

Fig. 4. Changes in the CO_2 concentration inside the test cage. Collection of CO_2 was done from the air ≈ 1 cm above the sensors.

from 1400 to 1600 hours (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. 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. Ae. 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 current study.

Three peaks of Ae. aegypti flight behaviors were observed at 0600–0700, 1100–1200 and 1500–1700 hours (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 0700, 1100, and 1700. A similar trimodal activity pattern was observed for Ae. aegypti by Atmosoedjono 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, whereas no nocturnal activity was observed in a rural test area. These authors attributed

the above-mentioned 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-mentioned theory also might be applicable to the nocturnal activity of Ae. albopictus. The night time activity of both Aedes species in the current 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 ≈0100-0500 hours, and no day-time activity was recorded (Fig. 6C). Most field studies reported that Cx. quinquefasciatus has a broad nocturnal activity pattern with several minor peaks ≈2200-0400 hours (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.

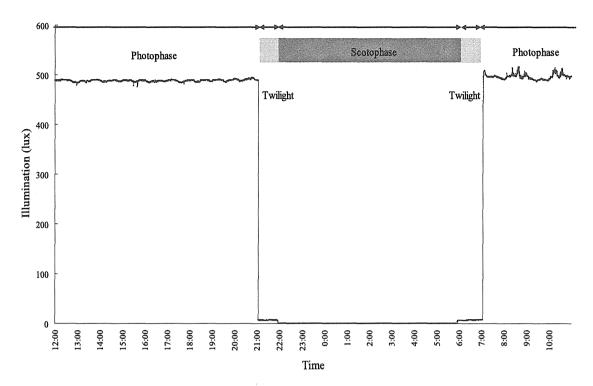


Fig. 5. Changes in the intensity of illumination in the laboratory.

7. A small activity peak at 2200 hours (just after the start of scotophase) and a larger activity peak at 0600-0700 hours (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 measures 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-mentioned fluctuation related the age composition of mosquito population. Our results, however, seem to show that Cx. tritaeniorhynchus intrinsically has a bimodal hostseeking pattern despite of their age, although the flight 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 current study greatly enhanced mosquito flight activity. In the absence of a stimulatory substance, background flight activity for nocturnally active spe-

cies (Fig. 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 that completes a circuit during the mosquito feeding. Recording of mosquito hostseeking behavior has been most successful with visual or video observations by 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 current 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 use CO₂, heat, and vision as major cues for orientation to hosts.

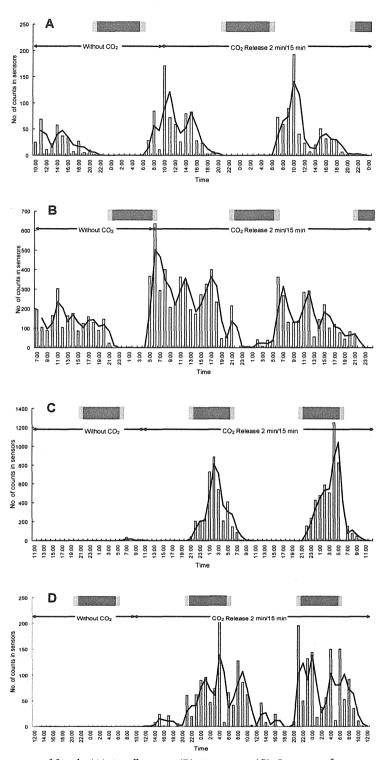
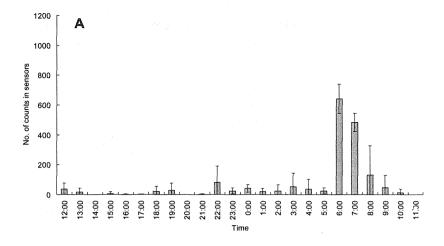
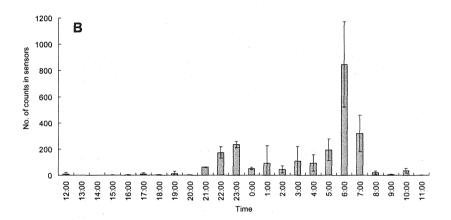


Fig. 6. Activity patterns of female (A) Ae. albopictus, (B) Ae. aegypti, (C) Cx. quinquefasciatus, and (D) An. stephensi detected and recorded by the automatic recording device. The solid line indicates a moving average of two successive counts in the sensors. Carbon dioxide was not released during the 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.





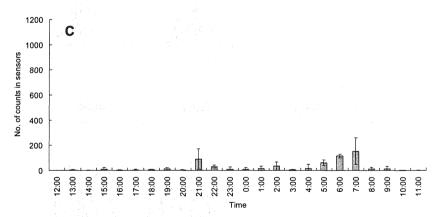


Fig. 7. Activity patterns of (A) field-collected nulliparous Cx. tritaeniorhynchus females, (B) laboratory-reared nulliparous Cx. tritaeniorhynchus females, and (C) laboratory-reared parous Cx. tritaeniorhynchus females detected and recorded by the automatic recording device. Each solid bar indicates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

Acknowledgment

We thank S. Saita, M. Hasegawa, and E. Kawashima (Department of Vector Ecology and Environment, Institute of

Tropical Medicine, Nagasaki University, Nagasaki, Japan), T.B.O. Argueta (Ministry of Public Health and Social Assistance, Verbena, Guatemala), K. Ikari (SunAim Inc., Saga,

Japan), and K. Shimabukuro (Teikyo University of Science, Yamanashi, Japan) for rearing and providing the experimental insects and for assistance to the study.

References Cited

- Atmosoedjono, S., P.F.D. van Peenan, R. See, and J. S. Sorono. 1972. Man-biting activity of Aedes aegypti in Djakarta, Indonesia. Mosq. News 32: 467–469.
- Bowen, M. F. 1992. Patterns of sugar feeding in diapausing and nondiapausing *Culex pipiens* (Diptera: Culicidae) females. J. Med. Entomol. 29: 843–849.
- Burkett, D. A., J. F. Butler, and D. L. Kline. 1998. Field evaluation of colored light-emitting diodes as attractants for woodland mosquitoes and other diptera in north central Florida. J. Am. Mosq. Control Assoc. 14: 186–195.
- Chadee, D. D., and R. Martinez. 2000. Landing periodicity of Aedes aegypti with implications for dengue transmission in Trinidad, West Indies. J. Vector Ecol. 25: 158-163.
- Chiba, Y., C. Yamakado, and M. Kubota. 1981. Circadian activity of the mosquito Culex pipiens molestus in comparison with its subspecies Culex pipiens pallens. Int. J. Chronobiol. 7: 153-164.
- Corbet, P. S., and S. M. Smith. 1974. Diel periodicities of landing of nulliparous and parous Aedes aegypti (L.) at Dar es Salaam, Tanzania (Diptera: Culicidae). Bull. Entomol. Res. 64: 111–121.
- Gouck, H. K., and C. E. Schreck. 1965. An olfactometer for use in the study of mosquito attractants. J. Econ. Entomol. 58: 589–590.
- Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. (Suppl. 1) 4: 1–39.
- Higa, Y., Y. Tsuda, N. Tuno, and M. Takagi. 2000. Tempospatial variation in feeding activity and density of Aedes albopictus (Diptera: Culicidae) at peridomestic habitat in Nagasaki, Japan. Med. Entomol. Zool. 51: 205–209.
- Ho, B. C., Y. C. Chan, and K. L. Chan. 1973. Field and laboratory observations on landing and biting periodicities of Aedes albopictus (Skuse). Southeast Asian J. Trop. Med. Public Health 4: 238–244.
- Jones, M.D.R. 1981. The programming of circadian flight activity in relation to mating and the gonotrophic cycle in the mosquito, *Aedes aegypti*. Physiol. Entomol. 6: 307–313.
- Jones, M.D.R., M. Hill, and A. M. Hope. 1967. The circadian flight activity of the mosquito Anopheles gambiae: phase setting by the light regime. J. Exp. Biol. 47: 503-511.
- Kline, D. L. 2002. Evaluation of various models of propanepowered mosquito traps. J. Vector Ecol. 27: 1–7.

- Kline, D. L., J. R. Wood, and J. A. Cornell. 1991. Interactive effects of 1-octen-3-ol and carbon dioxide on mosquito (Diptera: Culicidae) surveillance and control. J. Med. Entomol. 28: 254–258.
- Klowden, M. J., and A. O. Lea. 1984. Blood feeding affects age-related changes in the host-seeking behavior of Aedes aegypti (Diptera: Culicidae) during oocyte maturation. J. Med. Entomol. 21: 274–277.
- Mahanta, B., R. Handique, P. Dutta, K. Narain, and J. Mahanta. 1999. Temporal variations in biting density and rhythm of *Culex quinquefasciatus* in tea agro-ecosystem of Assam, India. Southeast Asian J. Trop. Med. Public Health 30: 804–809.
- Parker, M., A. L. Anderson, and M. Slaff. 1986. An automatic carbon dioxide delivery system for mosquito light trap surveys. J. Am. Mosq. Control Assoc. 2: 236-237.
- Pates, H. V., W. Takken, K. Stuke, and C. F. Curtis. 2001. Differential behaviour of Anopheles gambiae sensu stricto (Diptera: Culicidae) to human and cow odours in the laboratory. Bull. Entomol. Res. 91: 289-296.
- Pipitgool, V., P. Waree, P. Sithithaworn, and W. Limviroj. 1998. Studies on biting density and biting cycle of *Culex quinquefasciatus*, Say in Khon Kaen City, Thailand. Southeast Asia J. Trop. Med. Public Health 29: 333–336.
- Posey, K. H., D. R. Barnard, and C. E. Schreck. 1998. Triple cage olfactometer for evaluating mosquito (Diptera: Culicidae) attraction responses. J. Med. Entomol. 35: 330 – 334.
- Sonoda, H. 1971. Observations on the diurnal change of Culex tritaeniorhynchus (in Japanese). Jpn. J. Sanit. Zool. 22: 45–48.
- Takken, W., and D. L. Kline. 1989. Carbon dioxide and 1-octen-3-ol as mosquito attractants. J. Am. Mosq. Control Assoc. 5: 311–316.
- Takken, W., J. J. van Loon, and W. Adam. 2001. Inhibition of host-seeking response and olfactory responsiveness in Anopheles gambiae following blood feeding. J. Insect Physiol. 47: 303–310.
- Taylor, B., and M. D. Jones. 1969. The circadian rhythm of flight activity in the mosquito Aedes aegypti (L.). The phase-setting effects of light-on and light-off. J. Exp. Biol. 51: 59-70.
- Wada, Y. 1969. Ecological studies of Culex tritaeniorhynchus summorosus (Diptera: Culicidae) I. Biting rhythm of the mosquito. Jpn. J. Sanit. Zool. 20: 21–26.
- Yee, W. L., and W. A. Foster. 1992. Diel sugar-feeding and host-seeking rhythms in mosquitoes (Diptera: Culicidae) under laboratory conditions. J. Med. Entomol. 29: 784–791.

Received 5 January 2004; accepted 21 June 2004.

