alpha \leq 0.05$.

RESULTS

Olfactometer and bioassay results

The behavioral study showed that 16.3±1.4 (%±SE; *p-value* < 0.001) and 30.1±6.6 (%±SE; *p-value*= 0.003) of virgin females were attracted to confined males and confined females, respectively (Table 1). Males showed a lower response to confined females 10.9±3.1 (%±SE) and no response to confined males (Table 1). Different response patterns were observed for males and females of *Ae. aegypti*. Females flew directly toward confined males or females, while males showed an irritated flight pattern (similar to swarming) inside the test chamber when exposed to confined females.

A test of competitive sex preference was conducted in order to evaluate which sex had a stronger olfactory attraction. Males were confined in one port and an equal number of females in the other, and the competitive preference by the sex in the test chamber was observed (Table 2). Virgin females in the test chamber were significantly more attracted to confined females than confined males (p= 0.038), while males showed insignificant preference to a specific sex (p= 0.200). This test

Table 1. Bioassay of sex:sex response of seven to 14 day-old *Ae. aegypti* to biologically-produced aggregation pheromones in comparison to control (empty) port inside a dual port olfactometer for 20 min.

Tested sex	Confined sex in the Treatment port	# of mosquitoes used in the test chamber	% Response to treatment (Mean±SE)	% Response to control (Mean±SE)	df	t
Females	Males	3647	16.3±1.4*	4.0±0.6	31	9.224
	Females	957	30.1±6.6*	4.0±1.3	9	4.011
Males	Males	535	0.2±0.2	0±0	5	1.000
	Females	1351	10.9±3.0*	2.2±0.6	10	3.748

* Significant at α=0.05. df = Degree of freedom (number of replicates – 1).

Table 2. Competitive sex preference of seven to 14 day-old *Ae. aegypti* inside a dual port olfactometer.

Tested sex	Confined sex in the two ports	# of mosquitoes used in the test chamber	% Response to treatment (Mean±SE)	df	t
Females	Males	1109	12.8±2.7 a	11	2.364
	Females		20.5±2.5 b		
Males	Males	1195	5.6±1.4 c	11	1.362
	Females		8.3±1.4 c		

a and b= Significant at α=0.05; c=not significant. df = Degrees of freedom (number of replicates – 1).

Table 3. Response of seven to 14 day-old female *Ae. aegypti* to aggregation pheromone residuals on dead mosquitoes inside a dual port olfactometer for 20 min.

Treatments	# of mosquitoes used in the test chamber	% response to treatment (Mean±SE)	% response to control (Mean±SE)	df	t
Dead Males	550	16±3*	5±2	5	4.312
Dead Females	410	31±5*	2±1	4	4.761

* Significant at α=0.05. df = Degree of freedom (number of replicates – 1)

revealed that the female-female olfactory cue is stronger than the female-male olfactory cue.

The behavior of *Ae. aegypti* was studied in response to dead males and females in order to eliminate any acoustic component from the observed responses. Females in the test chamber were attracted to dead males (16±3% SE), *p*-value=0.008) and dead females (31±5% SE), *p-value*= 0.009) (Table 3). On the other hand, males did not show any response to dead mosquitoes. Females showed similar behavior in their response to dead or alive confined mosquitoes, while males responded only to live females. This reveals that females rely on olfactory cues to locate the swarm location, while males rely on acoustic signals produced by live insects.

Aeration, collection, and identification of volatiles associated with *Aedes aegypti*

The aeration technique was used to collect volatiles produced off *Ae. aegypti* bodies (Rohrig et al. 2008). Collected compounds were examined by Gas Chromatography Mass Spectrometry (GCMS). Comparison of the mass spectra of volatiles collected through mosquito aeration to those collected from a control chamber revealed three trace level peaks for females, only two of which were observed for males. The collected compounds were identified as 2,6,6-trimethylcyclohex-2-ene-1,4-dione (ketoisophorone) (CAS# 1125-21-9, t_R = 18.75), 2,2,6-trimethylcyclohexane-1,4-dione (the saturated analog of ketoisophorone) (CAS# 20547-99-3, t_R = 20.05), and 1-(4-ethylphenyl) ethanone (CAS# 937-30-4, t_R = 24.22) (Figure 1). These three

compounds will be denoted as compounds A, B, and C, respectively, for the rest of this paper. Compounds A, B, and C were found on females and only compounds A and C were found on males.

Bioassays of the identified aggregation pheromones

The attraction of *Ae. aegypti* to these chemicals was evaluated in the previously described olfactometer. Two types of responses were observed when females were exposed to the suspected pheromones: excitation and attraction. The excitation response was recognized as disturbance/anomalies in the mosquito flight pattern. The attraction response was recognized when females flew directly to the treated port. Compound A triggered an excitation effect on female flight pattern at a dose of 5 µg and no detectable response was observed below this dose. Compound B excited females with a lower dose of 2 µg. Only compound C attracted *Ae. aegypti* females (11.6% ± 1.9, *p*-value= 0.001) and (10.3% ± 1.6, *p*-value = 0.001) (%±SE) at concentrations of 5 and 1µg, respectively.

Male *Ae. aegypti* responded to compound A only. Males initiated a characteristic flight pattern similar to swarming when exposed to different doses of compound A. The number of males participating in the swarm increased in amount and in their duration of swarming as a function of increasing doses of compound A (Figure 2).

Large cage and preliminary field trial

The mosquito attraction of the isolated pheromones was evaluated in the large cages at the Center of Medical Agricultural and Veterinary Entomology (USDA-ARS-CMAVE) as described by Kline et al. (2003). The attraction of mosquitoes to each compound was evaluated using Biogents (BG) traps baited with 1 ml of one of the three suspected pheromones in comparison to a control of an unbaited BG trap. Traps baited with compounds A and C attracted slightly more *Ae. aegypti* females than the control trap. However, data were statistically insignificant. On the other hand, compound B showed significant repellency to *Ae. aegypti* L (*p-value*=0.003), which could be due to an overdose of attractant (Bernier et al. 2003).

DISCUSSION

Our observations indicate that males responded only to live confined mosquitoes, while females responded to both live and dead confined mosquitoes. Previous studies proved that males were more sensitive than females to acoustic signals produced by wing beats (Göpfert et al. 1999, Göpfert and Robert 2000, Gibson and Russell 2006). In concordance, our results demonstrated that *Ae. aegypti* females basically follow olfactory cues, while males rely on acoustic signals.

Identifying the mechanism of swarm formation is crucial to understand mating behavior in mosquitoes. Various factors are reported to be responsible for mosquito swarm initiation, including acoustic (wing beat), ocular, and environmental cues (Charlwood and Jones 1980, Marchand 1985). However, the initiation cues for swarm formation are still debatable. *Aedes aegypti* swarming mechanisms are postulated as illustrated in Figure 3. At the beginning of a swarm, an alpha male mosquito produces its species characteristic love song (Figure 3a). Wing beat frequencies are species specific and males are more sensitive to acoustic signals (Göpfert et al. 1999, Göpfert and Robert 2000, Gibson and Russell 2006). As a result, other males of the same species will be able to recognize the appropriate frequency and those conspecific males will respond initially to the swarm call (Figure 3b). As the number of males in the swarm increases, *Ae. aegypti* aggregation pheromones accumulate at the swarming site as a result of the cluster of the swarming males (Figure 3c). Based on the behavioral response reported earlier in this study, the attraction of females to swarming activity depends mainly on olfactory cues and the number of males participating in the swarm (Cabrera and Jaffe 2007). The clustering of aggregation pheromones produced by swarming males (Figure 3d) increases the swarm effectiveness to attract females to the swarm site (Figure 3e). Hence, more females will be attracted to the swarm site (Figure 3f).

Females must approach within a very near proximity in order to be located by a corresponding mate. Previous

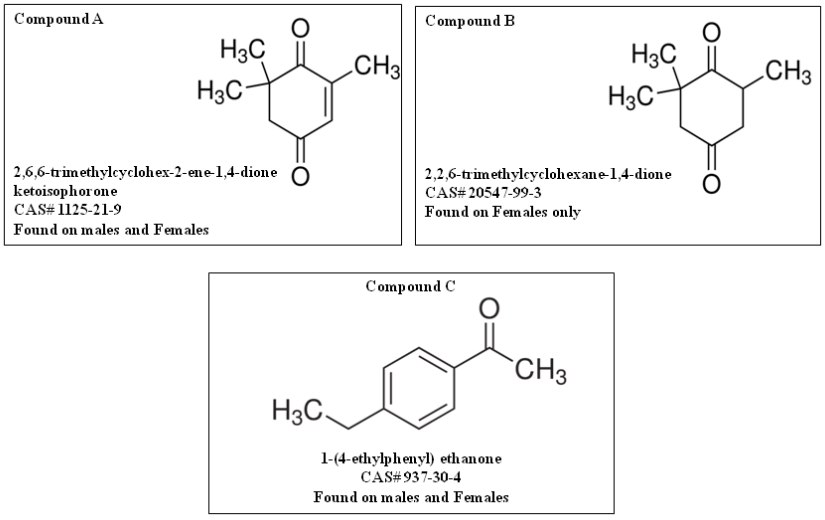

Figure 1. Chemical structure of the isolated *Aedes aegypti* pheromones (CAS, Chemical Book).

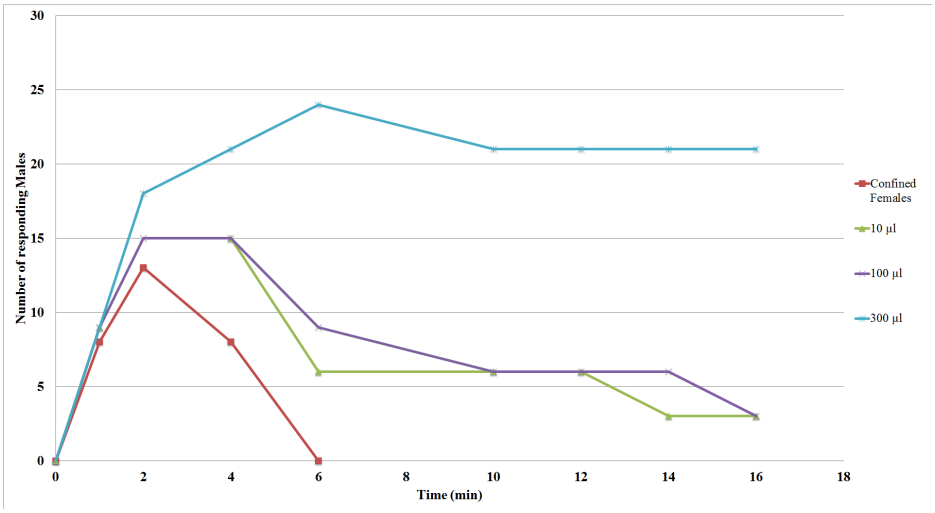

Figure 2. Behavioral response of *Aedes aegypti* males to different doses of ketoisopherone (Compound A) in comparison to their response to confined females.

studies showed that the ability of males to differentiate the acoustic signals produced by the wing beats of flying females from the background noise is limited to few centimeters (Hoy 2006). The swarm formation and associated pheromones play a significant role in getting males and females near enough in order to allow the recognition of acoustic wing beat frequencies. After recognition of female wing beat, males tune their wing vibration to higher levels (Cator et al. 2009), possibly to reach a threshold amplitude in order to compensate for the lower acoustic sensitivity of the females (Göpfert et al. 1999, Göpfert and Robert 2000). Once acoustic stimuli are recognized by females, both sexes adjust their wing beat frequency to produce a harmonic convergence (Cator et al. 2009) and mating takes place (Gibson and Russell 2006). Males were observed to copulate with females inside or near the swarm location (Cabrera and Jaffe 2007, Gibson et al. 2010). The ability of the mosquito sexes to tune their wing beat frequencies is the main criterion for mate selection (Charlwood and Jones 1980, Marchand 1985, Cator et al. 2009).

Identification of the volatile chemicals, such as aggregation pheromones involved in the mate-seeking process of *Ae. aegypti* and other mosquitoes, could provide a valuable tool for developing control strategies for mosquito borne diseases. The second part of our research targeted the isolation and identification of *Ae. aegypti* pheromones. The collected compounds were identified as 2,6,6-trimethylcyclohex-2-ene-1,4-dione (ketoisophorone) (CAS# 1125-21-9, t_R = 18.75), 2,2,6-trimethylcyclohexane-1,4-dione (the saturated analog of ketoisophorone) (CAS# 20547-99-3, t_R = 20.05) and 1-(4-ethylphenyl) ethanone (CAS# 937-30-4, t_R = 24.22).

The capability of female mosquitoes to locate human hosts has provided broad insights into using human-emitted volatiles, such as CO_2, kairomones, octenol, and L-lactic acid, for attracting mosquitoes for surveillance purposes (Bernier et al. 2007). Carbon dioxide is considered the standard attractant in the surveillance of mosquitoes and other anthropophilic insects. However, a drawback to the use of this compound is its unavailability and unfeasibility in remote locations, especially in underdeveloped areas. Therefore, discovery of suitable, environmentally safe, less-volatile replacements for CO_2 would be of great importance for vector control interventions. Nevertheless, the selection of proper chemicals for baits can allow for selective capture of targeted insect species (Bernier et al. 2007). The use of a species specific bait, such as an aggregation pheromone, can provide increased accuracy in the assessment of vectors present in the local areas for purposes of population surveillance and entomological risk assessment in epidemiological investigations. However, the adequacy of a projected mosquito lure to be used in field application is affected by many instrumental and operational factors, including but not limited to, the release container, working mixtures, composition, and adequate concentration (Bernier et al. 2007). Our findings demonstrated the attraction of *Ae. aegypti* to at least one of the three identified pheromones under laboratory conditions. This study may stimulate further field studies to provide practical chemical attractants for *Ae. aegypti*. In addition, investigating and identifying the aggregation pheromones of other mosquito species may reveal a novel approach for mosquito control.

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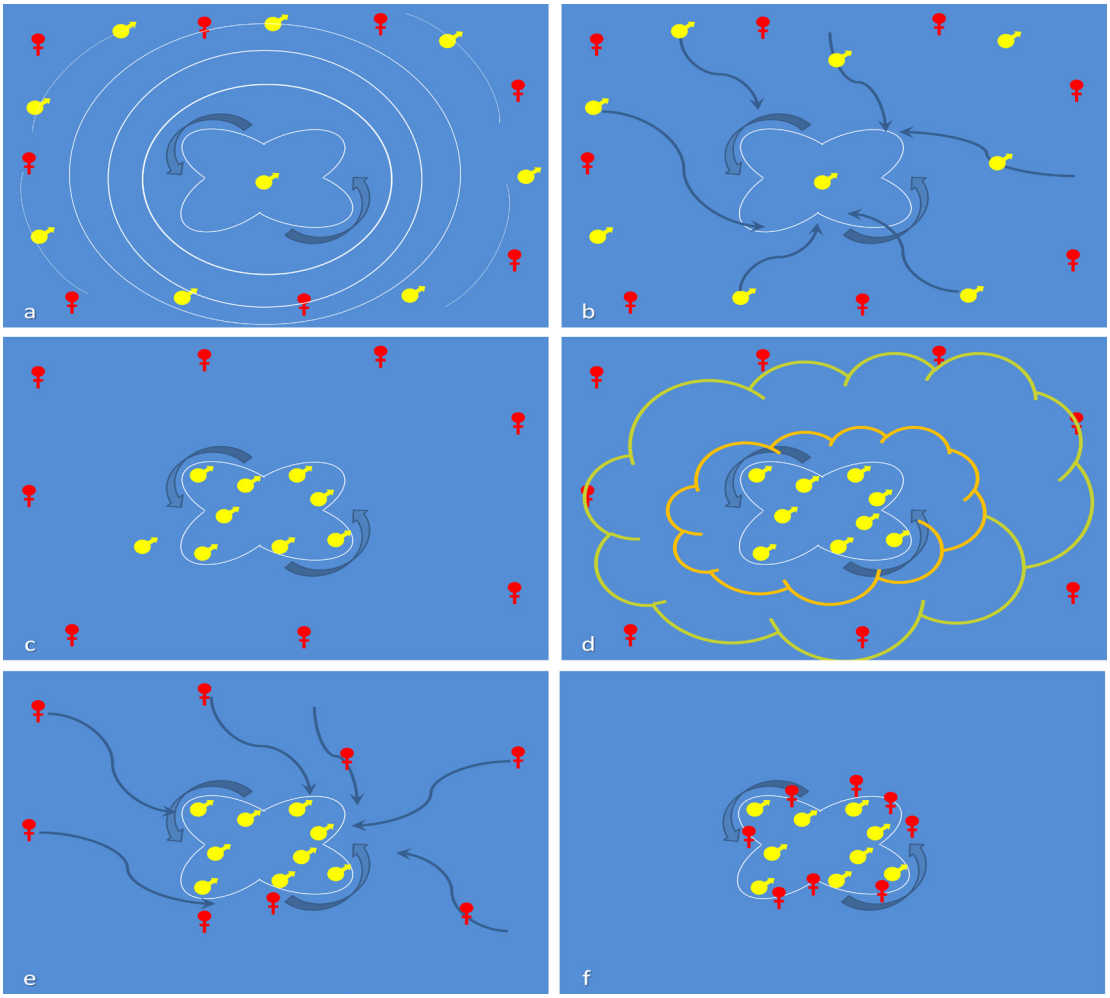

Figure 3. Graph simulating the mechanism of swarming and aggregation dynamics of *Aedes aegypti*: a. A male mosquito produces acoustic signals based on wing beat frequency. b. Other males are stimulated by the acoustic signals and begin aggregating around the male. c. Males aggregate in a swarm. d. The concentration of aggregation pheromones increases as the males swarm. e. Females are stimulated by the released pheromones and fly toward the swarming males. f. Females and males continue to aggregate towards the swarm.

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