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A Photoelectric Sensing Device for Recording Mosquito Host Seeking Behavior in the Laboratory

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ABSTRACT  A new automatic device for recording the host-seeking behavior of mosquitoes was designed using photoelectric sensors. The host-seeking rhythms of several species of mosquitoes were recorded under laboratory conditions. Use of CO₂, in addition to heating and black color to activate mosquito flight proved to be important for evaluation of the present recording device. The diel rhythms of non bloodfed Aedes albopictus, Ae. aegypti, Culex quinquefasciatus, Cx. tritaeniorhynchus, and Anopheles stephensi females recorded by the device corresponded well with known flight and activity rhythms in field for the same mosquito species. This simple automatic recording device provided accurate information on the flight behaviors of colonized and field collected mosquitoes.

KEY WORDS Anopheles, Aedes, Culex, host-seeking, photoelectric sensor
ACCURATE KNOWLEDGE OF the host seeking flight behavior of mosquitoes is important for controlling and regulating mosquito bites. Many previous studies of mosquito host-seeking behavior have involved field tests relying on landing collections at human or animal baits, or baited traps. The field collection of mosquitoes is one of the most reliable ways of studying mosquito flight behavior. Field studies, however, are labor intensive, expensive, time consuming, and sometimes place human volunteers at risk of disease transmission. Certain aspects of mosquito flight behavior can be studied in the laboratory and those results can be extrapolated to the field to help design more efficient and safe field experiments.

Extensive laboratory studies on mosquito behavior have been previously reported. These studies can be classified into several categories including: flight behavior (Taylor and Jones 1969, Chiba et al. 1981, Jones 1981), sugar-feeding behavior (Bowen 1992, Yee and Foster 1992), and biting or host-seeking behavior (Ho et al. 1973, Klowden and Lea 1984, Yee and Foster 1992, Takken et al. 2001).

Many laboratory and field studies on mosquito attractants such as heat, color, light, and chemical substances have been reported. Several key factors including carbon dioxide, 1-octen-3-ol, L-lactic acid, heat (ca 35 °C), black or dark color have been found to be essential for attracting mosquitoes (Takken and Kline 1989, Kline et al. 1991). These observations resulted in the development of a variety of laboratory olfactometers designed to evaluate mosquito flight behavior (Gouck and Schreck 1965, Posey et al. 1998, Takken et al. 2001) and a variety of mosquito traps (Parker et al. 1986, Burkett et al. 1998, Kline 2002) to evaluate mosquito flight behavior in the field. In addition, recording devices were designed to automatically record the flight activity and sugar-feeding behavior of mosquitoes in the laboratory (Chiba et al. 1981, Yee and Foster 1992). Many laboratory studies of mosquito host-seeking behavior, however, have relied on visual or video recording observations of mosquito flight behavior. The host seeking and blood feeding behaviors of mosquitoes in nature are known to be extremely complex. Host seeking behavior is affected by environmental conditions, mosquito physiological conditions, and host-produced cues. Blood feeding is affected by host physiology and environmental conditions. Simple recording of mosquito flight behavior, therefore, under represents the true complexity of
mosquito behavior. The introduction of variables such as host cues into flight experiments are needed for a more comprehensive study of mosquito host seeking behavior. In the present study, we designed an automatic recording device using photoelectric sensors and report the results of mosquito flight experiments in the laboratory.
Materials and Methods

**A general description of the automatic recording device.** The recording device was composed of 4 photoelectric sensors, an amplifier, a programmable controller unit, a power supply, and monitoring software (Keyence Corporation, Osaka, Japan) (Fig. 1). The photoelectric sensors had a small sensor head (55 x 30 x 10 mm) with a 21 x 21 mm window. The window was composed of a pair of infra-red light emission diode (LED) sensors with light receiving elements on both sides. Small objects (minimum detectable size is 0.5 mm in diameter) passing through the window were detected by the sensors and a signal was transmitted to the computer through the controller and monitoring software. Nocturnal activity was detected by the same system by using infra-red LEDs. The total cost for the recording device was ca $4,000 (ca $500 for a sensor and ca $2,000 for a programmable controller and monitoring software).

A water bath unit for the recording device was made from a white Styrofoam box (23 x 30 x 20 cm, 5 cm in thickness) which contained ca 3.5 liter of water (Fig. 2). The temperature of the water was maintained at ca 35 °C by a heater with thermo-regulator (Powerthermo ET-30, Kotobuki Kogei Co., Ltd., Osaka, Japan). The box was covered with a white plastic panel that contained the 4 photoelectric sensors, the bottom sides of which were sealed with black plastic plates. The top surface of the box, except for the sensors, was covered with a white plastic plate that helped maintain the temperature in the box. The recording device was placed in a cage (80 x 80 x 80 cm) with white plastic panels that buffered the mosquitoes inside the cage from the external stimulations, such as air streams, light, human movement, heat, and carbon dioxide (CO₂) (Fig. 3). Carbon dioxide at a flow rate of 500 ml/min was generated by a gas bomb and was released into the cage through a silicone tube, the opening of which was placed ca. 10 cm above the sensors. The CO₂ release was regulated (2 min on / 13 min off) by a solenoid valve (FSD-0408C, Flon Industry Co., Ltd., Tokyo, Japan) controlled by timer. The air inside the cage was ventilated by an electric fan located in the bottom corner of the cage. The concentration of CO₂ inside the cage was measured ca 1 cm above the sensors by a CO₂ detector (TECH-JAM® Co., Ltd.,
Osaka, Japan). The light intensity in the laboratory was measured with a photo recorder (T&D Corp, Nagano, Japan) before starting the tests.

**Recording mosquito flight patterns in the presence and absence of CO₂.** Colonized *Aedes albopictus* (Skuse), *Aedes aegypti* L., *Culex quinquefasciatus* Say, *Anopheles stephensi* Liston were used for this part of the study. Both *Aedes* species were collected in Singapore during 2000. *Culex quinquefasciatus* was collected in Vietnam during 2003. *Anopheles stephensi* was collected in India during the 1950s. Mosquitoes were maintained in the laboratory after collection at 27 °C, 70 % RH, and a 16L8D photoperiod regime. Sixty, ca. 10-day old non blood fed females were released into the test cage in the morning between 07:00 and 10:00 hr. The number of mosquitoes landing on the black plastic plate containing the photoelectric sensors was counted and recorded every 60 min for 72 h. Carbon dioxide was not released during the first 24 h of the experiment and was then released for 2 min every 15 min during the last 48 h of the experiment. Mosquitoes were provided a 1% sugar solution throughout the test. The test was carried out in a room maintained under a 16L8D photoperiod where the first and last 1 h of the scotophase were in twilight, 25-27°C, and > 60%RH regime.

**Recording the flight behavior of field-collected and colonized *Culex tritaeniorhynchus*.** Third and fourth instar larvae of *Culex tritaeniorhynchus* Giles were collected from a rice paddy field in Isahaya, Nagasaki, Japan on 14 July 2003. Larvae were reared to adults under the laboratory conditions described above. Sixty, ca. 7-day old non blood fed female mosquitoes were released into the test cage at 10:00 hr. The number of mosquitoes landing on the black plastic plate containing the photoelectric sensors was recorded as described above. The flight behavior of colonized *Cx. tritaeniorhynchus* (collected from Nishiarita, Japan in 1998) was recorded in the same manner and compared with the flight behavior of the wild-caught females. Sixty, ca 7-day old nulliparous females and sixty, ca 14-day old, parous (in their 2nd gonotrophic cycle) colonized females were used for this test. Three replicates were carried out for each recording for both wild and laboratory colonies.
Results and Discussion

The changes in CO₂ concentration in the air ca. 1cm above the sensors inside the cage are shown in Fig. 4. The CO₂ concentration rapidly increased to more than 5000 ppm (detective limit by the detector) immediately after the release, maintained a high value (> 5000 ppm) for 2-3 min, and recovered to the baseline concentration of ca. 2000 ppm rapidly after closing the solenoid valve. The CO₂ baseline concentration inside the cage was the balance of the release rate of CO₂ and the ventilation capacity with a fan and was slightly higher than that in the laboratory where the CO₂ baseline was 1600 - 1800 ppm.

Figure 5 shows the changes in the intensity of illumination in the laboratory. Intensity of illumination during the photophase, twilight phase, and the scotophase was ca. 490 lux, 7 lux, and 0 lux, respectively.

Diel changes in mosquito numbers recorded by photoelectric sensors for several laboratory colonies are shown in Fig. 6. There were significant increases in mosquito activity when CO₂ was intermittently released for all species examined in the study (2 Way ANOVA, p < 0.05). This indicates that CO₂ provided a strong stimulatory effect for all mosquito species tested. Slight amounts of flight activity were recorded for both *Aedes* species (Fig. 6A and B) even when CO₂ was not released, indicating that the presence of heat and color (the black plastic recording disk) may have stimulated flight activity in these normally diurnal species. The diel activity peaks in *Aedes* mosquitoes, however, were not clearly shown in the absence of CO₂. The stimulating effect of CO₂ was more prominently shown for *Cx. quinquefasciatus* (Fig. 6C) and *An. stephensi* (Fig. 6D) than for the *Aedes* mosquitoes. The difference in the reaction of mosquitoes to CO₂ might be attributable to the difference in the importance of attractant cues, such as heat, color and CO₂, to each mosquito species, that is to say, *Aedes* mosquitoes might use heat and visual information as attractant cues more than *Culex* and *Anopheles*. Two peaks of *Ae. albopictus* diel activity were observed, one from 10:00-12:00 and the second from 14:00-16:00 hr (Fig 6A). This corresponds to the diel host-seeking rhythms in field and laboratory for the same species in Singapore reported by Ho et al. (1973). Numerous studies have demonstrated that *Ae.*
Aedes albopictus rarely blood feed at night and usually exhibited a bimodal diurnal host-seeking rhythm (Hawley 1988). Yee and Foster (1992) reported diel sugar-feeding and host-seeking rhythms in *Ae. albopictus* in laboratory. *Aedes albopictus* host seeking behavior in their study, however, showed a higher activity throughout the night. Similar observations were reported by Higa et al. (2000). No *Ae. albopictus* night time host-seeking activity was observed in the present study.

Three peaks of *Ae. aegypti* flight behaviors were observed at 6:00-7:00, 11:00-12:00 and 15:00-17:00 hr (Fig 6B). These results correspond well with field observations for the same species in Trinidad (Chadee and Martinez 2000) where it was observed that landings on human bait were trimodal, with consistent peaks at 7:00, 11:00 and 17:00. A similar trimodal activity pattern was observed for *Ae. aegypti* by Atmostoedjono et al. (1972) in Indonesia and by Corbet and Smith (1974) in Tanzania. Chadee and Martinez (2000) reported an increasing number of *Ae. aegypti* females landing on human hosts during the night in an urban test area, while no nocturnal activity was observed in a rural test area. These authors attributed the above difference to the adaptation of insects to electrical lighting in the urban area. Their hypothesis was supported by Taylor and Jones (1969) who reported that both light-on and light-off had phase-setting effects to the flight activity of *Ae. aegypti* and the total amount of flight activity was correlated with the duration of light in the 24 h period. The above theory might also be applicable to the nocturnal activity of *Ae. albopictus*. The night time activity of both *Aedes* species in the present study, however, was very low, suggesting that the dark conditions in our study (Fig. 5) caused no phase-setting or stimulating effects to the insects.

*Culex quinquefasciatus* and *An. stephensi* both showed typical night time activity patterns in our study. A prominent activity peak was observed for *Cx. quinquefasciatus* at around 1:00-5:00 hr and no daytime activity was recorded (Fig. 6C). Most field studies reported that *Cx. quinquefasciatus* has a broad nocturnal activity pattern with several minor peaks around 22:00-4:00 hr (Mahanta et al. 1999, Pipitgool et al. 1998). A similar high and continuous nocturnal activity was observed for *An. stephensi* (Fig. 6D). In contrast to *Cx. quinquefasciatus*, however, multiple nocturnal activity peaks were observed for *An. Stephensi*. 
Changes in the diel flight activity of field-collected and colonized Cx. tritaeniorhynchus are shown in Fig. 7. A small activity peak at 22:00 hr (just after the start of scotophase) and a larger activity peak at 06:00-07:00 hr (just after start of photophase) were observed in the field-collected mosquitoes (Fig. 7A). The same general bimodal activity pattern was observed for the colonized Cx. tritaeniorhynchus females (Fig. 7B and C). The overall activity level, however, was significantly lower for the colonized parous mosquitoes (Repeated Measure ANOVA, df = 1, p = 0.0176). A similar bimodal activity pattern has been long recognized for Cx. tritaeniorhynchus in the field (Wada 1969, Sonoda 1971). Sonoda (1971) reported that the height of each bimodal peak fluctuated regularly according the population trend and the parity rate in the evening peak was lower than that in the morning peak, suggesting that the above fluctuation related the age composition of mosquito population. Our results, on the other hand, seem to show that Cx. tritaeniorhynchus intrinsically has a bimodal host seeking pattern despite of their age, although the fight activity levels seemed to be different between nulliparous and parous adults.

Heat, dark color, and carbon dioxide have long been demonstrated as effective mosquito attractants (Takken and Kline 1989, Pates et al. 2001, Kline 2002). The addition of attractants, especially carbon dioxide, in the present study greatly enhanced mosquito flight activity. In the absence of a stimulatory substance, background flight activity for nocturnally active species (Figs. 6C and D) was not recorded by our device. Several automatic devices for recording mosquito activity patterns have been developed since the 1960s. Most of these devices record flight. Jones et al. (1967) used a small recording chamber to monitor activity patterns of individual females. Chiba et al. (1981) recorded the circadian flight activity of mosquitoes with an actograph modified by adding a phototransister and far-red beam. Yee and Foster (1992) monitored the sugar-feeding rhythms of mosquitoes with a copper landing platform which completes a circuit during the mosquito feeding. Recording of mosquito host seeking behavior has been most successful with visual or video observations using animal or human bait. The automatic recording of mosquito activity patterns are most successful in the absence of a host because, once blood
fed, mosquito activity patterns change drastically. The recording device reported in the present study will provide an alternate method to record mosquito activity patterns in the presence of stimulatory effects such as CO₂. The diel activity patterns of non blood fed *Ae. albopictus, Ae. aegypti, Cx. quinquefasciatus, Cx. tritaeniorhynchus,* and *An. stephensi* females recorded by the device compared well with the published diel activities for these species in the field. The device reported here can be used to evaluate the activity patterns of field-collected mosquitoes as well as other hematophagous species that utilize CO₂, heat, and vision as major cues for orientation to hosts.
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Fig. 1. Schematic view of an automatic recording device to quantify mosquito activity patterns. The device is composed of four photoelectric sensors and amplifiers, a programmable controller unit, a power supplying unit, and monitoring software installed in a computer system. Mosquitoes passing through the sensor windows, composed of an infra-red LED with a light receiving element on both sides, are detected and a signal is transmitted to the computer.

Fig. 2. Schematic view of the water bath unit of the recording device. A Styrofoam box with 3.5 liter of water kept at 35 °C by a heater is covered with a white plastic panel containing 4 photoelectric sensors, the bottoms of which are covered with black plastic plates. The top surface of the Styrofoam box, except for the sensor plates, is covered with another white plastic plate to concentrate the heat onto the black plates.

Fig. 3. Schematic view of the mosquito holding cage and recording device. Carbon dioxide was released at the rate of 500 ml/min into the cage through the silicone tube. The release of CO₂ was regulated by solenoid valve controlled by timer. The air inside the cage was ventilated by the electric fan located at the bottom corner of the cage. Mosquitoes inside the cage were provided a 1% sugar solution during the test.

Fig. 4. Changes in the CO₂ concentration inside the test cage. Collection of CO₂ was done from the air ca 1cm above the sensors.

Fig. 5. Changes in the intensity of illumination in the laboratory.

Fig. 6. Activity patterns of female (A) *Aedes albopictus*, (B) *Ae. aegypti*, (C) *Culex quinquefasciatus*, and (D) *Anopheles stephensi* detected and recorded by the automatic recording device. The solid line indicates a moving average of two successive counts in the sensors. Carbon dioxide was not released during the first 24 h of the test and was
then released intermittently (for 2 min at 15 min intervals) during the final 48 h of the test.

**Fig. 7.** Activity patterns of (A) Field-collected nulliparous *Culex tritaeniorhynchus* females, (B) Laboratory-reared nulliparous *Cx. tritaeniorhynchus* females, and (C) Laboratory-reared parous *Cx. tritaeniorhynchus* females detected and recorded by the automatic recording device. Each solid bar indicates the standard deviation. Carbon dioxide was released intermittently (for 2 min at 15 min intervals) throughout the experiment.