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Comparative Study on Nocturnal Behavior of *Aedes aegypti* and *Aedes albopictus*

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ABSTRACT Nocturnal behavior of nonblood-fed females of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) was studied using an automatic recording device equipped with a photoelectric sensor. Carbon dioxide, heating, and the contrast of the black and white colors were used as attractive cues for mosquitoes. The nocturnal host-seeking activity positively correlated with the increasing light intensity in both species. *Ae. aegypti* was found to be more sensitive to light than *Ae. albopictus*. The threshold of light intensity for the activation of the nocturnal host-seeking activity was <0.1 lx (≈ 0.01 foot candle) in *Ae. aegypti* and >10 lx (≈ 1 foot candle) in *Ae. albopictus*. Complete darkness during the daytime deactivated the host-seeking activity of both species, irrespective of their increasing flight activity controlled by their intrinsic circadian rhythms. This finding suggested that visual cues are indispensable for host-seeking behavior. The eye parameter value, the product of the ommatidial diameter, and the interommatidial angle were significantly larger in *Ae. aegypti* than those in *Ae. albopictus*, indicating that the eye of *Ae. aegypti* is more adapted to a darker environment.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, nocturnal behavior, photoelectric sensor, eye parameter

SEVERAL STUDIES ON THE BEHAVIOR of *Aedes* mosquitoes have substantiated the evidence that visual information plays an important role in the behavior of *Aedes aegypti* (L.), such as resting, host seeking, and oviposition. Sippell and Brown (1953) reported that visual factors are roughly equivalent to the airborne emanations in attractiveness to the hosts in *Ae. aegypti*. Adult *Ae. aegypti* prefers to rest in darker locations and on dark, nonreflective surfaces such as clothing (Fay 1968, Fay and Prince 1970, Edman et al. 1997). Hecht and Hernandez-Corzo (1963) reported that both blood-fed and nonblood-fed females of *Ae. aegypti* displayed a stronger preference for black areas as resting places than the other nocturnal mosquitoes such as *Anopheles albimanus* Wiedemann, *Anopheles punctipennis* (Say), and *Culex quinquefasciatus* Say.

Ae. aegypti seems to have been a good subject for the study of the mosquito compound eye and vision (Brammer 1970, Brammer and Clarin 1976). However, studies on compound eye physics in relation to mosquito behavior have been surprisingly few (Allan 1994; Muir et al. 1992a, b). Muir et al. (1992b) calculated the eye parameter (p), which is the product of the ommatidial diameter (D) and the interommatidial angle ($\Delta\phi$) in female *Ae. aegypti* and concluded that the eye

parameter of *Ae. aegypti* was optimal for the dim light conditions that they prefer.

Nocturnal behavior of *Ae. aegypti* has been commonly observed through the data collected by field trapping and in laboratory behavioral studies. Chadee and Martinez (2000) reported an increasing number of *Ae. aegypti* females landing on human hosts during the nighttime in the urban areas, whereas no nocturnal activity was observed in the rural areas in Trinidad, West Indies. The authors attributed the difference to the adaptation of the mosquitoes to electrical lighting in the urban areas. Their hypothesis is supported by Taylor and Jones (1969), who reported that both light-on and light-off periods had phase-setting effects on the flight activity of *Ae. aegypti* and that the total amount of flight activity was positively correlated with the duration of light in the 24-h period. This theory might be applicable to the nocturnal behavior of *Aedes albopictus* (Skuse) (Yee and Foster 1992; Higa et al. 2000, 2001). However, few studies have been extensively conducted on the nocturnal behavior and visual factors for *Ae. albopictus* and there also seem to be several unresolved questions in this regard. In the present report, the nocturnal host-seeking behavior of nonblood-fed females of *Ae. aegypti* and *Ae. albopictus* in relation to the light intensity was observed in a laboratory by using an automatic recording device equipped with a photoelectric sensor (Kawada and Takagi 2004). The physical differences in the compound eyes of both these species also are discussed.

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Materials and Methods

General Description of Automatic Recording Device. The recording system described in detail by Kawada and Takagi (2004), composed of a recording unit, a water bath unit for attracting mosquitoes, and a test cage, was used for the experiment. Carbon dioxide (CO_2), heating, and the contrast of black and white were used as attractive cues for mosquitoes. The recording device was composed of four photoelectric sensors and amplifiers, a programmable controller unit, a power supply, and monitoring software (Keyence Corporation, Osaka, Japan). When an attracted mosquito passes through the sensor window, its presence is detected by the sensor and a signal is transferred to the computer via the controller and monitoring software. A water bath unit for the recording device was created from a white Styrofoam box (23 by 30 by 20 cm, 5 cm in thickness) that contained ≈ 3.5 liters of water. The temperature of the water was maintained at $\approx 35^\circ\text{C}$ by a heater with a thermoregulator. The box was covered with a white plastic panel that contained the four 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 by 80 by 80 cm) with white plastic panels that buffered the mosquitoes inside the cage from the external stimulations such as airstreams, light, human movement, heat, and CO_2 . The CO_2 was generated at a flow rate of 500 ml/min by a gas bomb and was released into the cage through a silicone tube, the opening of which was placed ≈ 10 cm above the sensors. The CO_2 release was regulated (2 min on/13 min off) by a solenoid valve controlled by a timer. The air inside the cage was ventilated by an electric fan located in the bottom corner of the cage.

Recording of Nocturnal Host-Seeking Behavior of *Ae. aegypti* and *Ae. albopictus* under Different Light Intensities during Scotophase. Laboratory colonies of *Ae. aegypti* and *Ae. albopictus* were used. Both species were collected in Singapore during 2000. The mosquitoes were maintained in the laboratory after collection at 27°C , 70% RH, and a photoperiod of 16:8 (L:D) h. Fifty 7–10-d-old nonblood-fed females were released in the test cage, and the number of mosquitoes landing on the black plastic plate through the photoelectric sensors was counted. The total number of landings was recorded every 60 min. The mosquitoes were provided a 1% sugar solution throughout the test. The test was carried out in a room maintained under a photoperiod of 14:10 (L:D) h (photophase, 0700–2100 hours; scotophase, 2100–0700 hours), 25 – 27°C , and $>50\%$ RH regime. The light intensity at the floor of the cage was measured by a digital illuminometer (YF-1065, KENIS, Tokyo, Japan). During the test, the light intensity in the scotophase was adjusted by two fluorescent lamps equipped with a dimmer placed ≈ 1 m above the test cage at 0, 0.1, 1, 10, and 100 lx. The light intensity in the light phase was ≈ 170 lx. Nocturnal

activity was evaluated by calculating the ratio of the number of mosquito landing counts in 5 h at the scotophase (2300–0400 hours) to those in 5 h at the previous light phase (1600–2100 hours).

Measurement of Eye Parameter of *Ae. aegypti* and *Ae. albopictus*. Female mosquitoes were killed by chloroform, decapitated, and prefixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate trihydrate buffer (CB), pH 7.3, for 1 h. After the prefixation, the samples were rinsed in 0.1 M CB for 30 min and postfixed with 2% osmium oxide in 0.1 M CB for 1.5 h. The samples were then rinsed in water and dehydrated in a graded series of ethanol and acetone and were finally rinsed with dehydrated acetone for 30 min. Acetone was replaced with propylene oxide, and the samples were immersed in resin solution (epoxy resin + propylene oxide) for 24 h. After the immersion, propylene oxide was evaporated in a drying box for one night. The samples were embedded in epoxy resin and stored in the box with desiccants (silica gel). Sections of $1\text{-}\mu\text{m}$ thickness were cut with an ultra microtome and stained with Azur II (Wako Pure Chemicals, Osaka, Japan). Digital photographs of the sections were taken using a light microscope. The facet diameter and interommatidial angle, the angle between the axes of adjacent ommatidia, were measured based on the photographs of the ommatidial longitudinal sections. The ommatidial axis was considered as a line through the midpoint of the rhabdom and the corneal lens, and the interommatidial angle was measured from the line drawings. Six to eight adjacent ommatidia were selected per sample, and the mean ommatidial diameter (D) and the mean interommatidial angle ($\Delta\phi$) were calculated. The eye parameter (p) was calculated as a product of D and $\Delta\phi$.

Results

Nocturnal Host-Seeking Behavior of Nonblood-Fed Females of *Ae. aegypti* and *Ae. albopictus* under Different Light Intensities during Scotophase. Typical patterns of the changes in the host-seeking activity of nonblood-fed females of *Ae. aegypti* and *Ae. albopictus*, when the light intensity during the scotophase was changed daily from 0 to 100 lx, are shown in Fig. 1. In both species, clear bimodal (*Ae. albopictus*) and trimodal (*Ae. aegypti*) patterns in diel activity were observed, although the overall number of mosquito counts by the sensors for *Ae. aegypti* was $\approx >10$ times larger than that for *Ae. albopictus*. No conspicuous activity was recorded under dark conditions (0 lx), whereas an increase in activity was recorded with an increase in light intensity during scotophase. Activation of the nocturnal activity by the light with different intensities during the scotophase for the nonblood-fed females of *Ae. aegypti* and *Ae. albopictus* is shown in Fig. 2. The activation of nocturnal host-seeking activity with an increase in light intensity during scotophase was more conspicuous in *Ae. aegypti* than in *Ae. albopictus*, when the increasing activity was expressed as the ratio of the number of mosquito counts in 5 h

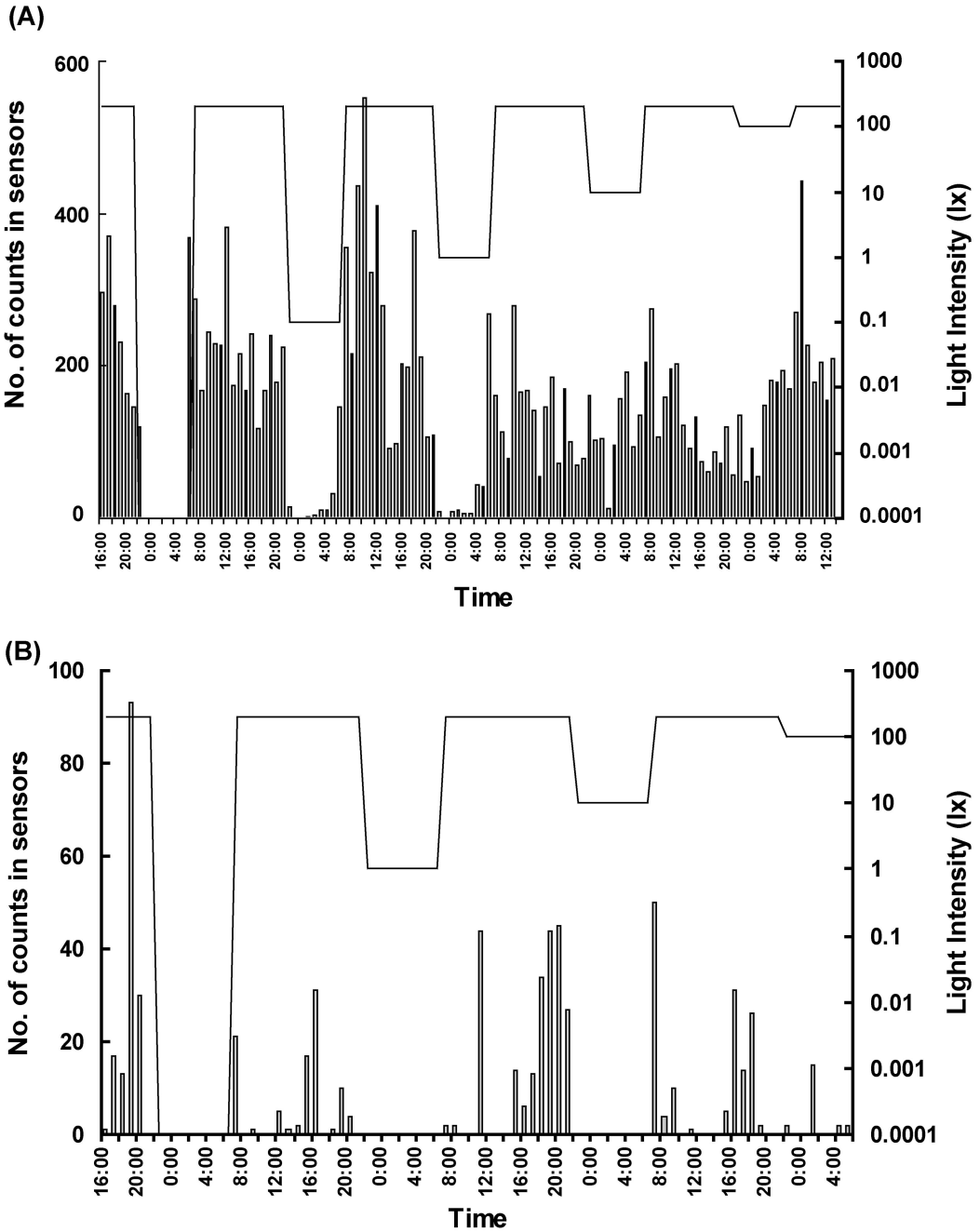


Fig. 1. Typical pattern of the changes in the nocturnal host-seeking behavior of female nonblood-fed (A) *Ae. aegypti* and (B) *Ae. albopictus* under different light intensities during scotophase. Solid line indicates the change in light intensity.

during scotophase (2300–0400 hours) to that in the light phase (1600–2100 hours) of the previous day. According to the statistically significant difference in the increasing nocturnal activity among the different light intensities, the threshold for the activation of host-seeking activity was estimated to be <0.1 lx in *Ae. aegypti* and >10 lx in *Ae. albopictus*. Figure 3 shows the host-seeking activity of *Ae. aegypti* and *Ae. albopictus*

when they were placed under continuous dark conditions for 2 d. In the continuous dark conditions, no other activity peaks were recorded except for the several minor peaks in the morning (0900–1100 hours) and evening (1900 hours) in *Ae. aegypti*. Almost no activity was recorded in *Ae. albopictus*. The host-seeking activity recovered in both species when the light was turned on on the third day in the morning.

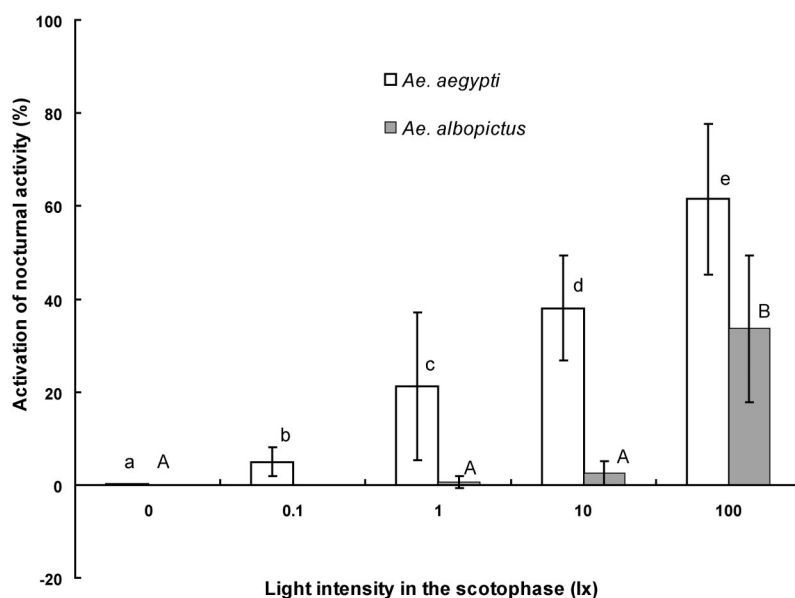


Fig. 2. Activation of the nocturnal activity by the light with different intensities during the scotophase for the nonblood-fed females of *Ae. aegypti* and *Ae. albopictus*. Activation of nocturnal activity (%) = $100 \times [\text{no. of mosquito counts in 5 h at scotophase (2300–0400 hours)}] / [\text{no. of mosquito counts in 5 h at previous light phase (1600–2100 hours)}]$. Solid bars on the graphs represent standard deviations. Values on the graphs followed by the same letter are not significantly different (Tukey's test, $P > 0.05$).

Eye Parameter of *Ae. aegypti* and *Ae. albopictus*. Cross sections of the compound eyes of *Ae. aegypti* and *Ae. albopictus* are shown in Fig. 4. Table 1 shows the D, $\Delta\phi$, and p values of the compound eyes of *Ae. aegypti* and *Ae. albopictus*. A significant difference in the ommatidial diameter (analysis of variance, $F = 9.16$, $df = 1$, $P = 0.0039$) and the interommatidial angle ($F = 89.3$, $df = 1$, $P = 0.0007$) was observed between the two species. The eye parameter was 2.08 ± 0.11 for *Ae. aegypti* and 1.59 ± 0.12 for *Ae. albopictus*.

Discussion

It was interesting to note that the overall host-seeking activity in *Ae. albopictus* was $<1/10$ of that in *Ae. aegypti* under the present test conditions. A similar difference was observed in the host-seeking activity between the two species in the laboratory experiment by Yee and Foster (1992), in which the total number of mosquito attacks on the host (a caged rat) during the light phase (0800–2400 hours) in *Ae. aegypti* ($n = 1168$) was approximately twice that of *Ae. albopictus* ($n = 627$). This behavior might simply explain the biting nature of both the species or suggest that additional attractive cues, such as movement of the host, are necessary for more effective orientation of *Ae. albopictus* to its hosts.

Ae. aegypti is highly sensitive to dim light and can be active even during the night when the light intensity is >0.1 lx (Fig. 3). In the dark, however, no conspicuous host-seeking activity was observed, with the exception of minor peaks that were recorded in the morning (0900–1100 hours) and evening (1900 hours)

in *Ae. aegypti*. The above-mentioned results indicate that both species could not effectively orient to the attractive source in the dark conditions, irrespective of their increasing daytime flight activity controlled by their intrinsic circadian flight rhythms as reported by Taylor and Jones (1969). The results also suggest that the diurnal host-seeking activities also might be affected by the daytime light intensity. Chadee and Martinez (2000) reported that the light intensity at both the urban and rural sites in Trinidad, West Indies, were similar during the period from 0500–1800 h, whereas significantly higher intensities, ≈ 0.3 –1 lx (3–10 foot candles), were measured at urban sites from 1900 to 0100 hours and that the increasing light intensities caused an increase in the nocturnal bites by *Ae. aegypti*. They concluded that the increase in the nocturnal feeding activity was caused by the adaptation of mosquitoes to light. The present results seem to agree with their findings. However, it seems that the nocturnal feeding might not be caused by the evolutionary adaptation to light, but by the intrinsic reaction of *Ae. aegypti* to light. Therefore, the potential risks of increased transmission opportunities of mosquito-borne diseases, such as dengue fever, exist wherever *Ae. aegypti* inhabits, irrespective of the intrinsic potency for adaptation to light in the mosquito population.

The activation of the host-seeking activity according to the increase in light intensity was lower in *Ae. albopictus* than in *Ae. aegypti*. The threshold light intensity for the activation of *Ae. albopictus* was thought to be >10 times higher than that for *Ae. aegypti*. The p values for both the species support the above-men-

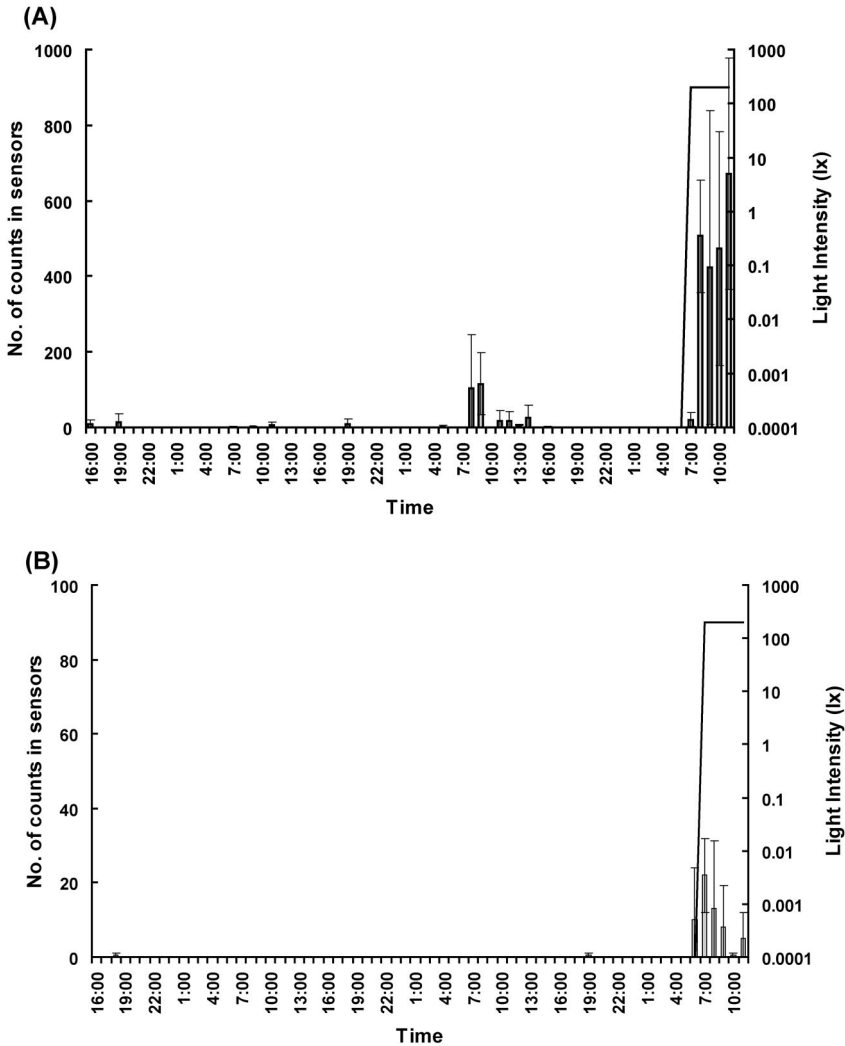


Fig. 3. Host-seeking behavior of female nonblood-fed (A) *Ae. aegypti* and (B) *Ae. albopictus* under continuous dark conditions for 2 d. Solid bars on the graphs represent standard deviations. Solid line indicates the change in the light intensity.

tioned difference. A large portion of the eye of bright-light insects has $0.45 \leq p \leq 1$; eye of several diurnal insects, $1 \leq p \leq 2$; eye of nocturnal insects, $2 \leq p \leq 3$; that is, the dimmer the insect's environment, the larger is its eye parameter (Snyder 1979). There are few reports on the eye parameters of mosquitoes: $p = 3.8$ for *Anopheles gambiae* Giles (Land et al. 1997, Clements 1999) and $p = 1.1$ for *Toxorhynchites brevipalpis* Theobald (Clements 1999). Muir et al. (1992b) calculated the eye parameter of the female *Ae. aegypti* as 1.6, which was the product of ommatidial diameter, interommatidial angle, and a correction factor. Based on the same data as Muir et al. (1992b), Clements (1999) also calculated the eye parameter, without a correction factor, as 1.9. The eye parameters for the above-mentioned three mosquito species seem to explain their behavioral periodicities based on the categorization of Snyder (1979). Moreover, the eye parameter for *Ae. aegypti* is thought to be relatively

larger among the diurnal species, indicating that it is optimal for the dim light conditions that they prefer. In the current study, the eye parameters were calculated by the same method as Clements (1999). There was a slight difference in the parameter for *Ae. aegypti* between our result (2.08) and that by Muir et al. (1992b) (1.9). It is noteworthy that there was a significant difference in both the ommatidial diameters and interommatidial angles between *Ae. aegypti* and *Ae. albopictus*. The eye parameter of *Ae. albopictus* (1.59) indicates that this species adapts to or prefers a brighter environment than *Ae. aegypti*. Several studies have demonstrated the physiological, ecological, and behavioral differences between *Ae. aegypti* and *Ae. albopictus* (Hawley 1988). The most interesting and important difference among them has been the ecological segregation of the two species. Generally, Asian *Ae. aegypti* is a domestic and endophagic mosquito and shows a greater preference for indoor

(A)



(B)



Fig. 4. Longitudinal section of the ommatidia of female (A) *Ae. aegypti* and (B) *Ae. albopictus*.

Table 1. Ommatidial diam, interommatidial angle, and eye parameter of *Ae. aegypti* and *Ae. albopictus*

Sample	No. of ommatidium used	Ommatidial diam(μm)	Interommatidial angle(°)	Eye parameter ^a
<i>Ae. aegypti</i>				
1	8	18.02	6.29	2.08 (0.11)
2	7	19.04	6.50	
3	6	18.92	6.40	
Avg (95% FL)		18.66 (0.63)	6.40 (0.12)	
<i>Ae. albopictus</i>				
1	7	16.28	5.17	1.59 (0.12)
2	8	17.56	5.50	
3	7	17.43	5.29	
Avg (95% FL)		17.09 (0.79)	5.32 (0.19)	

^a Eye parameter = ommatidial diam (μm) × interommatidial angle (rad).

breeding than does *Ae. albopictus* (Hawley 1988, Edman et al. 1997, Ishak et al. 1997). Higa et al. (2001) attributed the exophagy of *Ae. albopictus* to the environmental differences between the inside and outside of a house. However, they could not clearly identify the factors influencing the exophagy of *Ae. albopictus*. The present results might be one of the most appropriate answers to the exophagy differences.

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