Comparative attractancy of physical and chemical stimuli to aedine mosquitoes *

Yoshiyasu KUSAKABE** and Toshiaki IKESHOJI**

Department of Agrobiology, Faculty of Agriculture, The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

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Abstract: Trapping of both sexes of mosquitoes achieves more efficient control than manipulating the male population. For this purpose, various chemical and physical stimuli known to be attractive were reevaluated. Heat, black color, L-lactic acid, movement and sound were all attractive to both sexes of *Aedes aegypti* when tested individually under laboratory conditions. Black color, heat and lactic acid in this order contributed their attractancy in combinations with the other stimuli. In field tests at the fringe of a forest, however, only heat and sound proved to be of significant attractancy for female and male of *Aedes albopictus*, respectively. From these tests, it was suggested a powerful attractant from human emanations and optokinetic stimulus was necessary for further improvement of mosquito traps.

INTRODUCTION

Male mosquitoes are attracted to and effectively manipulated by acoustic sound (Ikeshoji *et al.*, 1985 for *Culex tarsalis*; Ikeshoji, 1986 for *Culex tritaeniorhynchus*; Ikeshoji and Yap, 1987 for *Culex quinquefasciatus*; Ikeshoji and Ogawa, 1988 for *Aedes albopictus*; Kanda *et al.*, 1987 for *Mansonia* spp.; Leemingsawat, 1989 for *Anopheles minimus* and *Anopheles maculatus*). However, mass trapping and autochemosterilization of both sexes rather than the male alone are more efficient for their control (Ikeshoji *et al.*, 1990).

Kanda *et al.* (1987) and Leemingsawat (1989) used a hamster and dry ice in addition to sound to attract females and more males. Animal traps are, however, not feasible for a control program because of their cost and handling difficulties. A good female trap may also replace the human bait trap employed in mosquito surveys.

For the practical purpose in mind, therefore, the attractancy of various known stimuli was reevaluated in the present paper. These include heat, humidity, lactic acid, carbon dioxide, visual factors such as black color and target movement, and sound (Clements, 1963). Except for sound, these stimuli have hitherto rarely been studied against males.

METHODS

Laboratory experiment. Thirty 3- or 4-day-old, laboratory-bred virgin males or mated females of *Aedes aegypti* were placed in a 30×30-cm wide and 60-cm deep acryl

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** 日下部良康, 池庄司敏明: 東京大学農学部害虫学教室 (〒113 東京都文京区弥生 1-1-1)

box, the bottom and end of which were screened and well ventilated by raising the box 30 cm above the floor. Seven stimuli: heat, humidity, lactic acid, color, carbon dioxide, movement and sound were tested individually and in different combinations against control. The first five stimuli were delivered through a 2-cm diam. glass tube which was passed between two holes on the side walls at 10 cm from the front and 20 cm from the bottom. The tube was sectioned in two; one half was treated with stimuli and the other kept as control, or *vice versa*. The tube was wrapped with the following materials of different shades to test color preference; a white filter paper (W), gray or black painted papers (G1-3, B1 and B4), a black blotting paper (B2), a black polyethylene sheet (B3) and a carbon black-coated paper (B5). Heat was delivered through the tube by circulating water heated to 30–50°C. For the heat *vs.* unheated experiments, two half length tubes were used. Humidity and L-lactic acid were treated onto the paper or sheet by pipetting 1.5 ml of water and the acetone solution of different concentrations, respectively. A regulated volume of CO₂ was released at a few cm height over the tube with a glass capillary connected to a gas bomb. To test optokinetic sense, a 4-cm square B2 paper with or without a 3-cm diam. dynamic speaker was tied on the 20-cm arm of a metronome and swayed horizontally at a speed of 8.7 cm/sec. A 400-Hz sinusoidal sound was generated at 5-sec intervals by a handmade generator (Ikeshoji *et al.*, 1990). The experiment was done under a 240 to 300 lx dim light at 27°C and 80–90% RH around 16:00 when flight activity was highest. Accumulated frequencies of alighting on the tube were counted by the naked eyes or a video camera at 1-m distance from the front acrylic wall for 5 min $\times$ 3 times for each observation and each experiment was replicated 3 to 7 times.

The light absorption spectra of the colored papers and the polyethylene sheet were measured with a Shimadzu TLC scanner, CS 920.

Field experiment. Different combinations of the attractive stimuli: heat, sound and dry ice were tested with black color. The trap consisted of an 80 $\times$ 90-cm black poly-

ethylene bag (B3), to the inside of which was taped a 90-cm dynamic speaker, a thin Samiconplate heater (20 $\times$ 30-cm wide, 230 V 48 W, Sakaguchi Dennetsu Co., Tokyo), and a 40°C temperature regulator (F1 type). The bag was hung at 1-m height from a bar crossing two tripods and set in half shade at the fringe of a bush back of the Universiti Sains Malaysia campus in Penang. The place was surrounded by few houses, a sheep pen and the brook at 20–30-m distances, and seemingly infested with inexhaustible number of *Ae. albopictus*. Out surface of the bag was sprayed with adhesive (Kinryu, Showa Denko Co., Tokyo). The 400-Hz sound was generated as above. One kg of dry ice was placed on the ground under the trap. Each test lasted only for 10 min, and a few tests were made daily at random times from 7:00 to 20:00. Trapped *Ae. albopictus* were counted (smaller numbers of *Mansonia uniformis* and *Cx. quinquefasciatus* trapped were not counted) and removed with a forceps each time.

RESULTS

Laboratory experiment

Optimum intensities of various stimuli. Heat was significantly attractive to *Ae. aegypti* female at 36 and 40°C against the ambient temperature of 27°C (Table 1). The high attractancy to male at 40°C was partly due to the unattractive white paper (W) used for the control as later shown in the color test of black paper B2 *vs.* W. Humidity was not attractive to male as dry control. Lactic acid was attractive at the dosage of 0.012 and 0.12 mg/cm² to female and male, respectively, but repellent at 1.2 mg/cm² to female. CO₂ was not attractive to both sexes at all dosages tested. When W, G1-3 and B1-4 papers or polyethylene sheet were tested to female against the most attractive B5 paper (refer Fig. 1 for the light absorption spectra), W attracted minimum, whereas all the others were equally less attractive to female than B5 control. However, B2 was highly attractive to male when tested against W. A 4-cm square B2 paper in a horizontal movement at 8.7 cm/sec speed was also slightly attractive to male, and so was this with sound to both female and male.

Table 1 Percentages of *Aedes aegypti* responded to various stimuli at different intensities against total numbers responded.

Stimuli	Intensity	Female	Male
Heat	30°C	52 ^{*1, a, b}	—
	36	59 ^{s, a, c}	—
	40	62 ^{s, b, d, e, f}	74 ^{*2, s}
	45	53 ^e	—
	50	51 ^{c, d, f}	—
Humidity	1.5 ml H ₂ O	—	62 ^{*3}
Lactic acid	0.012 mg/cm ²	59 ^{*4, s, a}	56 ^{*5}
	0.12	75 ^{s, a, b}	65 ^{s, a}
	1.2	40 ^{s, b}	43 ^a
	10 ml/min	52 ^{*4}	—
CO ₂	50	57	60 ^{*5}
	100	62	55
Color ^{*6}	W	3 ^{*7, s, a-f}	—
	G1	35 ^{s, a}	—
	G2	35 ^{s, b}	—
	G3	38 ^{s, c}	—
	B1	40 ^{s, d}	—
	B2	—	80 ^{*8, s}
	B3	42 ^{s, e}	—
	B4	33 ^{s, f}	—
Move	8.7 cm/sec	—	66 ^{*9, s}
Move+sound		67 ^{*10, s}	62 ^s

^{*1} Tested with black paper B2 against 27°C control. ^{*2} Tested with B2 against white paper W at 27°C. ^{*3} Tested with W. ^{*4} Tested with W at 40°C. ^{*5} Tested with W at 27°C. ^{*6} Color spectra are shown in Fig. 1. ^{*7} Tested at 40°C against B5. ^{*8} Tested at 40°C against W. ^{*9} Tested with B2 against non-moving control. ^{*10} Tested with B2 and sound against non-moving control. ^s Percentages are significantly different from 50% by Binomial Tests (one side test). ^{a-f} Percentages with the same letters in each test group are significantly different by Randomization Tests for Two Independent Samples.

Comparative attractancy of various stimuli.

Five stimuli were tested in different combinations at their optimum concentrations; heat at 36°C, humidity of 1.5 ml water, 0.12 mg/cm² of lactic acid, B2 paper (this was used instead of B5) and 50 ml/min of CO₂. When one stimulus was omitted from a combination of all five stimuli which was used as the

Fig. 1 Light absorption spectra of various visual targets.

control, the heat, black color or lactic acid-omitted combinations (combination 1, 3 and 4) reduced the attractancy significantly to female (in Fig. 2, S shows the mean percentage significantly different from 50% by binomial test, one side test). The humidity-omitted combination (2) reduced no attractancy, and significantly differed from the three combinations when tested by randomization test for two independent samples, one side test. The CO₂-omitted combination (5, 6), however, did not differ from the control. Among the four combinations excluding three stimuli (7–10), the heat, humidity and black color-omitted combination (10) reduced attractancy most. When the three four stimuli-omitted combinations or single stimuli (11–13) were tested, the heat (11) and the lactic acid (12) reduced attractancy significantly more than the black color (13). In other words, black color was most attractive, followed by heat and lactic acid.

Against male, attractancy was quite variable. Only the heat-omitted combination (14) reduced attractancy among the three one stimulus-omitted combinations (14–16). In contrast, two combinations omitting CO₂ (16, 17) somewhat increased attractancy. In other words, CO₂ was repellent under the present condition. In spite of the repellency

Fig. 2 Relative attractancy of five stimuli in various combinations to *Aedes aegypti*. The open circle shows the mean percentage and S shows its significance from 50% by binomial test, one side test.

Fig. 3 Numbers of *Aedes albopictus* caught in the field by traps with various combinations of stimuli for 10 min at different times of days.

of CO₂, the heat, humidity and CO₂-omitted combination (20) showed a reduced attractancy. In short, the heat-omitted combinations reduced attractancy most.

Field experiment

Daily activity of *Ae. albopictus*. The number of trapped mosquitoes in both sexes at different times of the day showed a bimodal pattern similar to *Ae. aegypti*, a small-

er peak in the morning and a large one in the afternoon (Trips *et al.*, 1973) (Fig. 3). Although different combinations of attractive stimuli were tested by the trap, the mosquito catches varied extensively even at the same time of day; the curves show their activity tendency as indicated by the maximum hourly catches. Therefore, only catches made at the hours of moderate and high activity, namely, from 9 to 11 and from 14 to 19,

Table 2 Numbers of mosquitoes caught by a black polyethylene trap with various combinations of stimuli.

	Combined stimuli				All
	Heat	Sound	Dry ice	None	
Heat		8, 1, 6, 2, 10, 9	6	10, 7	
Sound	2, 9, 1, 3, 1, 5		3, 13, 5	8, 4, 2, 2, 1	
Dry ice	0	9, 10, 4		3	
	2, 2	10, 4, 1, 12, 10	1	1, 2, 3, 0, 2, 5, 5, 7, 2	
None				0, 1, 2, 2, 0, 16, 5, 0, 0	
					11, 7, 3
All					3, 4, 4

Figures above and below the diagonal show the numbers of female and male, respectively.

were considered in the following discussion.

Mosquito catch in a combination of various stimuli. The catch by the black polyethylene sheet trap varied extensively depending on the combinations of stimuli and among repetitions at different days and times of day (Table 2). The numbers above and below the diagonal in Table 2 show the catches of female and male, respectively. When the female catches with the heat combinations ($\bar{x}=6.7$, $n=12$) (disregarding the other stimuli) were compared with those without heat ($\bar{x}=3.8$, $n=18$), the difference was significant at 5% level by Man-Whitney's *U*-test (two-way test). On the other hand, the male catches with the heat combinations ($\bar{x}=3$, $n=12$) were not significantly different from those without heat ($\bar{x}=4.8$, $n=18$). Likewise, when the female catches with the sound combinations ($\bar{x}=5.6$, $n=17$) were compared with those without sound ($\bar{x}=4.1$, $n=13$), the difference was not significant at 5% level. On the contrary, the male catches with the sound combinations ($\bar{x}=5.4$, $n=17$) were significantly different from those without sound ($\bar{x}=1.3$, $n=12$) at 1% level. The dry ice-combinations attracted neither more females ($\bar{x}=6.4$, $n=8$) nor more males ($\bar{x}=4.4$, $n=8$) than the combinations with-

out dry ice, females ($\bar{x}=4.4$, $n=22$) or males ($\bar{x}=4$, $n=22$), respectively.

Comparison between the catches of different sexes by the same stimulus combinations was also of interest. With the heat combinations, significantly more female ($\bar{x}=6.7$, $n=12$) was caught than male ($\bar{x}=3.0$, $n=12$). In contrast, the female catches did not differ from the male catches with either sound, dry ice or no-stimulus combinations.

DISCUSSION

Heat. Since Howlett first demonstrated in 1910 that warm air from a host was a signal for attack by mosquitoes, many researchers claimed convection current of heat as the most important stimulus for their attraction (Davis and Sokolove, 1975). In the present experiments, heat *per se* at 36–40°C or in combinations with other stimuli was reconfirmed to be the most important stimulus to *Ae. aegypti* in the laboratory and to *Ae. albopictus* in the field. In fact, the latter females were observed to be trapped mostly on and around the heat plate. However, heat works only at a short distance since the body surface temperature of human at 34°C

reaches over only 40 cm to ambient temperature where the thermoreceptor of *Ae. aegypti* responds to a temperature change of 0.05°C (Davis and Sokolove, 1975). As aedine males follow females to a host for mating, some stimuli emanating from the host have been thought to attract the males as well. In fact, there was an indication of attractancy in heat in the present laboratory experiment, but no statistical difference between the mean male catches of the heat-treated and untreated traps was observed in the field experiment. In conclusion, heat is of prime importance in female attraction but not in male.

L-Lactic acid. Though host odor has long been investigated and many compounds isolated as the candidate attractants, only L-lactic acid seems to show a moderate attractancy in laboratory experiments (Acree *et al.*, 1968; Carlson *et al.*, 1973). In the present laboratory experiment, this compound *per se* was certainly attractive at an optimum concentration and the combinations omitting this reduced attractancy to both sexes. Nevertheless, the attractancy could not be demonstrated in the field when 10 ml of the neat compound was treated to the lower part of the trap sheet (the data is not shown). Since the actual evaporating rate of lactic acid is hard to define under field conditions, various amounts should be evaluated to conclude the attractancy.

Black color. Importance of visual factors, particularly a black silhouette, in orientation of day active and some night active mosquitoes is known (Gillies and Wikes, 1982; Bidlingmayer and Hem, 1979). One of the many black colored traps used in population sampling is a black cylinder suction trap, which attracted more females than males of *Ae. aegypti* and *Cx. quinquefasciatus* (Wilton and Klotter, 1985). In the present laboratory experiment, black color, particularly B5 absorbing both UV and visual lights, was quite attractive to both sexes. The superior attractancy was also apparent with the black sheet B3 in the field experiment. The visually effective distance also varies depending on species from only 5 m of *Culex decens* to 9–18 m of *Cx. tritaeniorhynchus* and *Anopheles* spp. (Gillies and Wilkes, 1980), and 7.5 m of *Cx. quinquefasciatus* to

15–20 m of many other species (Bidlingmayer and Hem, 1980). Although the attractive distance was not determined in the present field experiment, the black sheet of the size used is believed to have worked to a moderate distance as above referred.

Carbon dioxide. Regardless of the *modus operandi* of CO_2 , either attraction or mere excitation, many types of mosquito traps using CO_2 *per se* or with other stimuli have been successfully employed for population sampling (Service, 1976). However, in the present experiment, CO_2 showed attractancy by itself to neither sex. Even under the field condition, no significant attractancy was detected, although the gas delivery was not regulated.

Movement. The optokinetic sense of an insect is keen, and the mosquito utilizes this sense in host finding. A moving mouse in a transparent airtight container attracted 3.7 times more *Ae. aegypti* than an anesthetized mouse in a similar one (Sippel and Brown, 1953). Field workers walking into aedine-infested area suspected that their movement was responsible for the initial attraction of host-seeking mosquitoes (Allan *et al.*, 1987). Similar observation was made in the present field experiment; the majority of the trapped *Ae. albopictus* were brought near the trap by persons approaching through the bush. But other human stimuli dispersed into the air current might have also played an important role in the response to the trap. In the laboratory experiment, the movement of a black target with or without sound showed slightly more attractancy than a still one; this may be worth testing in the field in the future.

Sound. There are many publications on male attraction to the wingbeat sound of female, and acoustic sound has been used for control trials as mentioned in the INTRODUCTION. In fact, acoustic sound was the only significant attractant to male in the present field experiment. Interestingly, a few males, 5 males in one case, were often observed caught around a female which was desperately vibrating her wings with her legs stuck to the adhesive trap surface.

In conclusion, five physical and chemical stimuli *per se* showed some attractancy to both sexes of (except female not tested to

movement) *Ae. aegypti*, but in combination tests, black color, heat and lactic acid proved superior attractants in this order. Under the field condition, however, only heat and sound when tested with a black sheet were significantly attractive to female and male of *Ae. albopictus*, respectively. Analysis of attractants in human emanations and movement may further improve trap efficiency.

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摘 要

Aedes 属に対する物理、化学的刺激の
相対的な誘引性

音響トラップによる雄蚊のみの捕獲より、雌雄蚊の駆除のほうが有利である。そこでネッタイシマカ、ヒトスジシマカに対して、既知7種の物理、化学的刺激の相対的な誘引性を比較した。その結果、熱、乳酸、黒色、動き、音は単独試験で、雌雄のネッタイシマカに対して誘引性を示した。しかし、混合刺激試験では、黒色、熱、乳酸のみが、この順に強い誘引性を示した。マレーシアの森林境界でのヒトスジシマカ捕獲試験では、熱と音のみがそれぞれ雌雄に対して有為な誘引性を示した。より強力なトラップ製作のためには、動き(動視覚刺激)と強力な誘引臭の開発研究が望まれる。